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Investigations into inflammation and central sensitization in CRPS

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A BURNING MATTER

Investigations into inflammation and central sensitization in CRPS

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof. dr. F.A. van der Duyn Schouten,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
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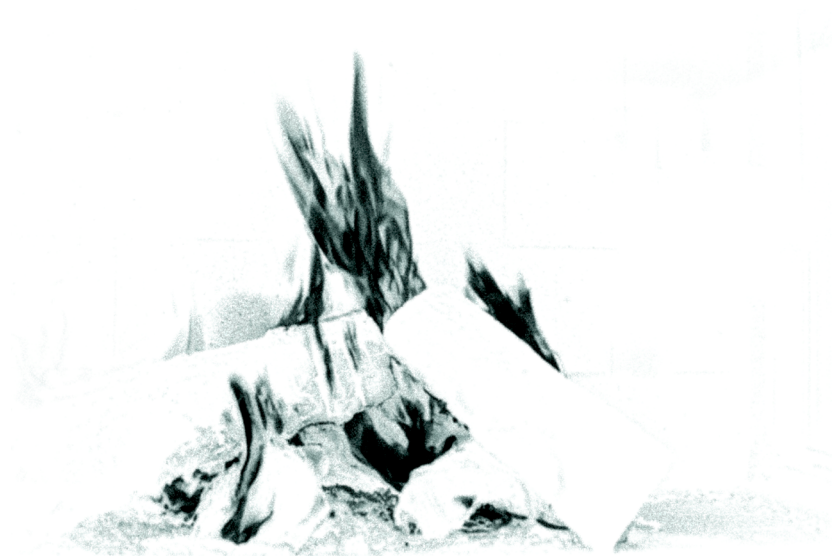prof. dr. S.A. Loer

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Contents

INTRODUCTION TO THIS THESIS

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"Hiram Weston, aged 42, Co. E, 18. th Mass., enlisted May, 1861. Healthy until wounded, in the Wilderness, May 5, 1864. He was moving at a double quick and was shot in the left arm [...] The ball passed over the nerves, and injured the ulnar nerve especially. [...] The pain which began has never left him."

"It is now days since this man was shot. [...] Immediately after the wounding, the whole limb swelled; but this rapidly subsided, and the hand was no larger than its fellow, until about the fortieth day, when it became rapidly oedematous. The pain has consisted all along of darting pangs from below or under the elbow, down into the hand, but not in the anterior surface of the forearm. In the hand, the pain is burning and tingling, or, as he phrases it, "prinkling". [...] It is intense, and is increasing. [...] The burning lies in the whole of the fingers, back and front, except the little finger, which is devoid of sensation; but it is worst in the palm. The entire hand is sore to touch everywhere; [...] The hand is swollen. The palm is red and dotted with patches of thickened epithelium. [...] The junctions of the fingers and the crease at the base of the thumb are ulcerated, and in two places there is pus under the palmar cuticle. The nails are laterally much arched, the skin at their bases is retracted, and at their extremities the line of union with the skin is deeply notched. The back of the hand is eczematous, and mottled in tint. The joints are exquisitely tender, and very stiff and swollen."

(Silas Weir Mitchell, 1864)

The above mentioned case is one of the first descriptions of what we would now call Complex Regional Pain Syndrome (CRPS). According to reports, a case of CRPS was reported for the first time by Ambriose Paré (1510-1590), describing severe pain and weakness following phlebotomy in his patient king Charles IX (1). One of the first case series in scientific literature was presented by Silas Weir Mitchell (1829-1914), an American civil war surgeon describing burning pain, sensory, trophic and motor disturbances in patients following nerve injuries (2). Mitchell and his colleagues, described enduring cases *"of suffering as yet undescribed, and so frequent and terrible as to demand from us the fullest description"*. Mitchell introduced the term "causalgia" for this complaint.

An important contribution to scientific research for this disease comes from the German Surgeon Paul Hermann Martin Sudeck (1866-1945), who in a lecture presented at the 29th Congress of the German Surgical society in Berlin on April 18th 1900, presented cases of rapidly developing patchy osteoporosis using x-ray findings, and related this to the development of acute inflammation: *"in acute inflammatory*

conditions of the bones and joints, the atrophy occurs with quite striking rapidity to a significant extent and, in fact, not only in the bone directly affected but also in the neighbouring bone parts which are functionally dependent on the diseased bone" (3).

Reflex Sympathetic Dystrophy or RSD (first introduced by the famous French surgeon Rene Leriche (1879-1955)) (4) has been one of the most frequently used terms of what we now call CRPS. Reflex Sympathetic Dystrophy refers to the notion that this complaint is related to increased activity of the sympathetic nervous system following trauma to sensory nerves. The discussion evolving around this description is illustrative for the manner in which the lack of a uniform, accepted pathophysiological mechanism has been handled in scientific discourse about CRPS. The changes in the name of this disease partially coincided with the current or local (medical) view of the complaint, reflecting to a certain extent the presumed underlying pathophysiological mechanism (e.g. algoneuro-dystrophy, chronic traumatic edema, post-traumatic osteoporosis, reflex neurovascular dystrophy, traumatic vasospasm; see for further details chapter 2). Taken together, about 79 different names can be found in English literature referring to what is now called CRPS. In an attempt to bring uniformity to the naming and classification of this disease, members of the scientific community under the umbrella of the International Association for the Study of Pain (IASP) introduced the descriptive term Complex Regional Pain Syndrome (CRPS) (5). A distinction as made between CRPS type I, previously called RSD, and type II in case of established major nerve damage (6). The most recent definition of this complaint as described in the IASP's Classification of Chronic Pain now reads:

> *"CRPS-1 is a syndrome characterized by a continuing (spontaneous and/or evoked) regional pain that is seemingly disproportionate in time or degree to the usual course of pain after trauma or other lesion. The pain is regional (not in a specific nerve territory or dermatome) and usually has a distal predominance of abnormal sensory, motor, sudomotor, vasomotor edema, and/or trophic findings. The syndrome shows variable progression over time."*
> (Definition of CRPS in the IASP's Classification on Chronic Pain (6))

As was the case for the pathophysiological perspectives and naming of this disease, so did the clinical diagnostic criteria of CRPS change in the course of time (7;8). The use of different diagnostic methods has most likely led to differences in included patient samples in studies and has influence epidemiological parameters of this disease such

Table 1: Occurrence of signs and symptoms in a sample of 681 CRPS I patients meeting the IASP (Orlando) criteria from 5 medical centers in the Netherlands. (11)

Signs and symptoms (Total n = 681)	n	Positive	%
Sensory			
Spontaneous pain	681	624	*91.6*
Increasing pain after exercise	675	643	*95.3*
Allodynia after light touch	676	260	*38.5*
Hyperesthesia	676	238	*35.2*
Hyperalgesia	675	350	*51.9*
Vasomotor			
Color change/difference	678	378	*55.8*
Temperature difference	673	367	*54.5*
Sudomotor/edema			
Transpiration disturbance	676	136	*20.1*
Edema	679	331	*48.7*
Trophic			
Hair growth change	642	159	*24.8*
Nail growth change	642	150	*23.3*
Trophic skin disturbance	643	180	*28.0*
Motor			
Limitation of movement	672	497	*74.0*
Dystonia	637	124	*19.5*
Tremor	637	75	*11.8*

as incidence and prevalence rates. Based on the most sensitive diagnostic criteria (9), it is assumed that approximately 4300 patients develop CRPS each year in the Netherlands, with a heterogeneous profile of presented signs and symptoms, disease durations and severity (table 1) (10).

Considerable progression in understanding of the disease mechanisms and development of treatment methods has been made in recent years. Currently it is assumed that different pathophysiological mechanisms might be at work in individual patients with CRPS, or co-occur in the same patient. An overview of these perspectives and related treatment methods (although not exhaustive) can be found in table 2. For further discussion on issues related to diagnosis, pathophysiology and treatment, please refer to chapter 2 of this thesis.

Despite the fact that considerable progression in the development of treatment methods has been made in recent years, for a large proportion of CRPS patients available therapies remain insufficient. As a consequence, patients are at risk of the condition to become chronic, and therewith suffer prolonged impairment of quality of life and ability to participate in society (19). For this challenging condition, research

Table 2: Disease mechanisms proposed to be involved in CRPS (12), related to presented signs and symptoms, pathological findings (13-18) and proposed treatment (7). Symptomatic treatment with analgesics and physical therapy is advised. The mechanisms are believed to overlap, whereby changes are suggested to develop in the presented order over time, however disease course and clinical presentation differs largely for the individual patient (Chapter 2).

Disease mechanism	Signs and symptoms	Suggested pathology	Proposed treatment related to mechanism
Immune mediated inflammation	Pain, swelling, impaired function, increased temperature, redness	IL-6, TNF-α, tryptase, IL-8, GFAP, MCP-1 ↑ IL-4, IL-10 ↓	Corticosteroids, TNF-α inhibitors
Neurogenic mediated inflammation	Pain, hyperesthesia, allodynia	SP ↑ CGRP ↑	Topical capsaicin
Oxidative stress	Increased pain sensitivity, swelling, impaired function, increased temperature, redness	Free radicals ↑	Free radical scavengers: DMSO, N-Acetylcysteine, vitamin C
Autonomic disturbances	Trophic changes (nail, hair, skin), sweating ↑, vasoconstriction	Sympathetic neurotransmitters ↑, sensitivity of α-adrenergic receptors ↑	
Vascular dysfunction	Cold extremity, discoloration	Endothelial dysfunction, hypoxia, NO synthase ↓, endothelin-1 ↑	α-1 adrenergic blockers, calcium influx blockers, ketanserin
Central sensitization	Allodynia, hyperalgesia, wind-up	Glutamate ↑, activation of NMDA receptors	NMDA receptor antagonist (ketamine)
Cortical reorganisation	Pain, hyperalgesia, movement disorders	Disturbance of cerebral representation and motor processing	Motor imagery, mirror therapy

is required to better understand factors underlying the disease mechanism, increase insight into the efficacy of established and novel treatment methods, and develop assessment tools for disease severity.

The aim of this thesis was to expand existing knowledge in these areas, whereby we focussed on the role of inflammation, oxidative stress, central sensitization and anti-inflammatory therapies in patients with CRPS.

Studies in this thesis

In **chapter 2,** a general introduction is provided about current knowledge on CRPS and suggestions for future research are presented.

In **chapter 3** a study describing co-occurrence of comorbidities with CRPS is presented, in order to assess the broader scope of disease burden in these patients and discuss co-morbid conditions in CRPS in light of current knowledge about disease mechanism in CRPS (20). In light of the changing views about the involvement of inflammation, a comprehensive assessment of anti-inflammatory treatment approaches of CRPS-1 will be presented.

In **chapter 4**, a systematic review is described evaluating the efficacy of anti-inflammatory treatment approaches for prevention of CRPS, reduction of pain, limitations in range of motion and overall clinical improvement in CRPS-1.

In **chapter 5** a study is presented about biomarkers related to features of inflammation in patients with CRPS-1. The aim of the study is to assess the role of oxidative stress in CRPS by measuring levels of specific oxidative stress markers in CRPS patients and compare these to age and gender matched healthy volunteers.

In **chapter 6 and 7**, clinical trials on pharmaceutical interventions for the treatment of CRPS are described.

In **chapter 6**, a study on the effects of activating the cholinergic anti-inflammatory pathway in patients with CRPS is presented. This proof-of-concept study was designed to test the hypothesis that augmenting the availability of ACh by means of the acetylcholinesterase inhibitor pyridostigmine would lead to a reduction of inflammation and affect autonomic changes to reduce signs and symptoms of CRPS.

In **chapter 7**, a randomized clinical trial evaluating the effect of intravenous administration of magnesium on level of impairment, activities, participation and quality of life in CRPS-1 patients is presented. In the central nervous system, magnesium has prominent anti-inflammatory properties and is involved in the inhibition of central sensitisation and is therefore hypothesized to have therapeutic value in CRPS.

Assessment tools to express severity of CRPS and require further validation. In **chapter 8** a method comparison study evaluating the CRPS Severity Score (CSS) in relation to the validated Impairment Level Sum Score (ISS) was performed, evaluating the concurrent validity and responsiveness of the CSS.

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General introduction
Complex Regional Pain Syndrome:
diagnosis, treatment and
future perspectives

2

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Roberto S.G.M. Perez
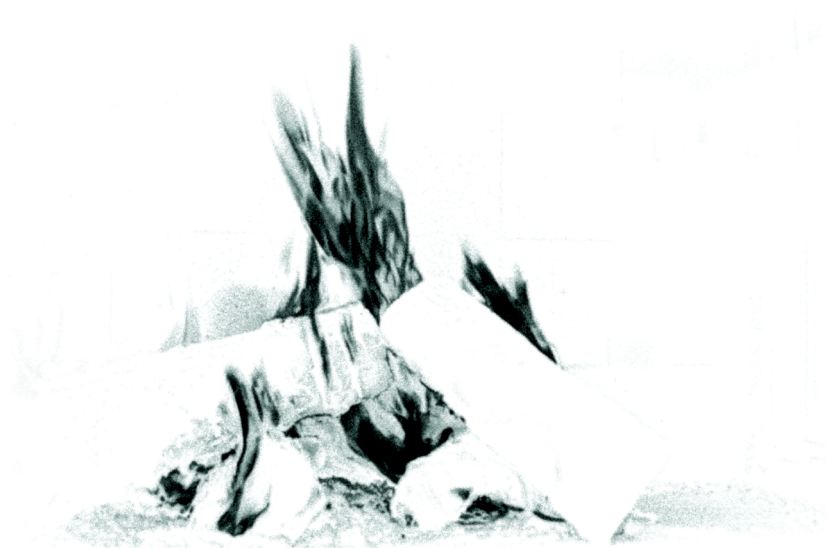
Abstract

Complex regional pain syndrome (CRPS) is a pain syndrome of the extremities that can result in severe disability. CRPS is diagnosed using diagnostic Budapest criteria based on signs and symptoms, whereby sensory, autonomic, vasomotor and motor-trophic disturbances are assessed. Many pathophysiological mechanisms are proposed in the development and disease course of CRPS, starting with exaggerated inflammation and resulting in vascular deregulation, central sensitization and cortical reorganization. Treatment is based primarily on reducing inflammation by medicinal anti-inflammatory therapy and increasing motor function by physiotherapy. Furthermore, pain reduction, normalisation of vasomotor and motor function and psychological interventions might be needed. Future research should focus on the efficacy of anti-inflammatory therapy, effective rehabilitation programmes, modulating neuropathic pain and in cortical reorganisation.

Keywords: Complex regional pain syndrome, diagnosis, treatment, future perspectives

Complex Regional Pain Syndrome (CRPS) is a painful disorder of the extremities, characterised by sensory, autonomic, vasomotor, motor and trophic disturbances (figures 1 and 2). CRPS mostly occurs after a trauma such as a fracture or an operation, but can also develop without a preceding event (1;2). In the Netherlands, approximately 4300 patients develop CRPS each year, whereby females are affected three times more than males and the highest incidence is found between the age of 61 and 70 (3).

Figure 1: Patient with CRPS (florid clinical type) of left hand: characteristics: red (vasomotor), swollen (sudomotor), increased hair growth (trophic disturbance).

Figure 2: patient with CRPS and dystonia of the left foot.

Diagnosing Complex Regional Pain Syndrome

The diagnosis CRPS is based on clinically observed signs and symptoms reported by the patient. Additional laboratory or radiologic assessments provide insufficient basis for diagnosing CRPS, but should be used to exclude other pathologies (such as an unresolved fracture or active infection) (4). Several sets of diagnostic criteria have been proposed over the past decades, some are still being used concurrently. The criteria by Veldman et al. (5) are based on the identification of a limited amount of signs and symptoms which are present predominantly in the acute phase of CRPS. The International Association for the Study of Pain (IASP)-Orlando criteria (6) allow for the diagnosis to be made almost exclusively based on anamnestic information and appear to be more sensitive than the Veldman et al. criteria (7). More specific criteria have

been developed by Bruehl and Harden (8), requiring both anamnestic and observed information regarding sensory, vasomotor, motor, sudomotor and motortrophic disturbances. An adapted version of the latter criteria set has been validated internationally, resulting in a diagnostic tool that combines good specificity with excellent sensitivity for diagnosing CRPS: the Budapest criteria (see table 1) (9). These criteria have recently been adopted by the IASP as the international standard for diagnosing CRPS.

To maximize the comparability of studies of CRPS and ensure agreement between clinicians involved in diagnosing and treating CRPS, a uniform and internationally accepted criteria set such as the Budapest criteria is necessary. Uniform diagnosis and assessment of CRPS could be further improved by identification of disease markers of CRPS type 1 (CRPS-1) and development of objective assessment tools.

Table 1: Diagnostic Criteria for CRPS-1.

Budapest clinical diagnostic criteria for CRPS

1.   Continuing pain, which is disproportionate to any inciting event

2.   Must report at least one symptom in *three out of four of the* following categories:

- *Sensory:*
  reports of hyperesthesia and/or allodynia

- *Vasomotor:*
  reports of temperature asymmetry and/or skin color changes and/or skin color asymmetry

- *Sudomotor/edema:*
  reports of edema and/or sweating changes and/or sweating asymmetry

- *Motor/trophic:*
  reports of decreased range of motion and/or motor dysfunction
  (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)

3.   Must display at least one sign at time of evaluation in *two or more* of the following categories:

- *Sensory:*
  evidence of hyperalgesia (to pinprick) and/or allodynia
  (to light touch and/or deep somatic pressure and/or joint movement)

- *Vasomotor:*
  evidence of temperature asymmetry and/or skin color changes
  and/or asymmetry

- *Sudomotor/edema:*
  evidence of edema and/or sweating changes and/or
  sweating asymmetry

- *Motor/trophic:*
  evidence of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or
  trophic changes (hair, nail, skin)

4.   There is no other diagnosis that better explains the signs and symptoms.

Pathophysiological mechanisms of Complex Regional Pain Syndrome

Neurogenic and immune-mediated inflammation, disproportional oxidative stress, autonomic dysfunction, vasomotor dysfunction, increased neuronal excitation, central sensitisation, cortical reorganisation and psychological predisposition have been proposed as possible disease mechanisms for CRPS. This variety in pathophysiological perspectives combined with possible simultaneous occurrence of different mechanisms in a single patient, might provide an explanation for the heterogeneity of phenotypes described in CRPS literature in recent years.

Neurogenic inflammation

A trauma can easily result in micro-injury of small nerve fibres, which in turn triggers the release of neuropeptides, such as substance P (SP) and calcitonine gene-related peptide (CGRP) in the periphery (10;11). This excessive release of neuropeptides, called neurogenic inflammation, induces vasodilatation and increases vascular permeability leading to plasma extravasation and attraction of immune mediators to the site of injury, resulting in an inflammatory response. Neurogenic as well as immune-mediated inflammation contribute to generation of pain, whereby features of neuropathic sensitisation such as allodynia or hyperesthesia, are observed. In patients with CRPS, elevated levels of CGRP have been found, suggesting a contribution of neurogenic inflammation to the development and maintenance of in this condition (12).

Immune mediated inflammation

Especially during the early stages of the disease course, CRPS signs and symptoms resemble the classical clinical presentation of inflammation: rubor, calor, dolor and functio laesa. Increased levels of inflammatory markers and markers of mast cell activity have been found in blister fluid (e.g. Interleuking [IL]-6, tumor necrosis factor [TNF]-alpha and tryptase) and serum (IL-8, soluble TNF receptors, SP) obtained from the affected extremity of patients with CRPS, compared with both the unaffected extremity and healthy controls (10;13;14). Indications for central inflammatory activity can be found in increased pro-inflammatory cytokines (IL-6) and decrease of anti-inflammatory cytokines (IL-4, IL-10) in cerebrospinal fluid (15). Furthermore, markers of increased glial cell activation (glial fibrillary acidic protein [GFAP] and monocyte chemoattractant protein 1 [MCP1]) found in CRPS, might provide support for neuronal-driven immune activation (16).

Oxidative stress

Excessive, non-self-limiting release of free oxygen radicals leading to oxidative stress has been proposed as a pathophysiological mechanism underlying CRPS. Studies based on animal models have shown that infusion with free radical-inducing agents results in features that resemble clinical signs of CRPS, such as swelling, impaired function, increased temperature, redness and increased pain sensitivity (17). A role for increased oxidative stress is also supported by the observation of elevated levels of oxidative markers in the serum and saliva in patients with CRPS-1 (18). Furthermore, leukocyte accumulation in the affected extremity has been observed in patients with CRPS, probably resulting from increased vascular permeability owing to oxidative stress (19). Tissue hypoxia, as shown by the poor oxygenation of skin in patients with CRPS (20), lends further support to this observation. The efficacy of free radical scavengers such as dimethylsulfoxide and N-Acetylcysteine (21;22), in the treatment of CRPS-1 and the preventive effect of vitamin C after fractures (23;24) in the development of this disease provide indirect evidence for the oxidative stress hypothesis.

Autonomic disturbances

Hyperactivity of the sympathetic nervous system has long been thought to be the primary pathophysiological mechanism of CRPS-1, resulting in increased vasoconstriction, increased sweating and trophic disturbances. However, studies have shown lower levels of sympathetic neurotransmitters in the affected limb (25-27) compared with the unaffected limb, indicating decreased sympathetic activity. Presumably, increased sensitivity of α-adrenergic receptors, probably resulting from reduction of sympathetic neural traffic, would explain this phenomenon (28). The extent in which autonomic disturbances are observed can differ depending on the stage of the disease course (29).

Vascular dysfunction

An alternative hypothesis for the vasomotor instability observed in CRPS patients can be found in endothelial dysfunction resulting in hypoxia, decrease of NO synthase and increase of endothelin-1 (30). This dysfunction leads to clinical features such as a cold affected extremity and discoloration (pale, blue skin), and other features of CRPS associated with oxygen deprivation.

Neuronal excitation and central sensitization

Clinical features displayed in CRPS, such as allodynia, hyperalgesia and wind-up, have been related to the process of central sensitisation (31). This process is triggered by the release of SP, CGRP and glutamate after tissue damage, which in turn activates the normally dormant N-methyl-D-aspartic acid (NMDA) receptor (32). Elevated levels of glutamate found in serum and cerebrospinal fluid of patients with CRPS are suggestive of the involvement of NMDA receptor responses (33). Central sensitisation may also influence the spinal motor circuitry, resulting in movement disorders associated with CRPS, such as dystonia, tremor or myoclonia (34).

Cortical reorganisation

Pain and sensory disturbances in CRPS often spread from the location of the initial trauma to a larger area, sometimes even to another extremity, which might indicate plastic changes of the central nervous system owing to neurogenic inflammation (35). In patients with CRPS, reorganisation of the primary somatosensory cortex (S1) has been observed, which correlated with the amount of pain and hyperalgesia experienced by patients (36;37). Cerebral representation and motor processing in the brain are also described to be disturbed, possibly leading to movement disorders in CRPS-1 and distorted visualised representation of the affected limb (38-41).

Psychological factors

Psychological disturbances have often been proposed to be involved in complex conditions, such as chronic pain and CRPS. However, little evidence has emerged to support this hypothesis. No relation has been found between psychological dysfunction, disease-related fear or personality and the development of CRPS (42-44). One study reports that stressful life events are more common in patients with CRPS than in controls (45), but other studies could not confirm this finding (42;43;46;47). Once patients have developed CRPS, pain-related fear and fear of re-injury are proposed to be risk factors for a poor prognosis to pain reduction and functional improvement (48;49).

Treatment options

Treatment of CRPS-1 is challenging, because of the wide variety of symptoms and the variable disease course exhibited by patients. A multimodal approach consisting of pharmacologic treatment and physiotherapy, sometimes in combination with invasive therapy or psychological support, is required. An overview of systematic reviews and guidelines providing an evidence-based approach to treatment of CRPS is presented in tables 2 and 3.

Pharmacologic treatment

Analgesics

Pain medication according to the WHO analgesic ladder has been suggested in therapeutic guidelines, although evidence supporting the efficacy of paracetamol and nonsteroidal anti-inflammatory drugs (NSAIDS) is limited. Tramadol has been shown to be effective in neuropathic pain disorders, therefore it can be considered for severe pain accompanying CRPS, although evidence for its effects in CRPS is lacking (4;50;51;55;61-63).

Treating CRPS with strong opioids should only be considered as crisis management for a limited period of time (51;55). Furthermore, gabapentin has proven efficacy in CRPS-1 (50;51;55;62;64), however, amitryptilin and carbamazipine or newer TCA's such as duloxetine or venlafaxine, can also be considered, because of their shown effectiveness in other neuropathic pain disorders (50;55;62).

Intravenous administration of the NMDA receptor antagonist ketamine can be considered; however the full scope of its therapeutical potential (including a risk-benefit assessment) has not been established yet (52;60;65;66). Lidocaine patches have been proposed for treatment of localised sensory deficits, such as allodynia in CRPS (67).

Anti-inflammatory therapy

The free radical scavenger dimethyl sulfoxide (DMSO) has been shown to be effective in patients who have had CRPS-1 for less than one year (58). N-Acetylcysteine (NAC) shows comparable efficacy to DMSO, but proved superior to DMSO for primary cold CRPS in subgroup analyses (68). Vitamin C has established efficacy in the prevention of CRPS after wrist fractures (23;24). Furthermore, corticosteroids can provide significant pain reduction in CRPS; however there is no consensus on the dosage or duration of treatment (50;58;62;63). Both types of interventions have so far only been evaluated in early-stage CRPS (51;55).

Table 2: Systematic reviews on interventions for CRPS-1.

Review	Topic
Kingery *Pain; 1997; 73; 123-139*	Oral corticosteroids, DMSO, calcitonin intranasal and subcutaneous, regional blocks, intravenous ketanserin and phentolamine and epidural clonidine in CRPS, comparing with other neuropathic pain disorders.
Stanton-Hicks et al. *Clin. J. Pain; 1998; 14(2); 155-166*	NSAIDs, opoids, antidepressants, calcium blockers, corticosteroids, bisphosphanates, capsaicin, adrenergic drugs, local anaesthetic blocks, neuromodulation, physical therapy, psychiatric and psychological measures and treatment for children with CRPS.
Raja et al. *Anesthesiology ; 2002 ; 96 ; 1254-1260*	Oral, topical and intravenous analgesics, bisphosphonates, free radical scavengers, cortocosteroids, alfa blockers, blockades, epidural and intrathecal therapies, physiotherapy, neuromodulation, psychotherapy and invasive treatment options.
Foroufanzar et al. *Eur. J. Pain; 2002; 6; 105-122*	Blocks, intravenous ketanserin, calcium regulating drugs, free radical scavengers, corticosteroids, complementary therapies and prevention of CRPS.
Cepeda et al. *Clin. J. Pain ; 2002 ; 18(4) ; 216-233*	Local anaesthetic sympathetic blockade.
Harden *American Journal of Physical Medicine & Rehabilitation; 2005 March; S17-S28*	Tricyclic antidepressants (TCA), anticonvulsants, anti-inflammatory drugs, opoids, clonidine, nifedipine, calcitonin, bisphosphanates, adrenergic antagonists, topical treatments, local anesthetic block therapies.
Brunner et al. *Eur. J. of Pain; 2009; 13(1):17-21*	Bisphosphonates.
Daly and Bialocerkowski *Eur. J. Pain; 2009; 13(4); 339-353*	Physiotherapy.
Fischer et al. *Pain; 2010; 151; 251-256*	Anti-inflammatory drugs.
Perez et al. *J. of Pain and Symptom Management; 2001; 21; 511-525*	Anti-inflammatory drugs, free radical scavengers, beta blockers, calcitonin, stellate ganglion blocks, intravenous treatment with lidocaine and ketanserine, clonidine, bisphosphonates.
Collins et al. *Pain Medicine; 2011; 11(11); 1726-1742*	NMDA receptor antagonists.

Calcium regulating drugs

The use of bisphosphonates has been proposed as a treatment option for CRPS. Although limited support their ability to reduce pain (associated with bone loss) has been reported, additional research with regard to dosage, frequency and duration of treatment is required (21;50;53;55;56;62;69;70).

Vasodilatory medication

For treatment of patients with CRPS and vasomotor disturbances, α-1 adrenergic blockers, phenoxybezamine and terazosin, or calcium influx blockers such as nifedipine, can be considered (4;55;71). No reduction in temperature asymmetry was found for NO regulating medication (e.g. tadalafil, isosorbinedinitrate) in primary cold CRPS, although tadalafil is superior to placebo in reducing pain for this subgroup (72;73). Likewise, intravenous administration of ketanserine is reported to reduce pain in CRPS (50;74).

Spasmolitics

Movement disorders in CRPS-1, such as dystonia, myoclonia and tremor, might benefit from treatment with baclofen or benzodiazepines (4;50;63). Treatment with anti-cholinergics has not shown to be beneficial for CRPS-related movement disorders over a longer period of time (34).

Physical therapy

An important modality for treatment of CRPS is physical therapy directed at increasing control over pain and improving skills (57;75). Motor imagery and mirror therapy have been proven effective, both of which are applied to counter disturbed cortical motor processing, resulting in improvement of pain and function (41;76;77).

Transcutaneous electrical nerve stimulation (TENS) might be beneficial for treatment of pain in a subgroup of patients and might therefore be a suitable adjunctive non-invasive therapy. However evidence for the latter is limited (4).

Invasive treatment

Spinal cord stimulation with an implantable generator can be considered for patients with chronic CRPS; however, the high incidence and severity of complications warrant careful patient selection (78). Spinal cord stimulation should not be considered in early stages of CRPS (79). Studies of intrathecal administration of baclofen show that patients with CRPS-dystonia can experience marked improvement in pain and disability levels, paralleled by improvement in quality of life (80;81). However, as with other intrathecal approaches, complications can be severe, and, therefore, should be limited to patients refractory to conventional therapy and be conducted by physicians with ample experience with intrathecal devices (81).

Psychological treatment

Although studies with regard to psychological interventions for CRPS are limited, treatment by a psychologist can be considered in cases where disease burden is high or there is a discrepancy between noted pain behaviour and observed signs and symptoms of CRPS (63). Graded exposure is a promising therapy to reduce fear of pain and to regain functionality of the affected extremity (49).

Table 3: Guidelines for the treatment of CRPS-1.

CBO Guidelines Complex Regional Pain Syndrome type 1: 2006 *http://www.cbo.nl/thema/Richtlijnen/Overzicht-richtlijnen/Overig/*
Dutch guidelines for CRPS considering analgesics, local and intravenous anaesthetics, anticonvulsants, antidepressants, capsaicin, free radical scavengers, oral muscle relaxants, local botulin, intrathecal baclofen, corticosteroids, calcitonin, bisphosphanates, calcium channel blockers, invasive treatment, paramedical intervention, treatment options for children with CRPS and recommendations for prevention of CRPS.
AWMF: Leitlinien der Deutschen Gesellschaft für Neurologie: 2008 (German) *http://www.awmf.org/leitlinien/detail/ll/030-116.html*
German guideline for treatment of CRPS considering bisphosphanates, calcitonin, corticosteroids, free radical scavengers, physio- and occupational therapy, analgesics, psychotherapy, blockades, spinal cord stimulation and intra-thecal treatment.
RSDSA Complex Regional Pain Syndrome: Treatment Guidelines : 2010 *http://www.rsds.org/3/clinical_guidelines/index.html*
American guidelines on effects of anti-inflammatory drugs, anticonvulsants, neuromodulators, antidepressant, anti-anxiolytics, opioids, NMDA receptor antagonists, anti-hypertensiva and α-adrenergic antagonists, calcitonin, bisphosphonates, topical treatment, psychological interventions and invasive interventions.
Evidence-Based Interventional Pain Medicine According to Clinical Diagnoses: **Chapter 16. Complex Regional Pain Syndrome: 2010 Van Eijs et al.** *Pain Pract.; 2011; 11(1); 70-87*
Dutch guidelines for anaesthesiologists on physical therapy, psychological support, anti-inflammatory therapy, analgesic therapy, vasodilatory therapy, spasmolytic therapy, regional blocks, intrathecal and epidural treatment and neurostimulation.

Future perspectives

In recent decades, much research effort has been directed to unravelling the underlying mechanisms, and improving strategies for its prevention and treatment, alongside the unification of diagnostic procedures. An important issue to be addressed is the identification of prognostic factors for disease development, which could lead to a more targeted approach and therewith improve the prognosis of patients with CRPS. Prospective cohort studies on the development of CRPS are necessary to gain a better understanding of prognostic factors related to disease onset and disease course (82).

The recently developed CRPS severity score (CSS) as a derivation of the Budapest diagnostic criteria (83) may be helpful in improving the systematic follow up of patients; however, validation of this tool is ongoing. CRPS remains a clinical diagnosis, and the patient population is very heterogeneous. Although it has been proposed that CRPS comprises different disease subtypes or stages of the disease, this has not led to further subcategorisation of this disease (8). A targeted approach based on the identification of CRPS subtypes and specific mechanisms prevailing in an individual patient is therefore still warranted.

The many available treatment options suggest that the optimal therapy for CRPS has not yet been identified. Given the heterogeneous nature of CRPS, an optimal therapy seems unlikely; therefore, a mechanism-directed approach to treatment of CRPS appears preferable. With regard to interventions targeting inflammation, comparative studies of established interventions (for instance Prednisolon and DMSO) and novel anti-inflammatory agents such as intravenous administration of immunoglobulin (84), should be performed. Furthermore, alternative approaches such as targeting the cholinergic anti-inflammatory pathway (85), whereby activating the parasympathetic nervous system to inhibit inflammatory activity and autonomic dysregulation in CRPS, could be pursued. Promising interventions addressing sensory disturbances related to peripheral and central sensitization, such as NMDA receptor antagonists (86) and the N-type calcium channel blocker ziconotide (85), are worthwhile targets for further research.

Therapy directed at the stimulation of adaptive cortical reorganisation involving brain-training programs, such as mirror therapy (76) and motor imagery (77), merit implementation in daily practice. Continuing this line of thought, a strong point can be made for increasing patients' awareness and knowledge regarding mechanisms underlying development of chronic pain and CRPS (88). Further research within the field of exercise and occupational therapy should be focused on the distinction between pain and time-contingent approaches. Positive initial results have been found for pain exposure physical therapy (PEPT), which is based on progressive-loading exercises and desensitisation beyond the patients' pain limits (89). Cognitive behavioural aspects are taken into account with the goal to motivate patients to use the affected limb in daily activity despite experiencing pain. Likewise, for patients with CRPS with pain-related fear, research is ongoing into the effects of graded exposure therapy (GEXP), comprising provision of information about CRPS, observational learning and 'flooding' of feared movements and activities.

Repetitive transcranial magnetic stimulation (rTMS), whereby the motor cortex is stimulated in order to treat neuropathic pain, has been shown to provide short term pain relief in patients with CRPS-1 (88). However, issues related to placebo response and ways to prolong its efficacy need to be addressed.

For each of the interventions discussed in this article, long-term studies of sufficient sample size, with specific attention to patient selection, timing, and dosage of therapies are required in order to establish risks and benefits for CRPS patients.

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Co-morbidities in Complex Regional Pain Syndrome type 1

3

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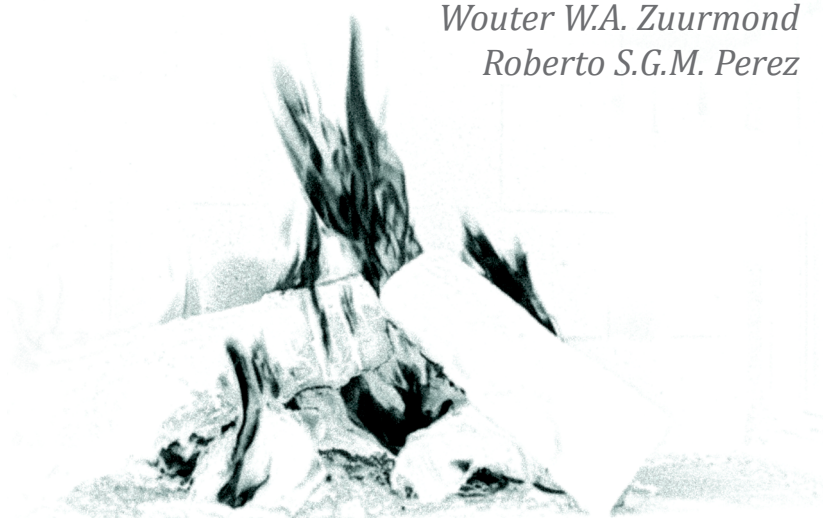
Abstract

The prevalence of co-morbidities was analyzed in a large group of Complex Regional Pain Syndrome type 1 (CRPS1) patients diagnosed with the Budapest criteria. A subgroup analysis was performed to compare disease prevalence between warm and cold type CRPS1. A cross-sectional study was performed to explore differences in the prevalence of co-morbidities between CRPS1 patients and non-CRPS patients. Co-morbidities were self-assessed and questionnaire-based in both groups. 669 CRPS1 patients and 180 non-CRPS pain patients were included. The most occurring categories of disorders among CRPS1 patients were muscle-bone and skin disorders (67%), gastrointestinal disorders (49.3%) and neurological disorders (25.3%). In the subgroup analysis, osteoporosis was associated with warm CRPS1 (OR: 5.94, 95%CI: 1.90–18.58) and constipation with cold CRPS1 (OR: 2.18, 95%CI: 1.20-3.98) but adjustment for age and disease duration resulted in non-significant results. In comparison with non-CRPS pain patients, a higher prevalence of gastrointestinal disorders (OR: 1.60, 95%CI: 1.14-2.24) was observed in the group of CRPS1 patients. Including only disorders recorded by patients as pharmacologically treated, no significant difference was found between the two groups. On the basis of our findings we can conclude that CRPS1 patients present a high burden of muscle-bone and skin disorders and that CRPS1 is associated with gastrointestinal disorders. No difference seems to be present among warm and cold type CRPS1. Future comprehensive population based prospective research is needed to confirm our findings.

Introduction

Complex Regional Pain Syndrome (CRPS) is a disabling condition of the extremities characterized by autonomic, sensory, trophic and motor disturbances resulting in pain, swelling, color changes, limited mobility and change in temperature of the affected extremity [5;12;31]. A multifactorial nature is proposed to explain the pathophysiology of the disease, whereby aberrant inflammatory response to trauma leading to vasomotor dysfunction, central sensitization and maladaptive neuroplasticity are suggested as underlying biological mechanisms [17]. Variation in susceptibility to perturbed regulation of any of the proposed pathogenic pathways may account for the clinical heterogeneity of the syndrome and may explain the differences across patients and even within a patient over time [4;5]. In the past decade, several epidemiological, genetic and clinical studies have been performed in an attempt to identify factors that modulate the risk of developing CRPS and are prognostic for either positive or negative outcome. Recently, a study by *De Mos et al.* showed that a history of migraine asthma, osteoporosis, menstrual cycle related disorders and neuropathies is associated with increased risk of developing CRPS [20;23]. In a recent prospective study where the occurrence of CRPS1 after fracture was studied in 596 patients, *Beerthuizen et al. [1]* suggested that patients affected by musculoskeletal diseases and rheumatoid arthritis at the time of the trauma are more susceptible to develop CRPS1. Moreover, a group of chronic medical conditions, namely arthritis, neck/back disease, osteoporosis, heart disease, migraine, bowel disease and depression/anxiety has been described to be significantly associated in an independent manner with the occurrence of chronic pain [9]. Resulting from observations as stated above, we hypothesize that individuals affected by specific medical conditions may be at greater risk of developing CRPS1, while the presence of CRPS1 itself, could induce physical/mental deregulations potentially leading to disease.

The identification of co-morbidities may assist in the development of both preventive and treatment strategies and can be informative to gain more insight in disease mechanisms of CRPS1. Therefore, as a first step, the aim of our study is to describe the occurrence of other medical conditions in patients with CRPS1, identify diseases occurring with a higher prevalence in CRPS1 patients compared to non-CRPS pain patients and evaluate if prevalence differences of co-morbidity are influenced by CRPS subtypes (warm vs cold type).

Methods

Data were collected from a NEN-7511 certified central web-based database (ProMISe®). The sample comprised a group of CRPS1 patients and a group of non-CRPS pain patients visiting the outpatient clinic of four University Medical Centers participating in the TREND consortium (VUMC, LUMC, EMC, and MUMC) (http://www.trendconsortium.nl/) in the period 2003-2011. CRPS1 patients were diagnosed by a specialist, and standardized diagnostic forms were filled out to assess if patients fulfilled the Budapest criteria (criteria with the highest specificity) for CRPS [13]. The group of non-CRPS pain patients consisted of patients that did not fulfill the IASP criteria [18] (criteria with the highest sensitivity) for CRPS, for which an alternative explanation for the amount of pain and dysfunction was found, and the CRPS diagnosis was excluded by the specialist.

Data stored in the database were collected using the TREND Symptom Inventory (TSI) questionnaire. The TSI questionnaire is a validated Dutch questionnaire developed to evaluate demographic characteristics, symptoms, general health status, medication use and history of surgery of CRPS1 patients and non-CRPS pain patients [8]. Three sections of the questionnaire - *medication use*, *other medical conditions, and surgery* - were used to assess co-morbidities whereby medication use, medical conditions and underlying diseases that have led to surgery were self-reported by patients. A description of the prevalence of all categories of diseases occurring in the group of CRPS1 patients is shown. In a cross-sectional study the prevalence of diseases in CRPS1 patients is compared with that of non-CRPS pain patients.

Categorization of data. Categorization of co-morbidities was performed following the classification structure of the Cumulative Illness Rating Scale for Geriatrics (CIRS-G) [15]. A set of subcategories such as headache and migraine, osteoporosis, asthma, menstrual cycle related disorders were added to the main group of categories listed in the CIRS-G to investigate the *a priori* hypothesis about associations between these medical conditions and CRPS that have been suggested in literature [20]. Allergies and diabetes were investigated separately because of the possible association with CRPS1 due to the similarities in the assumed underlying disease mechanisms (hypersensitivity/inflammation and impaired circulation/sensory disturbances, respectively). Gastrointestinal disorders were computed with and without the inclusion of constipation and subsequently excluding patients using opioids and antidepressants, which have constipation as a side effect. Neurological disorders were computed with and without general headache and migraine.

As an alternative approach to identify the presence of co-morbidities, medications listed by the patients were categorized according to their general use in clinical practice. In order to correctly attribute medications listed by the patients to the associated illness (i.e. either CRPS or co-morbidities), a validity analysis was performed based on the reported reason for use of a specific medication and acceptability of the provided reason. This was done to control for listed medication whereby no disease was reported and with different therapeutic options. Drugs commonly prescribed for other medical conditions but also used for CRPS1 (for example, vasodilators, antidepressants) whereby no reason for use was indicated, were not coded as related to the treatment of co-morbidities. A systematic decision tree was adopted for the coding of information reported in this section with the intent to identify data, which could lead to non-univocal interpretation that were therefore classified as "not valid" (see Fig. 1).
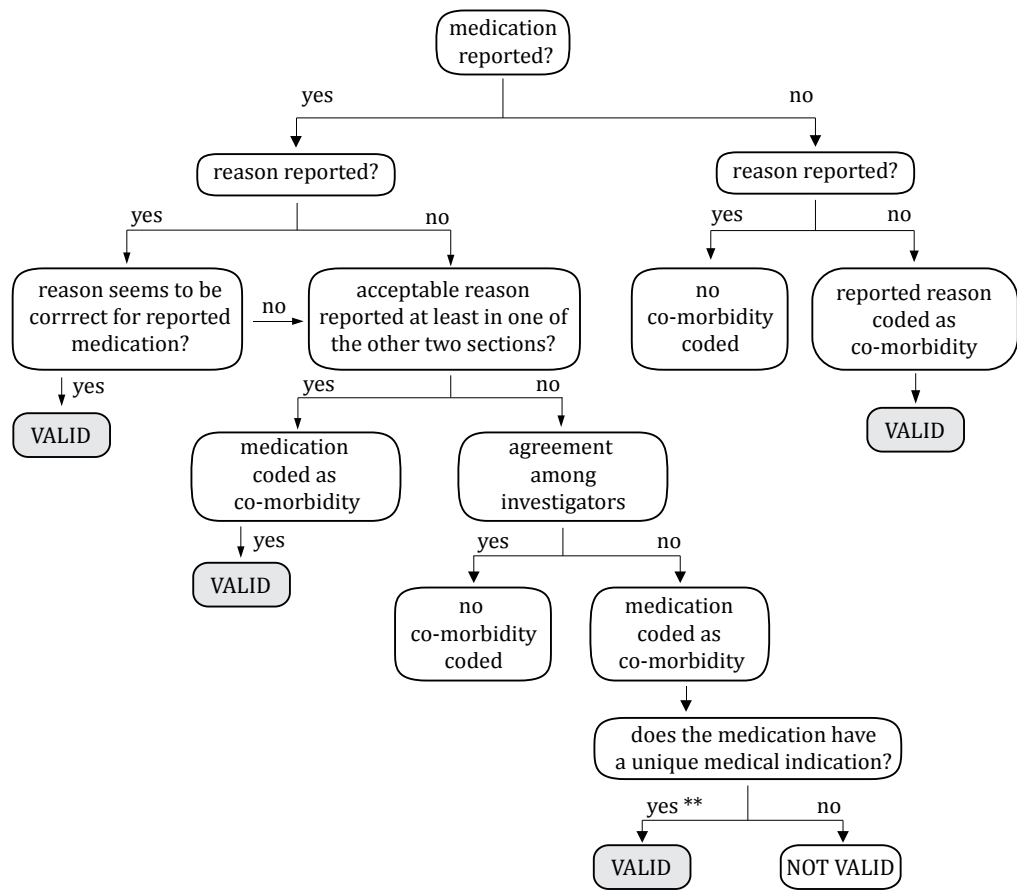
Figure 1. Decision tree to establish cases valid co-morbidities.
* Excluded medication that could be used for the treatment of CRPS1
** Proton pump inhibitor and H2 inhibitor, drugs acting on intestinal motility (laxatives, antispasmodics), antibiotics, anti-allergic, insulin and oral anti-diabetic drugs

For reported surgical procedures, co-morbidity was coded when another disease/reason for surgery not related to CRPS1 was indicated. Abortion, curettage and cosmetic surgery where not coded as co-morbidities.

Two investigators performed the categorization of diseases independently, and agreement was reached for data with non-univocal interpretation.

Analysis

The number of patients with a specific disease as a proportion of the total number of patients in the analyzed group, was seen as the prevalence of the condition within that group. Using data registered by TREND patients in all three sections of the questionnaire we obtained a "general prevalence" for each disorder. The "general prevalence" of constipation was further implemented adding data registered in the questionnaire under the specific question: "*How frequently do you suffer from constipation?*". Since response options in the TIS involve frequencies, the response options "often" and "always", were considered as presence of co-morbidity, while the answers "never" and "sometimes" were not registered as a co-morbidity by the investigators. The so computed "general prevalence" of each group of disorders was used for the general analysis.

In order to control for misinterpretation of TREND data, besides the general analysis we performed a validation analysis. Within the validation analysis, a "validated prevalence" of disease was computed by the exclusion of data coded by the investigators as not valid (see Fig.1). In an attempt to control the misclassification of diseases, a sensitivity analysis was performed were a "treatment-based prevalence" was obtained using only co-morbidities for which a medication use was reported by the patient.

Additional analysis

Warm/cold comparison. In order to evaluate the relation between co-morbidities and clinical sub types of CRPS1, an additional analysis comparing warm and cold type CRPS1 was performed. Subgroups warm and cold were identified within CRPS1 patients on the basis of registered signs or symptoms regarding abnormal temperature characteristics of the affected extremity.

Statistics

Standard comparative statistics were used to compare frequencies (Chi-square test) or means (Student's *t*-test and Mann-Whitney *U* test) and identify potential confounders. The significance of odds ratios was assessed with 95% confidence intervals (CIs). Logistic regression analysis was performed to adjust for confounders. Significance was established at $p<0.05$. Data were analyzed with IBM SPSS statistics 20. Given that this is an exploratory study, we made no attempt to control for multiple comparisons.

Results

General characteristics. A total of 669 CRPS1 patients and 180 non-CRPS pain patients were included in this study. The mean age at onset was 40 years for CRPS1 patients and 45 years for non-CRPS pain patients (p=0.002). Both groups consisted predominantly of females; no significant difference for gender was found. The median of disease duration was longer for CRPS1 patients than for non-CRPS pain patients (19 months IQR:5-72 vs. 9 months IQR:3-27, $p<0.001$). The median number of co-morbidities was 3 for both groups (p=0.133). The number of patients using opioids and antidepressants was higher among CRPS1 patients than non-CRPS pain patients (243 vs. 28, $p<0.001$) (Table 1).

Table 1. General characteristics of patients.

	CRPS1 patients	Non-CRPS pain patients	p
N	669	180	
Gender (% female)	86.3%	82.3%	0.170
Mean age at time of research	45.8 (SD:14.8)	48.7 (SD: 14.18)	0.017
Age at onset (mean)	40.6 (SD:15.5)	45.8 (SD:15.8)	0.002
Disease duration (months; median, IQR)	19 (IQR:5-72)	9(IQR: 3-27)	<0.001
Median number of co-morbidities	3 (IQR: 2-5)	3(IQR: 1-4)	0.133
N of pts using opioids and antidepressants	243	28	<0.001

N= number   SD= standard deviation
Pts= patients   IQR= interquartile range

Disease prevalence

In the group of CRPS1 patients categories of disorders with the highest prevalence were muscle-bone and skin disorders (67%), gastrointestinal disorders (49.3%) and neurological disorders (25.3%).

Among the a priori-tested group of disorders, headache had a prevalence of 7.3%, migraine of 5.8%, osteoporosis 3.6%, asthma 6%, menstrual cycle related disorders 1.6%, allergies 9.3% and diabetes 4.3%.

In the group of non-CRPS pain patients muscle-bone and skin disorders occurred with the highest prevalence (74.4%). 37.8% of non CRPS pain patients had gastrointestinal disorders and a quart of them suffered from a cardiovascular disease.

As described in table 2, a large group of patients in both groups had more than three co-morbidities (44% CRPS1 patients and 35% non-CRPS patients).

Table 2. Number of co-morbidities in CRPS1 and non-CRPS patients.

N extra diseases	CRPS1 patients	non-CRPS patients
0	6,1%	6,7%
1	15,2%	20%
2	17,2%	17,2%
3	17,2%	21,1%
>3	44,2%	35%

Chi square not significant.

Comparison

General analysis. Results of the general analysis showed that CRPS1 patients had a higher prevalence of gastrointestinal diseases than non-CRPS pain patients (OR: 1.60, 95%CI 1.14-2.24; *p*=0.006).

Results of the logistic regression analysis showed that the association remained significant after adjustment for age and disease duration (Table 3).

The association was non-significant after additional adjustment for the use of opioids and antidepressants (OR: 1.39, 95%CI 0.97 – 1.98, *p*=0.067).

The prevalence of constipation, the principal side effect of those drugs on the gastrointestinal tract, was higher for CRPS1 patients (OR: 1.65, 95%CI: 1.01-2.72, *p*=0.046). However, after adjustment for age, disease duration (OR: 1.56, 95%CI: 0.85 – 2.83, *p*=0.144) and, subsequently, for drugs having constipation as a side effect, this association became non-significant (OR:1.22, 95%CI: 0.73 – 2.05, *p*=0.451).

After the exclusion of patients using opioids and antidepressants from both groups (remainders: 426 CRPS1, 152 non-CRPS), the prevalence of gastrointestinal

disorders, including constipation, was significantly higher in CRPS1 patients (OR: 1.57, 95%CI: 1.06-2.33; p=0.024) also after adjustment for age and disease duration (OR: 1.71, 95%CI: 1.04-2-82, p=0.034) (data not shown in Table 3).

Validation analysis. No significant difference was found with regard to the percentage of valid data among CRPS1 and non-CRPS1 patients (p=0.630). Gastrointestinal disorders remained significantly higher in CRPS1 patients compared to non-CRPS pain patients (p=0.020) in the validation analysis (Table 3). This association remained significant also after the exclusion of patients using opioids and antidepressants (p=0.010).

Sensitivity analysis. When only co-morbidities for which a medication use was reported by the patient, with the exclusion of cases with non-univocal interpretation were analyzed, no significant differences were found between CRPS1 patients and non-CRPS pain patients.

Table 3. Associations between CRPS1 and other medical conditions.

	CRPS1	Non-CRPS	General analysis OR (95%CI)	Validation analysis a OR (95%CI)	Adjusted OR b
			CRPS1 vs. non-CRPS pain patients cases: N = 669; controls: N = 180		
Cardiovascular	164	45	0.97 (0.66-1.42)		
Gastrointestinal	330	68	1.60 (1.14-2.24)*	1.50 (1.06-2.1)*	1.65 (1.08-2.52)*
Genitourinary	165	37	1.26 (0.84-1.89)		
Hematopoietic	7	1	1.89 (0.23-15.48)		
Respiratory	81	18	1.23 (0.72-2.12)		
Eyes, ears, nose, throat, larynx	166	39	1.19 (0.80-1.77)		
Liver, biliary, pancreatic	46	13	0.94 (0.50-1.80)		
Muscle, bone, skin	448	134	0.69 (0.47-1.00)		
Neurology	169	38	1.26 (0.84-1.88)		
Endo-metabolic	59	20	0.77 (0.45-1.32)		
Psychiatry	96	30	0.83 (0.53-1.31)		
Headache	49	7	1.95 (0.86-4.38)		
Migraine	39	7	1.52 (0.67-3.48)		
Other headache	13	1	3.54 (0.46-27.2)		
Osteoporosis	24	11	0.57 (0.27-1.19)		
Asthma	40	5	2.22 (0.86-5.72)		
Allergic manifestations	62	9	1.94 (0.94-3.98)		
Diabetes	29	10	0.70(0.34-1.43)		
Menstrual cycle related disorders	11/577**	2/148**	1.14(0.31-6.47)		

OR = Odds ratio   ** within the female group
CI = confidence interval   a Excluding cases with non-univocal interpretation.
N = number   b Adjusted OR for age and disease duration through logistic regression analysis.
* p < 0.05

Warm/cold affected extremity subgroup analysis. For 664 patients out of 669, information about temperature abnormalities were available. Twenty-eight of them never had temperature abnormalities in the affected extremity. Among those who experienced temperature abnormalities, temperature characteristics of the affected extremity were retrieved for 554 patients. Of 554 patients, 154 (23%) had a "warmer" extremity, 262 (39.2%) a "colder" extremity and 138 (20.6%) had a combination of both (Table 4).

Compared to the "warm" CRPS1 group, the age at onset was significantly younger (37 years, SD:13.8 vs. 49.5 years, SD:14.7 *p*<0.001) and the disease duration was significantly longer (24 vs. 6 months, *p*<0.001) for the "cold" CRPS1 group. No significant difference was found about the number of patients using opioids and antidepressants between the two groups.

Patients with warm CRPS1 had a higher prevalence of osteoporosis (n "warm"= 13, n "cold"= 4, OR: 5.94, 95%CI: 1.90–18.58, *p*=0.001), while cold CRPS1 patients had a higher prevalence of constipation (n "warm"= 16, n "cold"= 53, OR: 2.18, 95%CI: 1.20-3.98, *p*=0.010). However, both associations disappeared upon adjustment for age and disease duration (OR= 2.45, 95%CI: 0.66 – 9.08, p=0.180 and OR= 2.04; 95%CI: 0.94-4.42, p=0.070, respectively).

Table 4. General characteristics of "warm" and "cold" CRPS1 patients.

	Warm		Cold		p
Number	154		262		
Gender(%female)	126	(81.8%)	237	(90.5%)	0.010
Reported as a symptom	55		85		0.560
Age at onset	49.5	(SD:14.7)	37	(SD:13.8)	<0.001
Disease duration (months)	6	(IQR:3-21)	24	(IQR:6-78)	<0.001
Mean number of co-morbidities	3	(SD: 2)	3	(SD: 2)	0.6

SD= standard deviation
IQR= interquartile range

Discussion

In this study we analyzed the occurrence of co-morbidities within a relative large population of CRPS1 patients and we subsequently made a comparison with a group of pain patients not suffering from CRPS1.

Among CRPS1 patients, 67% suffered from muscle-bone and skin disorders, and half of them reported to be affected with a gastrointestinal disease. Between the a priori-tested co-morbidities, suggested in the literature to occur more frequently with CRPS than in controls from the general population [20], neurological disorders

occurred with a higher frequency (a quart of the whole group) while the prevalence of migraine, osteoporosis, asthma, menstrual cycle related disorders and allergies did not reach the 10% respectively.

The most prevalent diseases in CRPS1 patients were muscle-bone and skin disorders. Nevertheless, muscle-bone and skin disorders were also well represented in the group of non CRPS pain patients, suggesting their possible association with a chronic pain state rather than with a specific pain disease. This hypothesis is supported by the study of *Dominick et al.* [9] where an association between arthritis and neck/back disorders with the occurrence of chronic pain was found. However not being able to perform a reliable comparison with data from the general population, the external validity of our finding is limited. On the other hand, in a recent study by *Beerthuizen et al.* [1], patients with musculoskeletal disorders and rheumatoid arthritis were found to be more susceptible to develop CRPS1 after a fracture [1].

The most important finding in our study is the significantly higer prevalence of gastrointestinal disorders in CRPS1 patients in comparison with non-CRPS pain patients. The association between gastrointestinal disorders (including constipation) and CRPS1, remained significant after the exclusion of patients using opioids and antidepressants, drugs that could have influenced the prevalence of these disorders. Gastrointestinal disorders have been reported in relation to CRPS in literature. *Goebel* found an increased small bowel permeability in both CRPS and fibromyalgia patients [10]. Here authors suggest that the development of a systemic disease could be enhanced by the stimulation of the immune systems exerted by luminal products as a consequence of the increased leakiness of the intestinal epithelial layer [7;10;16]. A genetic study has recently shown an association between CRPS patients with fixed dystonia and the human leukocyte antigen (HLA) HLA-B62 and HLA-DQ8 [24]. HLA DQ8 is well known to be one of the principal genetic factors associated with the occurrence of celiac disease and has also been associated with liver disease and upper functional gastrointestinal disorders not related to the celiac disease [3].

Moreover, neurogenic inflammation may play a role in both CRPS and irritable bowel syndrome (IBS). Most inflammatory changes that occur in CRPS are mediated by neuropeptides such as substance P (SP) and calcitonine gene-related petptide (CGRP). Serum concentration of CGRP and SP are higher in patients with CRPS than in healthy controls individuals. Neurogenic inflammation may also contribute to visceral hypersensitivity in IBS. The content of SP is increased in the inflamed colon of patients with ulcerative colitis [11] and in animal models of intestinal inflammation [6;27].

The association with gastrointestinal disorders was not further confirmed in a subsequent sensitivity analysis where only co-morbidities that were, as reported by patients, pharmacologically treated, were taken into consideration. The reason can be related to the fact that those medical conditions rarely require a continuous pharmacological treatment.

Differences among warm and cold CRPS have already been described in literature [19;22;30]. In our study, warm CRPS1 patients had a higher prevalence of osteoporosis as compared to cold CRPS1 patients that could be explained by the predominant role of inflammation in the "acute" phase of the syndrome [14;21]. The higher incidence of constipation in cold CRPS1 could be related to the possible effect exerted by the autonomic imbalance on gastrointestinal motility [2;29]. However, these associations were influenced by the age of the patients and the duration of the disease.

In order to inspect a misclassification of co-morbidities, two investigators performed the coding procedure independently. Moreover, all relevant information available for a patient was used to code a single item or clinical condition. We also performed a validation analysis excluding data with non-univocal interpretation limiting the effect of investigators interpretation. In order to avoid overestimation of gastrointestinal disorders, we performed sub-analyses excluding patients using opioids and antidepressants, since those drugs frequently induce constipation.

Several limitations in our study should be acknowledged. First, being a cross sectional study, only associations among factors can be described. Nevertheless, our results are helpful to have a better portrait of the general medical condition of CRPS1 patients, while longitudinal studies are suggested to further investigate the casual link among the here described associations. Second, prevalences of co-morbidities for the CRPS1 and non-CRPS group were based on patient-reports. However, researchers have demonstrated patient self reports to be a generally reliable data source of co-morbidities when compared with medical records [25;26;28]. Another shortcoming of the present study could be the relatively small number of non-CRPS pain patients, and the fact that they may have been too similar to CRPS1 patients. Non-CRPS pain patients were initially presented at the participating clinics as potential CRPS1 patients, but a pain specialist later excluded the diagnosis of CRPS1. A more specific group of pain patients should be considered for a better comparison in a future study.

On the basis of our findings we can conclude that CRPS1 is associated with gastrointestinal disorders and that CRPS1 patients present an high burden of muscle-bone and skin disorders. No difference seems to be present among warm and cold type CRPS1. Future case-control studies where both patient records and medically assessed co-morbidities are systematically evaluated should be conducted to confirm our findings.

Appendix 1.

Classification of disease used in this study:
1. Cardiovascular (excluding hypercholesterolemia, including cerebrovascular accident)
2. Gastrointestinal (esophagus, stomach, duodenum, intestines, hernias)
3. Genitourinary (kidneys, ureters, bladder, urethra, prostate, genitals, uterus, ovaries. Included menstrual disorders)
4. Hematopoietic (blood, blood cells, marrow, spleen, lymphatics)
5. Respiratory (lungs, bronchi, trachea, below the larynx. Excluded allergic asthma)
6. Eyes, ears, nose and throat and larynx (excluded allergic manifestations)
7. Liver (including biliary and pancreatic trees)
8. Musculoskeletal/tegument (muscles, bone and skin. Excluded allergic manifestations)
9. Neurological (brain, spinal cord and nerves. Included spinal hernia. Excluded cerebrovascular accident)
10. Endocrine/metabolic (excluded breast and hypercholesterolemia)
11. Psychiatric illness

Additional categories:
1. Headache
    - migraine
    - other headache
2. Osteoporosis
3. Asthma (excluded allergic asthma)
4. Allergic manifestations
5. Diabetes
6. Menstrual cycle related disorders

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Anti-inflammatory treatment of Complex Regional Pain Syndrome

4

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CRPS-1 – definition, pathophysiology, treatment

Complex regional pain syndrome type 1 (CRPS-1) is a disabling condition characterized by sensory, autonomic, (vaso)motor and trophic disturbances, of which pain, swelling, colour changes, limited mobility and change in temperature of the affected extremity are the most predominant [4,33]. CRPS-1 mainly develops after fractures, operations or a small trauma but also occasionally develops without a triggering event [26].

Different mechanisms are thought to play a role in the development of CRPS-1, providing a possible explanation for the heterogeneity seen within this patient population. One of the mechanisms proposed to be involved in the origin and maintenance of CRPS-1 is an exaggerated inflammatory response to tissue injury [2]. Scientific evidence supports the involvement of inflammatory processes in CRPS-1, whereby elevated cytokine levels [17], elevated activity of mast cells, neurogenic inflammatory reactions [2,18] and markers of oxidative stress [6] were found.

Systematic reviews addressing anti-inflammatory therapy provide limited evidence and contradictory conclusions [7,20,27]. However, in recent years additional studies have been published targeting the inflammatory component of CRPS-1. In light of the changing views about the involvement of inflammation, a comprehensive assessment of anti-inflammatory treatment approaches of CRPS-1 is warranted. The goal of the present topical review is to evaluate the effect of anti-inflammatory therapy on prevention, pain, range of motion and overall clinical improvement in CRPS-1.

Retrieving studies of anti-inflammatory CRPS-1 treatment

The Embase, Cochrane, Medline and Pubmed databases were scanned for relevant literature up to December 2009 (for search string, see Appendix 1). Reference lists of retrieved articles were screened for additional articles. Methodological quality of the articles was rated by two reviewers using the Delphi list [34]. Scores ≥ 7 indicate high quality, scores $4 \leq 6$ indicate moderate quality and scores ≤ 3 indicate poor quality. Included articles were evaluated on outcome (pain, range of motion and clinical improvement) and type of medication. A distinction was made between articles reporting about CRPS-1 after peripheral trauma (PT), and those evaluated about CRPS-1 after central nervous trauma (CNT), as different diagnostic criteria for the latter have been used in literature.

Results

Twenty-four eligible articles were found. Data for two clinical trials were reported in four separate articles and were therefore analyzed as two independent studies [29,32,35,37]. In total, twenty-two independent studies were analysed in this review [1,3,5,8-12,14,19,21-24,28,29,31,32,35,37-40], sixteen of which were not evaluated in previous reviews [1,9-11,19,21-24,28,29,31,32,36]. The anti-inflammatory modalities found in our search were corticosteroid treatment [1,3,5,9,10,14,19,21,22,24,31,39], free radical scavengers [8,11,12,23,28,29,32,35-38] and the combination of corticosteroids and free radical scavengers [40]. Characteristics of included studies are presented in table 1.

Pain

Twelve studies evaluated pain reduction following anti-inflammatory treatment [1,8,11,19,21,24,28,29,31,32,38,39,40].

Free radical scavengers

No effect of DMSO on pain reduction was found in a randomized placebo controlled trial (RCT) of high quality (PT) [38], however a case series showed DMSO to significantly reduce pain (PT) [23]. Mannitol showed no significant pain reduction compared to placebo in a high quality RCT (PT) [28]. No significant differences were found in pain reduction between N-Acetylcysteine and DMSO in a high quality RCT (PT), however there was significant improvement for both interventions over the course of the trial [29,32].

Corticosteroids

One RCT of high quality revealed significantly more pain reduction for oral prednisolone than the prostaglandine inhibitor piroxicam (CNT) [19]. In addition, significant pain reduction was reported in two case series after treatment with prednisolone (PT, CNT) [1,21]. Furthermore, a case series showed 73% of patients experiencing pain reduction and 18% remaining pain free one year after treatment with intravenous blocks with lidocaine and methylprednisolone (PT) [39].

In contrast, a placebo controlled RCT of poor quality evaluating low dosages of oral prednisolone showed no effects on pain reduction (PT) [24]. Likewise, a high quality RCT on blocks with lidocaïne and prednisolone showed no effect on pain reduction compared to placebo (PT) [31].

Table 1: Study characteristics
* Studies with scores ≥ 7 were considered of good quality, scores between 3 and 7 indicate moderate quality and studies with scores ≤ 3 are considered to be of poor quality.

References	Diagnostic term/ include patients	Design	Quality score*	N	Intervention	Primary outcome	Results
Glick ('73) [9]	Reflex dystrophy syndrome	Case series	3 (0,0,0,1,0,0,1,1)	17	Prednisolone during 10-70 weeks in a dosages from 15 to 40 mg/day	Clinical improvement: (graded as: excellent-very good-good-fair-poor-no improvement).	Results: excellent (N=4), good very (N=3), good (N=3), fair (N=2), poor (N=2), no improvement (N=2), withdrawal because of side-effects (N=1).
Glick and Helal ('76) [10]	Post-traumatic neurodystrophy (SHS included)	Case series	2 (0,0,0,0,0,0,1,1)	21	Prednisolone for 3-4 months starting at 15-40 individual increase of dosage (total dosages not reported).	Clinical improvement (graded as: very good-good-fair-poor).	Results: very good (N=10), good (N=3), fair (N=5), poor (N=3).
Kozin et al. ('76) [21]	Reflex sympathetic dystrophy (SHS and development of RSD after MI and cancer included)	Case series	3 (0,0,0,1,0,0,0,1,1)	11	Prednisone dose and period of time dependent on patient, ranging from 2 to 14 weeks, dosages from 60 to 80 mg/day	Grip strength, swelling by ring size, joint tenderness by dolorimeter score (per joint: max. score of 20, measured joints: 7-15).	Significant improvement in ring size (mean change -2.6% (range 7.7% to + 3.0%); $p<0.05$), and dolorimeter score (mean change -78 (range -3 to -224; ($P<0.02$)).
Kozin et al. ('81) [22]	Reflex sympathetic dystrophy (nerve injury included)	Comparative non-randomized study	3 (0,0,0,1,0,0,0,1,1)	55	Stellate ganglion blockade versus 60-80 mg prednisone for 2-4 days (occasionally up to 2 weeks), where after rapidly tapered.	Subjective response (excellent, good, fair, poor, fair).	Prednisone: excellent 40%, good 23%, fair 9%, poor 29%. Stellate ganglion blockade: fair 15%, poor 85%.
Christensen et al. ('82) [5]	Reflex sympathetic dystrophy	Randomized placebo controlled trial	5 (1,0,1,1,0,0,0,1,1)	23	Prednisone orally, 10 mg three times daily until clinical remission, maximum of 12 weeks	Clinical improvement score consisting of pain, oedema, volar sweating and finger knitting ability (max score 20)	Significant better clinical improvement for prednisone (prednisone: mean score from 8.5 (range 4-18) to 0.7 (range 0-3), placebo: mean score from 8.2 (range 6-11) to 5.9 (range 0-9); $P<0.01$)

Refer-ences	Diagnostic term/include patients	Design	Quality score*	Intervention	N	Primary outcome	Results
Goris ('85) [11]	Reflex sympa-thetic dystrophy	Non-ran-domized comparative trial	2 (0,0,0,1,0,0,0,1,1)	Topical DMSO 50% 5 times/day for 2 weeks or mannitol 10% iv 1 liter/day during 1 week or mannitol 10% 20 ml oral 5 times/day for 2 weeks	9	Pain, oedema, hyper-hydrosis. Function	Full pain relieve in 8/9 patients, full recovery of function in 6/9 patients, all treated with DMSO
Goris et al. ('87) [12]	Reflex sympa-thetic dystrophy	Randomized controlled cross-over study	3 (1,0,0,1,0,0,1,0,0)	Topical DMSO 50% in water versus pla-cebo (plain water) five times a day, 1 week of DMSO and one week placebo.	20	Subjective clinical evaluation by patient and researcher and range of motion (ROM)	Subjective clinical improvement for DMSO: 13/20 patients (patient based) and 16/20 (researcher based), P<0.001. ROM improvement: 15/17 patients, average improve-ment of 100o for DMSO: 8/17 patients, average of 41o for placebo, P=0.035
Lan-gendijk et al. ('93) [23]	Reflex sympa-thetic dystrophy	Case series	3 (0,0,0,1,0,0,0,1,1)	Topical DMSO 50% cream, five times a day, until RSD score <10.	38	RSD scoreb(on a 0-100 scale), VAS pain scorec	Significant improvement of RSD score (mean 83.5; SD 13.2 to ≤10; P<0.01) and VAS pain score (mean 5.3; SD 2.9 to 0.9; 1.3); P<0.01)
Braus et al. ('94) [3]	SHS in hemi-plegic patients after stroke	Randomized placebo-con-trolled trial	4 (1,0,1,1,0,0,0,1,0)	Methylprednisolone 32 mg during 14 days and a 14 day tapering period. Placebo group continued as open study after 14 days (except for 2)	36	SHS-scoreb (on a 0-14 scale)	Relevant improvement (SHS-score <4) for 31/34 patients treated with methylprednisolone. 17/34 were first treated with placebo and phys-ical therapy resulting in temporary relief

References	Diagnostic term/ include patients	Design	Quality score*	Intervention	N	Primary outcome	Results
Geertzen et al. ('94) [8]	Sympathetic reflex dystrophy of the hand	Randomized actively controlled trial	5 (1,0,1,1,0,0,0,1,1)	DMSO lotion 50% in water applied three times/day for 3 weeks versus regional intravenous ismelin blocks 2/week during 3 weeks	26	VAS pain score, VAS daily activities, oedema, discoloration, ROM, abduction/adduction of fingers and a total score of all above (on a 0-70 scale, shown in figure) during 9 weeks	Patients treated with DMSO improved more on mean total score (42-15) then ismelin blocks (43-27) (information derived from figure)
Grundberg ('96) [14]	Reflex sympathetic dystrophy	Case series	3 (0,0,0,1,0,0,0,1,1)	Intramuscular methylprednisolone 80 mg every 2 weeks, with a maximum of four injections, average 2.3 injections	47	Grip strength, pinch strength, PIP motion, swelling (graded as: no-moderate-severe)	Average grip strength improvement of 22 lbs, pinch grip 4 lbs improvement; average improvement of PIP motion from 39° to 75°. Swelling decreased in all patients (base line: 47 patients with moderate to severe swelling; after treatment: no swelling in 26 patients and mild swelling in 21 patients)
Zuurmond et al. ('96) [38]	Reflex sympathetic dystrophy	Randomized controlled trial	7 (1,1,1,1,0,1,1,1,0)	DMSO 50% in fatty cream versus placebo fatty cream during 2 months (times/day not reported)	30	RSD score(on a 0-5 score), VAS pain score	Significant improvement of RSD score (DMSO: median improvement 4 (range 0-5), placebo: median improvement of 3 (range 0-5), P<0.01). No significant improvement of VAS pain score (DMSO: median improvement 2.9 (range -2.8 to 7.0), placebo: 1.0 (range -3.9 to 9.0)
Zyluk ('98) [39]	Post-traumatic reflex sympathetic dystrophy	Case series	2 (0,0,0,1,0,0,0,1,0)	Single intravenous block of 80 mg methylprednisolone and 20 ml 1% lidocaine	36	Pain (severe/moderate), swelling, discoloration, temperature, hyperhidrosis, loss of finger flexion, summarized in total score (graded as poor-moderate-good)	Pain relieve in 73%, 18% of the patients pain free. Clinical improvement qualified as good in 69%, moderate in 22% and poor in 9% of the patients

References	Diagnostic term/ include patients	Design	Quality score*	Intervention	N	Primary outcome	Results
Zollinger et al. ('99) [37] Zollinger et al. ('00) [35]	Wrist fractures, conservatively treated	Randomized controlled trial	8 (1,1,0,1,1,1,1,1) 9 (1,1,1,1,1,1,1,1,1)	Vitamin C 500 mg/day versus placebo during 50 days after trauma	115	Development of reflex sympathetic dystrophy	Significantly lower ratio of RSD (vitamin C: 7%, placebo 22%, P<0.04)
Perez et al. ('03) [29]	Complex regional pain syndrome type 1	Randomized actively controlled trial	8 (1,1,0,1,1,1,1,1)	Topical DMSO 50% cream 5 times/day, versus N-acetylcysteïne (NAC) 600 mg three times/day	146	ISSd, WSQ, QRSD, gait analysis, EuroQol, COOP/WONCA, SF-36	DMSO and NAC equally effective. Both resulted in decrease of ISS (reduction DMSO: 9.05; SD 6.97, NAC: 8.31; SD 8.13)
van Dieten et al. ('03) [32]	Reflex sympathetic dystrophy according to the Veldman criteria	Randomized comparitive controlled trial	8 (1,1,0,1,1,1,1,1)	Topical DMSO 50% cream 5 times/day, versus NAC 600 mg three times/day	131	Cost-effectiveness, ISSd, mean utility	DMSO provides the best cost effectiveness profile, see Perez et al. ('03).
Taskaynatan et al. ('04) [31]	Complex regional pain syndrome type I	Randomized placebo controlled trial	7 (1,1,0,1,1,1,1,0)	Bier block with lidocaïne 10 ml 2% and methylprednisolone 40 mg once a week versus 100 ml saline, three times in total	22	VAS pain, ROM (distance between fingertip and distal palmar crease in cm), oedema (measured by a volumeter in grams)	No significant difference in improvement of: mean VAS pain (active treatment: 5.7; SD 1 to 4.2; SD 1.3, placebo: 4.8; SD 1.1 to 3.5; SD 0.9), mean ROM (active treatment: 2.8; SD 0.3 to 2.7; SD 0.4, placebo: 2.5; SD 0.6 to 2.5; SD 0.6), mean oedema (active treatment: 1522; SD 134 to 1516; SD 133, placebo 1522; SD 137-1520; SD 137)

References	Diagnostic term/ include patients	Design	Quality score*	Intervention	N	Primary outcome	Results
Bianchi et al. ('06) [1]	CRPS according to criteria of Kozin, not reacting on regular physiotherapy	Case series	3 (0,0,0,1,0,0,0,1,1)	Prednison 60 mg (N=2), 50 mg (N=1) or 40 mg (N=28) for 2-4 days tapered to 30-40 mg for 2-4 days and at last tapered to 5-10 mg for 2-3 days. Two cycles in patients with poor results (N=4)	31	Pain: VAS scalec. Swelling function (on a 0-2 scale), clinical severity (on a 0-22 scale)	Improvement after 1 year for all measured variables after 1 cycle (upper limb: median VAS 9 (range 3-10) to 0 (range 0-1), functional ability 2 (range 1-2) to 0 (range 0-0), lower limb: VAS median 7.5 (range 6-9) to 0 (range 0-1), functional ability 1 (range 0-2) to 0 (range 0-2), P<0.001). Clinical severity score for affected limb: 16.8 (range 10.1-22) to 2.0 (range 0-4.5). Clinical improvement after 1 year and two cycles of treatment (median score 19 (range 18-21) to 8 (range 1-10), P<0.01).
Kalita et al. ('06) [19]	Complex regional pain syndrome developed after stroke	Randomized actively controlled study	8 (1,1,0,1,1,1,1,1)	Prednisolone oral 40mg/day versus piroxicam oral 20mg/day for 14 days	60	CRPS scoreb (on a 0-14 scale): sensory aspects separately described (on a 0-5 scale), Barthel index (daily activity scale from 0 to 20)	Significant improvement of: CRPS score (prednisolone: mean 10.73; SD 1.95 to 4.27; SD 2.83, piroxicam: mean 9.83; SD 2.34 to 9.37; SD 2.89, (P>0.0001), sensory component (prednisolone: mean 3.98; SD 0.85 to 1.13; SD 1.35; P<0.0001). No significant difference in Barthel index (prednisolone: mean 1.97; SD 4.94 to 9.87: SD 4.43; piroxicam: 2.57; SD 4.32 to 7.07; SD 5.56) after 1 monts
Lukovic et al. ('06) [24]	Complex regional pain syndrome type I (first stadium)	Randomized placebo-controlled trial	3 (0,0,0,1,0,0,1,1,0)	Oral prednisone 5 mg + diverse physical agents versus placebo + diverse physical agents, until stable remission	60	Treatment duration, VAS pain scoresc, swelling (severe, moderate, absent), skin color (normal, pale, cyanotic), motor function (1st-3rd degree functional impairment)	No significant difference for VAS pain (prednisone: 6.0; SD 1.5 to 0.2; SD 0.4, placebo: 5.9; SD 1.5 to 0.3; SD 0.7), severe swelling (prednisone: 12/30 to 0/30, placebo: 13/30 to 0/30), function (prednisone: 29/30 patients with 1st degree impairment after treatment; placebo: 27/30 patients with 1st degree impairment after treatment)

References	Diagnostic term/include patients	Design	Quality score*	Intervention	N	Primary outcome	Results
Zollinger et al. ('07) [36]	Wrist fractures	Randomized controlled trial (comparative and placebo)	9 (1,1,1,1,1,1,1,1)	Vitamin C 200 mg, 500 mg, 1500 mg or placebo during 50 days after trauma	416	Development of CRPS-1	Significant lower ratio of CRPS (vitamin C (all dosages): 2.4%, placebo: 10%; P=0.002, vitamin C 500 mg: 2%, placebo: 10%; P=0.007, vitamin C 1500 mg: 2%, placebo: 10%; P=0.005). Vitamin C 200 mg is not significantly more effective then placebo (vitamin C 200 mg: 4%, placebo: 10%)
Perez et al. ('08) [28]	Complex regional pain syndrome type I	Randomized placebo controlled trial	9 (1,1,1,1,1,1,1,1)	Mannitol 10% 1 l or placebo (1 l NaCl 0.9% iv) every day during 5 days	41	VAS pain scorec, function level, quality of life (QOL), hand function/foot function, dynometer, AROM	No significant improvement of pain (mannitol: 53.1; SD 17.5 to 49.7; SD 25.3, placebo: 48; SD 23.6 to 45.1; SD 31.8), AROM (mannitol: -0.5 (IQR -5.0 t0 20), placebo -5.0 (IQR -10.0 to 15.0), social functioning; mannitol: 0.0 (IQR -12.5 to 12.5), placebo: 0.0 (IQR -25.0 to 12.5)
Zyluk and Puchalski ('08) [40]	Complex regional pain syndrome type I, less than 4 months	Case series	2 (0,0,0,1,0,0,0,1,0)	Mannitol 10% iv 2x 250ml and 8 mg dexametason/ day every day for 1 week	70	VAS pain scorec, finger flexion (distance in cm from finger tip to distal palmar crease), CRPS scoreb (on a 0-10 scale)	Significant improvement of: VAS pain score (mean 6.7 (range 5-9) to 2.3 (range 1-5), (P<0.05), finger flexion (6 cm (rage 3-10) to 0.4 cm (range 0-5), P<0.05), CRPS score (7.6-2.2, P<0.05)

SHS = shoulder hand syndrome.

a Methodological quality is rated according to a Delphi list [34]. Studies receive a score "0" or "1" on the following criteria: randomization, blinded medication, similar study groups, properly specified in- and exclusion criteria, blinded researcher, blinded care taker, blinded patient, clear purpose of the study and intention-to-treat analysis (e.g. 0,0,0,1,0,0,0,1,1 = quality score of 3). Scores ≥ 7 indicate high quality, score 4 ≤ 6 indicate moderate quality and scores 3 or lower indicate poor quality.

b RSD score, SHS score and CRPS score are compound scores to assess disease severity, based on pain, temperature and volume differences between the extremities and loss of function.

c VAS pain score is measured on a scale from 0-10.

d ISS is a validated compound score to assess the severity of symptoms of CRPS-1, based on pain, temperature and volume differences between the extremities and loss of function measured on a scal from 5 to 50.

Combined free radical scavenger and corticosteroid treatment

In one case series a combination of intravenous mannitol and dexametason was reported to provide significant decrease in pain (PT) [40].

Range of motion

Ten studies addressed the effects of anti-inflammatory treatment on range of motion (ROM) [1,8,11,12,14,24,28,29,31,32,40]. Outcome was either reported as subjective improvement [1,11,12] or as objectively measured effects [8,14,24,28,31,40] (see table 1).

Free radical scavengers

Treatment with DMSO provided a significant subjective improvement of ROM compared to placebo in one randomized controlled cross over study of poor quality (PT) [12]. In another non-randomized trial on topical DMSO compared to intravenous mannitol a decrease of subjective joint stiffness in both patient groups was found (PT) [11]. One high quality RCT showed no improvement of ROM between N-Acetylcysteine and DMSO (PT), however there was a significant improvement with both free radical scavengers over the course of the trial [29,32]. One RCT of high quality on intravenous mannitol did not show improvement of range of motion (PT) [28].

Corticosteroids

Improvement of ROM was reported in two case series. One or two treatment cycles of corticosteroids showed a significant improvement of ROM after one year (PT) [1]. Similarly, intramuscular corticosteroids (PT) [14] were reported to provide increase in proximal interphalangeal joint movement in 68% of the patients at one year follow-up. However, RCTs reported less positive effects for improvement in range of motion, whereby only limited effects of oral prednisolone were observed when compared to piroxicam in a high quality RCT (CNT) [19]. Another high quality trial on bier blocks with lidocaine and prednisolone (PT) [31] and an RCT of poor quality on oral administration of low-dose corticosteroids (PT) showed no improvement on range of motion when compared to placebo [24].

Combined free radical scavenger and corticosteroid treatment

The combined treatment of patients with CRPS-1 with the scavenger mannitol and dexametason (PT) [40] yielded a significant improvement of finger flexion in a case series.

Overall clinical improvement: compound scores and subjective global assessment

Clinical improvement was studied in thirteen studies [1,3,5,9,10,12,19,22,23, 29,32,38-40], using either compound scores based on validated measurements of pain, range of motion, edema and temperature difference between the affected and unaffected extremity [1,3,5,19,23,29,32,38-40] and subjective assessment as outcome [9,10,12,22].

Free radical scavengers

One placebo controlled cross-over RCT of poor quality reported overall clinical improvement for DMSO expressed as subjective clinical wellbeing by the patient and the physician (PT) [12]. Positive results for clinical improvement as determined with compound scores were found for topical DMSO in two RCTs of high quality compared to placebo (PT) [38] and in one RCT of moderate quality compared to regional intravenous ismelin blocks (PT) [8] as well as in one case series (PT) [23]. A high quality RCT comparing N-Acetylcysteine to DMSO (PT) [29,32] revealed significant improvements in clinical compound scores for both interventions, without significant differences between both arms of the study.

Corticosteroids

All studies evaluating the use of corticosteroids reported significant positive results on overall clinical improvement. This included three case series, of which one showed improvement of clinical scores after one or two cycles of corticosteroids (PT) [1], and two case series showed good to excellent clinical results in respectively 59% (PT/ CNT) [9] and 62% of CRPS-1 patients (PT/CNT) [10]. Two RCT's of moderate quality comparing corticosteroids to placebo ((CNT) [3], (PT) [5]) and a high quality RCT comparing corticosteroids to piroxicam (CNT) [19] reported significant differences in favour of corticosteroid treatment.

Combined free radical scavenger and corticosteroid treatment

A case series evaluating the effect of a combination of mannitol and dexametason showed a significant improvement on a compound score (PT) [40].

Prevention of CRPS-1

Two RCTs addressed primary prevention of CRPS-1 using the free radical scavenger vitamin C (PT) [35-37]. A significant preventive effect of vitamin C was found in both studies. While 22% of the patients in the control group, only 7% of the patients in the vitamin C group, developed CRPS-1 [35,37]. Similar results were observed in another study (control 10.1%, vitamin C 2.4%) [36].

What to do now?

Our results suggest that anti-inflammatory therapy may be beneficial for CRPS-1. Pain reduction and improvement of range of motion were found after treatment with the free radical scavengers N-Acetylcysteine and DMSO, as well as after treatment with corticosteroids. In all evaluated studies both free radical scavengers (DMSO, N-Acetyl-cysteine) and corticosteroids showed improvement of clinical outcome. In addition, the free radical scavenger vitamin C showed substantial preventive effects. These results are in line with current hypotheses about the involvement of an inflammatory process in CRPS-1 [2,17,30] and are comparable to other reviews evaluating anti-inflammatory interventions for CRPS-1 [7,20,27].

Glucocorticosteroids and free radical scavengers differ in pharmacological mechanism. Glucocorticosteroids reduce manifestations of inflammation by suppression of mediators, such as cytokines and chemokines. Furthermore, regulation of immune cells alters as a result of corticosteroid treatment, which may lead to reduction of phagocytosis, antigen response, cytokine production and cellular immune response. On the other hand, free radical scavengers reduce inflammatory reactions by neutralizing free radicals that are produced during the inflammatory cascade, thereby limiting ongoing tissue damage.

In the studies included in this review both pathways result in decrease of symptoms in patients with CRPS-1, which is in line with the pathophysiological mechanisms proposed to be involved in CRPS-1. Abberant and neurogenic inflammation after trauma associated with elevated cytokine levels [2,17], elevated activity of mast cells [18] and increased cell markers of oxidative stress have been reported [6]. Furthermore, ischemia-reperfusion injury leading to excessive free radical production has been proposed to play a role in CRPS-1 [13].

Interestingly, the effects of both interventions were not uniformly beneficial. Although significant pain reduction was observed for DMSO and N-Acetylcysteine in the course of treatment [29], no difference was found between both interventions. In addition, DMSO exhibited no effects on pain reduction in another high quality placebo controlled trial [38] and intravenous mannitol provided no effects on any outcome measurements [28]. Intravenous corticosteroids [31] and low dose corticosteroids [24] also showed no effects on pain reduction or ROM. Our findings suggest that these treatment modalities may not be equally effective for all features exhibited by CRPS-1 patients. Furthermore, the mode of administration (i.e. intravenous, oral, topical) may be of influence on the efficacy of the intervention. In addition, these treatments were applied in heterogeneous groups of CRPS-1 patients, without accounting for possible differences related to prevailing pathophysiological mechanisms in individual patients. Arguments in favour of a phenotype or mechanism based approach to CRPS-1 have been made by some researchers [15,25]. Unfortunately, descriptions of clinical profiles of patients included in the studies were insufficient to allow for phenotype-based subgroup comparisons in the present review.

Restrictions

Studies of limited methodological strength were also included in the present topical review to obtain a comprehensive overview of effects of anti-inflammatory therapies. This may, however, have led to overestimation of the effects, because low quality studies tend to report more positive results. Different non-validated or subjective measurement instruments were used in the evaluated studies, limiting the reliability and comparability of results.

Articles of our own group [28,29,38] were evaluated in the present review. To exclude bias the quality assessment was not performed by the authors involved in these studies. In addition, the applied methodological scoring list used left little room for interpretation bias. Furthermore, all evaluated studies addressing prevention of CRPS-1 were performed by the same research group, and the number of patients that actually developed CRPS-1 was limited. Replication of these findings in other settings may therefore be warranted.

Needs for the future

Further research on anti-inflammatory therapy in patients with CRPS-1 is clearly indicated. Inclusion of homogeneous patient groups using internationally accepted diagnostic criteria [16] and the use of standardized measurement instruments for pain, physical function as well as for quality of life may help improve the interpretation and comparability. Research targeted at well-defined subgroups of CRPS-1 patients with a clear inflammatory profile may add to a more mechanism based approach.

Considering the positive results for both free radical scavengers and corticosteroids, studies comparing both treatment modalities as well as combining free radical scavengers and corticosteroids may be of interest. Further research may explore other forms of anti-inflammatory therapy, for instance anti-TNF-α and immunoglobulins.

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Oxidative Stress in Complex Regional Pain Syndrome (CRPS): No Systemically Elevated Levels of Malondialdehyde, F2-Isoprostanes and 8OHdG in a Selected Sample of Patients

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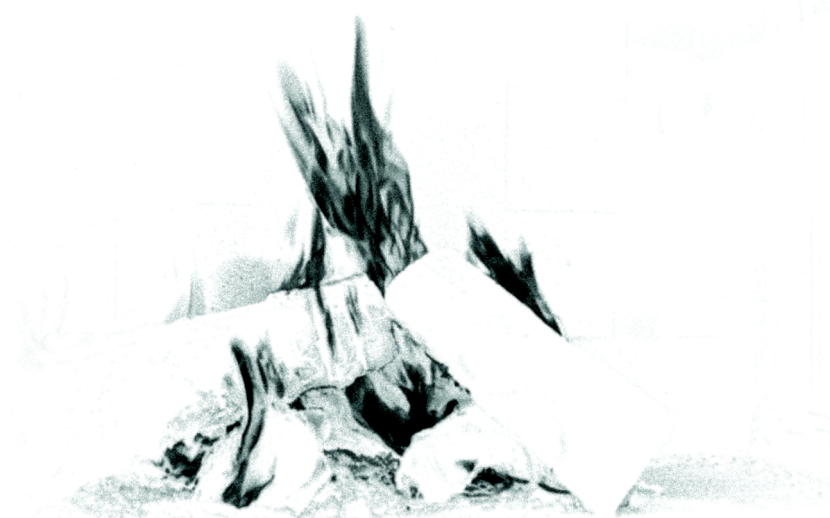
Abstract

Exaggerated inflammation and oxidative stress are involved in the pathogenesis of Complex Regional Pain Syndrome (CRPS). However, studies assessing markers for oxidative stress in CRPS patients are limited. In this study, markers for lipid peroxidation (malondialdehyde and F2-isoprostanes) and DNA damage (8-hydroxy-2-deoxyguanosine) were measured in nine patients (mean age 50.1 ± 17.1 years) with short term CRPS-1 (median 3 months) and nine age and sex matched healthy volunteers (mean age 49.3 ± 16.8 years) to assess and compare the level of oxidative stress. No differences were found in plasma between CRPS patients and healthy volunteers for malondialdehyde (5.2 ± 0.9 μmol/L vs. 5.4 ± 0.5 μmol/L) F2-isoprostanes (83.9 ± 18.7 pg/mL vs. 80.5 ± 12.3 pg/mL) and 8-hydroxy-2-deoxyguanosine (92.6 ± 25.5 pmol/L vs. 86.9 ± 19.0 pmol/L). Likewise, in urine, no differences were observed between CRPS patients and healthy volunteers for F2-isoprostanes (117 ng/mmol, IQR 54.5–124.3 vs. 85 ng/mmol, IQR 55.5–110) and 8-hydroxy-2-deoxyguanosine (1.4 ± 0.7 nmol/mmol vs. 1.4 ± 0.5 nmol/mmol). Our data show no elevation of systemic markers of oxidative stress in CRPS patients compared to matched healthy volunteers. Future research should focus on local sampling methods of oxidative stress with adequate patient selection based on CRPS phenotype and lifestyle.

Keywords: CRPS; oxidative stress; inflammation; MDA; F2-isoprostanes; 8OHdG

1. Introduction

Complex Regional Pain Syndrome (CRPS) is a disabling condition that is usually preceded by a trauma and characterized by excessive pain, sensory disturbances, changes in temperature and color of the affected area and disturbed motor function [1,2]. An exaggerated inflammatory response is suggested to be a key mechanism in the development of CRPS, resulting in oxidative stress and elevated levels of pro-inflammatory mediators [3–6]. It is hypothesized that oxidative stress in CRPS is due to increased exposure to free oxygen radicals and insufficient anti-oxidative defenses [7], and formed the basis for treatment of CRPS with anti-oxidants [8–10]. Free radicals can be generated by various sources, for example exposure to toxins such as cigarette smoke, inflammation as believed in CRPS, but increased levels of free radicals can also result from the physiological aging process [11]. Since direct detection of free radicals is impracticable due to its highly volatile nature [12], several indirect methods have been proposed to examine the levels of oxidative stress *in vivo*. Lipid peroxidation is a mechanism induced by oxidative stress, in which free radicals abstract a hydrogen atom from a methylene carbon in their side chain. Products of this mechanism are, among others, malondialdehyde (MDA) and F2 isoprostanes [11,13]. MDA is the most studied marker for oxidative stress [13], but F2-isoprostanes better reflects oxidative stress because this marker is more specific and is not influenced by dietary intake [14–16]. Furthermore, high levels of free radicals may react with DNA, leading to generation of 8-hydroxy-2'-deoxyguanosine (8OHdG), which has been widely used as a biomarker for oxidative DNA damage. 8OHdG originates from oxidation of the deoxynucleotide pool and is stable and not metabolized in the systemic circulation, and therefore reliable as a marker for oxidative stress [12,17,18]. Assessment in urine is in general preferred because of the short half-life time low stability of the markers during storage and measurements of plasma [11].

Eisenberg *et al.* found elevated levels of MDA and anti-oxidants in serum and saliva of patients with CRPS, therewith establishing a possible involvement of oxidative stress in the disease mechanism of this condition [5]. These findings may provide an explanation for the effectiveness of the free radical scavengers in the treatment of CRPS [9,19]. Dimethylsulfoxide and N-Acetylcysteine are free radical scavengers that are first choice treatment for CRPS in Dutch guidelines, and their therapeutic effect is described in German guidelines for the treatment of CRPS [8,20]. These laboratory findings may be of value for CRPS and stimulate research on therapeutic options based on aberrant inflammation in CRPS.

However, the patients in the study by Eisenberg *et al.* may not be representative for the population of CRPS patients. The studied patient sample consisted of 17 male and 14 female patients, whereas the prevalence of CRPS is highest in females (3–4:1) [21]. Another concern could be the disease duration of included patients, since inflammatory signs and symptoms are most dominant in short term CRPS [22] whereas Eisenberg *et al.* included predominantly patients with longer disease durations. In addition it is not clear, whether or not lifestyle related factors were corrected for in this study (whereby smoking is considered the most important). From population based studies it is known that CRPS patients compared to matched non-CRPS controls are more often smokers and this may bias results. Furthermore the reliability and accuracy of markers for oxidative stress may have influenced the results. Although MDA is a widely marker for oxidative stress, F2 isoprostane is considered to be a more discriminative marker to measure lipid peroxidation [11]. The stability and influence of external factors between various types of specimen differs, whereby markers for oxidative stress are more stable in urine [16]. Saliva on the other hand, is easily influenced by external influences leading to less reliable analysis.

The aim of the present study was to compare levels of highly specific oxidative stress markers, *i.e.*, F2-isoprostanes and 8OHdG in a representative sample of non-smoking female CRPS patients with a short duration of CRPS to age and gender matched healthy volunteers.

2. Results

2.1. Patient Characteristics

Nine female CRPS patients, mean age 50.1 years (SD 17.0, age range 19–66), median disease duration 3 months (IQR = 1.5; 2–3.5), and nine age and sex matched healthy volunteers (mean age 49.3 years (SD 16.8, age range 23–76) participated in the study. Age differences between patients and matched healthy volunteers ranged from 1–10 years (median 3 years). Five patients with an affected upper extremity and four patients with an affected lower extremity were studied. CRPS patients reported pain scores of 5.2 (mean over a week) and 6.2 (during use of the affected extremity). A vivid inflammatory profile with a warmer affected extremity, edema, pain and functio leasa was found in four patients (Table 1). One patient did not provide a urine sample.

Table 1. Characteristic and markers of oxidative stress of Complex Regional Pain Syndrome (CRPS)-1 patients and healthy volunteers.

	CRPS-1 patients $n = 9$	Healthy volunteers $n = 9$	*p*-value
Age *	50.1 (17.0)	49.3 (16.8)	0.92
Duration CRPS (months) **	3.0 (2.0–3.5)	-	-
Affected extremity	-	-	-
upper/lower	5/4		
Temperature affected extremity (warm/cold/no difference)	4/3/2	-	-
Swelling (yes/no)	4/5	-	-
Reduced range of motion (yes/no)	9/0	-	-
Pain score * — Pain yes/no	9/0		
Pain score * — mean over 1 week	5.3 (2.3)	-	-
Pain score * — during movement	6.2 (3.3)		
Impairment levels sum score * (ISS)	26.1 (11.1)	-	-
CRPS Severity Score * (CSS)	10.3 (2.6)	-	-
Plasma	-	-	-
MDA* (µmol/L)	5.2 (0.9)	5.4 (0.5)	0.66
F2 isoprostanes * (pg/mL)	83.9 (18.7)	80.5 (12.3)	0.65
8OhdG * (pmol/L)	92.6 (25.5)	86.9 (19.0)	0.60
Urine	$n = 8$	$n = 9$	-
F2 isoprostanes ** (ng/mmol)	117 (54.5–124.3)	85.0 (55.5–110.0)	0.61
8OHdG* (nmol/mmol)	1.4 (0.7)	1.4 (0.5)	0.85

* mean and SD (independent sample *t* test). ** median and IQR (Mann Whitney).

2.2. Oxidative Stress Markers

No significant difference was found between CRPS patients and healthy volunteers for MDA (mean 5.3 µmol/L *vs.* 5.4 µmol/L; $p = 0.66$), F2-isoprostanes (mean 83.9 pg/mL *vs.* 86.9 pg/mL; $p = 0.65$) and 8OHdG (mean 92.6 pmol/L *vs.* 86.9 pmol/L; $p = 0.59$) in plasma (Table 1).

Also in urine no significant differences were found between CRPS patients and healthy volunteers for F2-isoprostanes (median 117 ng/mmol *vs.* 85 ng/mmol; $p = 0.61$) and 8OHdG (mean 1.4 nmol/mmol *vs.* 1.4 nmol/mmol; $p = 0.85$).

Sub-analyses comparing patients with a warm and/or swollen affected extremity to patients with less pronounced inflammatory signs and symptoms showed no significant differences of MDA, F2 isoprostanes and 8 OHdG ($p > 0.5$). However, there was
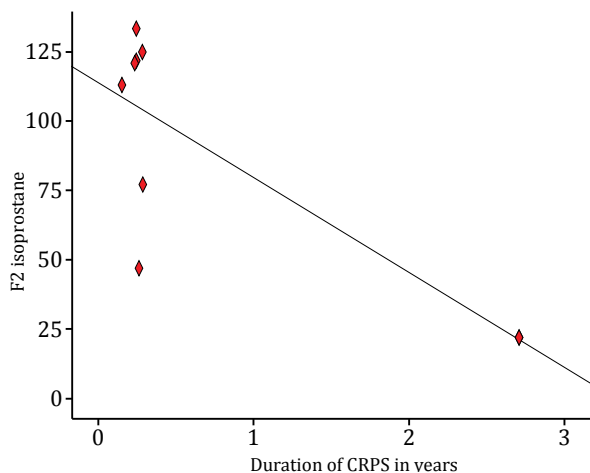
Figure 1. Correlation between levels F2 isoprostane and duration of CRPS. R = −0.72; (p = 0.04).

a strong negative correlation (−0.72; p = 0.04) between duration of CRPS with levels of F2-isoprostanes in urine, indicating higher levels of oxidative stress in shorter duration of CRPS (Figure 1) Statistically significant correlations between age and marker for oxidative stress were only found for MDA in plasma (0.60; p = 0.02), 8OHdG in plasma (0.56; p = 0.02) and F2 isoprostanes in urine (0.49; p = 0.05). Levels of 8OHdG in plasma and urine were highly correlated (0.80; p = 0.00), however for F2-isoprostanes in urine and plasma these correlations were not found (0.29; p = 0.26).

3. Discussion

In the present pilot study, levels of markers of lipid peroxidation (MDA and F2 isoprostanes) and DNA damage (8OHdG) in plasma and in urine of female CRPS-1 patients were not found to be elevated compared to age and sex matched healthy volunteers. This is in contrast with previous findings whereby elevated levels of MDA were found in serum and saliva [5] in a sample of 31 CRPS patients. This was an unexpected finding since the clinical profile of the included patients in our study was typical for a picture of inflammation (dolor, rubor, calor and functio laesa). Furthermore, a study by Schinkel *et al.* revealed systemically elevated levels of IL-8 and sTNFR in acute CRPS patients [6]. Notwithstanding these findings, the systemic inflammation as expressed by the level of oxidative stress with the markers used in our study did not reveal increased levels of oxidative stress. However, it is too premature to conclude that the well-established theory of tissue injury, inflammation and oxidative stress leading to the development and persistence of CRPS [23-25] should be abandoned. Studies evaluating local cytokines levels and mast cells in blister fluid of the affected extremity

reveal elevated levels of TNF-alpha, IL-6 and tryptase as an indicator for increased mast cells in CRPS patients [24–26]. Moreover, diminished oxygen metabolism shown in muscles of affected extremities of 11 CRPS patients [27] and increase of skin lactate as a marker for hypoxia in 11 CRPS patients [28] provided a basis for a localized exaggerated oxidative response in CRPS.

In accordance with our findings, however, is the fact that in several studies evaluating systemic markers no elevated levels of markers for inflammation and oxidative stress were found. Levels of cytokines were found not to be elevated in plasma of nine patients with CRPS, whereas levels of markers of inflammation were increased in blister fluid obtained from the affected CRPS extremity were increased in these same patients [25]. The same result was found in patients with severe CRPS with multiple affected extremities [29].

Another explanation for the disagreement between studies may be the heterogeneity of the population and the selection of a representative patient population. Comparing our present findings to the study by Eisenberg *et al.* [5], several differences already described could have contributed to the differences between both studies. Furthermore, the groups in our study were carefully matched for age in order to minimize differences between the groups (mean 49.3 *vs.* 50.1 years of age, median difference of 3 years), because it is well known that ageing is related to increase of oxidative stress [30-31]. In our studied patient sample, age was strongly correlated to F2-isoprostanes, MDA and 8OHdG. Importantly, in the study by Eisenberg *et al.* more male than female patients were included, no adjustments were performed for smoking and the manner in which age matching was performed was not clearly described.

Additionally, laboratory procedures and specimens differ between studies as well. In the present study both urine and plasma was analyzed, whereby oxidative stress markers in urine are most stable. Moreover a highly specific mass-spectrometry (LC-MS/MS) procedure was used because it has been shown that immuno-assay analyses overestimate levels of F2-isoprostanes and 8 OHdG [28,30]. In the studied samples no correlations were found between levels of F2-isoprostanes in urine and plasma indicating the importance of correct and reliable procedures and difference of stability of markers for oxidative stress. Taken together, the differences in methods and patient populations between both studies may explain the conflicting results that were found.

A limitation of this study is the lack of reference values for products of lipid peroxidation and DNA damage in the general population, leading to the need of match-

ing healthy volunteers, leading to a possible selection bias. The small sample size can also relate to the fact that no significant differences between the groups were found. However, when differences are as small as in our study one should consider its clinical relevance. Notwithstanding these results, discriminative markers for oxidative stress as a screening method for CRPS may be of value for understanding the complex pathology of CRPS and may be helpful in diagnostics and phenotyping of CRPS.

One should keep in mind that measurements have been performed in body fluids representing systemic values of oxidative stress (urine and plasma), while CRPS has a regional distribution [6,25]. Although no support for systemically elevated levels of free radicals was found in this study, one should therefore be cautious in dismissing therapies based on systemic intake of free radical scavengers (*i.e.*, N-acetylcystein and vitamin C) as these may exert effects more on a local level. Furthermore, a substantial body of evidence is available for the efficacy of preventing CRPS by increasing vitamin C intake. Nonetheless, considering the fact that both N-acetylcystein and vitamin C may influence multiple biological processes, the efficacy could be related to other mechanisms than attributable to their scavenging properties.

Studies performed to analyze local inflammation and oxidative stress [4,6,25,26] do show positive results in patients with CRPS. It has been shown that most promising markers for research of inflammation in CRPS are pro- and anti-inflammatory cytokines [24,31]. Therefore, another approach should be to assess and compare local inflammatory and oxidative stress markers in individuals and extend research when positive results are found to specify patients with an inflammatory profile of CRPS.

The high correlation found between disease duration and levels of F2-isoprostanes in urine may indicate that patients with a very short disease duration have the most profound inflammatory profile as was suggested in earlier studies [1]. However, these correlations should be considered with care, because of the small sample size potentially biasing our outcomes. Also in our study, one measurement significantly influenced the magnitude of the correlation. Nonetheless, these should be kept in mind when selecting patients for studies evaluating levels of oxidative stress or inflammatory markers in CRPS. On the other hand, studies evaluating anti-inflammatory therapy for patients with CRPS may want to focus on patients with short time CRPS to prevent elaborate tissue injury due to exaggerated inflammation and oxidative stress.

Future research should focus on local assessments of markers of oxidative stress and inflammation, whereby selection of CRPS patients with a short disease duration, an inflammatory phenotype and specific methods of analysis are important. Further

research is relevant to elucidate understanding of this complex disease and it may lead to an objective measurement to improve diagnostics, therapy and phenotyping in CRPS.

4. Methods

4.1. Patients and Healthy Volunteers

Nine female patients with CRPS-1 according to the IASP "Budapest" criteria visiting the pain department of the VU University Medical Center in Amsterdam were enrolled. Blood samples from the unaffected upper extremity and a sample of morning urine were provided. Control samples were obtained from gender and age matched volunteers (<10 years difference). Patients and healthy volunteers did not smoke and did not use free radical scavengers in the week prior to sampling. Blood was collected in EDTA containers and directly centrifuged at 1500 × g. Plasma was stored at −80 °C in aliquots. Urine was transferred into polypropylene tubes for storage at −20 °C.

4.2. Measurement of Plasma Malondialdehyde

The concentration of total (free and protein-bound) plasma MDA in EDTA-plasma was determined after reaction with thiobarbituric acid (TBA) [32]. To 50 μL of plasma 25 μL of 0.2% butylatedhydroxytoluene (BHT) as anti-oxidant and 0.4 mL 1 mol/L sodium hydroxide for alkaline hydrolysis were added. The mixture was incubated at 60 ° C for 60 min in a shaking water bath. After cooling to room temperature, 1.5 mL of 1% potassium iodide in 10% trichloroacetic acid was added, and the mixture was placed on ice for 10 min and centrifuged at 1500 × g for 10 min at 4 °C. To 0.5 mL of the supernatant 0.25 mL 41.6 mmol/L TBA was added, and the mixture was heated at 95 °C for 30 min. After cooling to room temperature and centrifugation (1500 × g, 10 min) 50 μL of the supernatant was injected on a symmetry C-18 column (Waters 4.6 × 100 mm, 3.5 μm) eluted at 1 mL/min by using 70% (v/v) 25 mmol/L KH_2PO_4 (pH 6.8) and 30% (v/v) methanol. Detection of the MDA-TBA adduct was performed with fluorescence detection (excitation at 515 nm and emission at 553 nm). For quantification the intensities of the MDA-TBA peak areas were compared to standards constructed with tetraethoxypropane (Sigma, St Louis, Missouri, USA). The intra-run and inter-run variations were 3.5% and 8.7%, respectively.

4.3. Measurement F2-Isoprostanes in Plasma

The total, *i.e.*, free and esterified, concentration of iPF2α-VI was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). In brief, 0.1 mL of 2 ng/mL deuterated internal standard (8iPF2α-d4; Cayman chemical, Ann Arbor, Michigan, USA) was added to 0.5 mL EDTA-plasma. Butylated hydroxytoluene (BHT) was added to a final concentration of 0.05% to prevent arachidonic acid from auto-oxidation during sample preparation. Then 0.5 mL of 2.6 mol/L KOH was added, and the samples were incubated for 60 min at 40 °C for alkaline hydrolysis. Afterwards, 250 µL formic acid (20%) was added to adjust the pH at 4.5, and the samples were cleaned up using Oasis mixed-mode anion exchange cartridge (3 cc/60 mg; Waters). The column was successively washed with 2 mL of 2% NH_4OH, 2 mL 10% methanol 20mM formic acid 40:60, 2 mL 100% hexaan and 2 mL hexaan:ethylacetate 70:30. The fraction containing F2-isoprostanes was eluted with 2 mL of 0.6% acetic acid in ethylacetate and then dried under a stream of nitrogen and successively dissolved in 100 µL 10% acetonitrile containing 0.1% formic acid. A volume of 20 µL was injected on a reverse-phase XTerra MS C18 column (Waters, Milford, Massachusetts, USA; 3.5 µm, 2.1 × 100 mm). F2-isoprostanes were quantified by a API 5000 triple quadruple mass spectometer (AB Sciex Technologies, Toronto, Canada). To calculate the iPF2α-VI concentration, the analyte to internal standard peak area ratio with transitions 353.2 and 357.7 respectively to 115.0 were compared with a standard curve up to 8 ng/mL iPF2α-VI (Cayman chemical, Ann Arbor, Michigan, USA). The intra-run and inter-run variations were 8.1% and 11.3%, respectively.

4.4. Measurement F2-Isoprostanes in Urine

The concentration of iPF2α-VI in urine was determined by LC-MS/MS. In brief, 0.1 mL of 10 ng/mL deuterated internal standard (8iPF2α-d4; Cayman chemical, Ann Arbor, Michigan, USA USA) was added to 1 mL urine. The sample was then subjected to solid phase extraction (Oasis HLB, Waters, Milford, Massachusetts, USA) as previously described [12,33]. The eluate was taken to dryness under a stream of nitrogen at room temperature, and afterwards redissolved in 100 µL 10% acetonitrile of which, 40 µL was injected on a reverse-phase XTerra MS C18 column (Waters, Milford, Massachusetts, USA; 3.5 µm, 2.1 × 100 mm). Urinary F2-isoprostanes were quantified using a Quattro Micro (Waters) mass spectrometer. To calculate the iPF2α-VI concentration, the analyte to internal standard peak area ratio was compared with a standard curve from 2 to 16 ng/mL iPF2α-VI (Cayman chemical, Ann Arbor, Michigan, USA). The intra-run coefficient of variation (CV) was 4.8% and the inter-run CV was 10.1%.

4.5. Measurement of 8-Hydroxy-2-Deoxyguanosine in Plasma

Plasma levels 8-OHdG were determined by adding 0.5 mL of 1 nmol/L internal standard ($^{15}N_5$ 8-OHdG, Buchem, Apeldoorn, The Netherlands) in 3.4% phosphoric acid to 0.5 mL EDTA-plasma. The analytes were then extracted using Oasis mixed-mode anion exchange 96-wells plate (60 mg; Waters, Milford, Massachusetts, USA) [34]. Each well was successively washed with 1.5 mL 5% NH_4OH, 1.5 mL 10% methanol and 1.5 mL 100% methanol. The fraction containing 8OHdG was eluted with 1 mL methanol containing 2% (*v/v*) formic acid, dried under a steam of nitrogen at room temperature and redissolved in 100 μL 5% methanol containing 0.1% (*v/v*) acidic acid. A volume of 12 μL was injected on a reverse phase HSS T3 column (1.8 μm, 2.1 × 100 mm; Waters, Milford, Massachusetts, USA). The eluate components were separated at a flow rate of 0.45 mL/min using a gradient of milliQ water and methanol containing 0.1% acetic acid and were measured on an AB Sciex API 5000 mass spectrometer in positive ion multiple reaction monitoring acquisition mode. To calculate the 8OHdG concentration, the analyte to internal standard peak area ratio with transitions 284.2 to 168.2 and 289.2 to 173.2 respectively were compared with a standard curve ranging 0.2–4.0 nmol/L 8-OHdG. Intra- and inter-assay CVs were 5.8% and 7.2%, respectively.

4.6. Measurement of 8-Hydroxy-2-Deoxyguanosine in Urine

Urine levels 8OHdG were determined similarly as determined in plasma, however: urine samples are 10 times diluted in MilliQ water, the standard curve ranges from 1.0–16.0 nmol/L [12]. Intra- and inter-assay CVs were 4.1% and 5.3%, respectively.

4.7. Statistics

Analyses were performed using SPSS 20. Patient characteristics, levels of F2-isoprostanes, MDA and 8OHdG were compared between patients and healthy volunteers using the independent sample *t* test and Mann-Whitney-U tests. For evenly distributed outcomes values were described as mean and with standard deviation, otherwise outcomes were expressed as median and interquartile ranges (with presentation of 1st and 3rd quartile). Correlations were calculated using Pearson's correlation between measured markers for oxidative stress and age and disease duration. A $p < 0.05$ was considered significant.

5. Conclusions

Altogether, this study does not confirm a role for systemically elevated levels of MDA, F2-isoprostanes and 8OHdG in patients with CRPS in plasma or urine. Although results of this study are based on a small sample size, the selection of patients was representative for the population of CRPS and analytical procedures were highly reliable and specific.

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Pyridostigmine: activating the cholinergic anti-inflammatory pathway in Complex Regional Pain Syndrome; a pilot study

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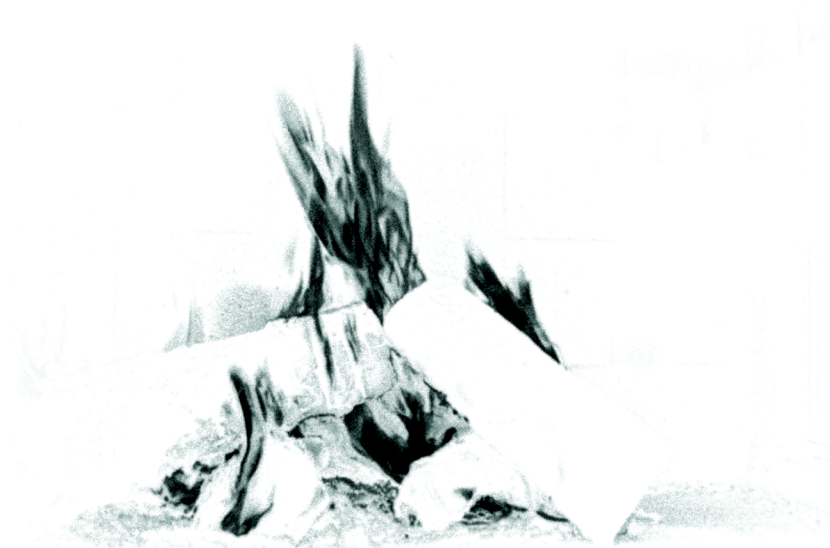
Abstract

Exaggerated inflammation involving excessive oxidative stress, neurogenic inflammation and autonomic disfunction are involved in the pathophyiological process of CRPS. The cholinergic anti-inflammatory pathway is a fast endogenous feedback mechanism that influences inflammation by regulating Acetylcholine (ACh) availability at the α7-nicotinic-ACh receptors (α7nAChR).

Objective:

This proof of concept study was designed to test the hypothesis that augmenting the availability of ACh would lead to a reduction of signs and symptoms of CRPS.

Methods:

Ten patients with CRPS were treated with the acetylcholinesterase antagonist pyridostigmine, consisting of a titration phase, 2 treatment phases (A) and 2 control phases (B) following an A-B-A-B sequence. Autonomic status and inflammatory profile were assessed at baseline. Primary outcome measurements were pain (BOX-11) and impairment (ISS). Functional limitations (RSQ, WASQ and health related quality of life (SF-36)) were assessed as secondary outcome measurements.

Results:

Significant pain reduction was found in five out of nine evaluated patients (1.1 to 21.5%), relevant reduction of impairment in three out of ten patients (5 to 7), improvement in functional limitations (1.0 to 2.6) and quality of life (11 to 34) in respectively six and three out of eight evaluated patients. One out of the two patients with definite parasympathetic dysfunction and one patient with probable parasympathetic dysfunction were reported as responders to pyridostigmine. Inflammatory profile was not related to a positive response.

Conclusions:

Most patients showed limited improvement on one or more outcome measures. These results warrant further research of the possibilities of activating the cholinergic anti-inflammatory pathway in CRPS-1 patients.

Key words: CRPS, pyridostigmine, cholinergic anti-inflammatory pathway

Introduction

Complex regional pain syndrome (CRPS-1) is a pain syndrome of the extremities that most commonly develops after trauma (1;2). Treatment with free radical scavengers (DMSO cream) or corticosteroids is currently shown to be effective in treating features of inflammation in CRPS-1 patients (3-5). However, approximately one third of patients remain disabled five years after the initial trauma, leading to unemployment and low quality of life. Therefore, new therapeutic pathways should be explored to reduce the disease burden of individual patients who do not respond to conventional therapy (6).

The predominant pathophysiological mechanism in CRPS is exaggerated inflammation involving excessive oxidative stress and neurogenic inflammation (7-9). This sustained inflammation leads to maladaptive neuroplasticity and vasomotor disturbances, resulting in signs and symptoms such as pain, allodynia, temperature asymmetries, motor disturbances and trophic changes of the affected limb (10-13). The exaggerated inflammatory response in CRPS may be explained by either excessive immune activation involving increased production of pro-inflammatory mediators (TNF-alpha, IL-6, SP and CGRP, free radicals) or due to a decreased or insufficient inhibitory regulation (14). Autonomic endogenous subsystems such as the cholinergic anti-inflammatory pathway have been proposed to play a key role in regulation of inflammation (15). Following this line of thought, disturbances in the autonomic subsystem can ultimately lead to deregulation of the inflammatory/anti-inflammatory balance (16). Indications of autonomic disturbances in CRPS are provided by data showing altered sympathetic neurotransmitters levels in the affected limb, coinciding with increased sensitivity of alpha-adrenergic receptors (17-20). Furthermore, increase of heart rate and decreased heart rate variability in patients with CRPS-1 has recently been reported as signs for autonomic disturbances (21).

The cholinergic anti-inflammatory pathway is a fast endogenous feedback mechanism (22) that can influence inflammation by regulating Acetylcholine (ACh) availability at the α7-nicotinic-ACh receptors (α7nAChR) (15;23). Activating α7nAChR expressed on immune cells (e.g. macrophages), inhibits inflammation by reduction of release of pro-inflammatory cytokines (eg. TNF-α). Therapeutic options for affecting this pathway can be found in activating α7nAChRs (eg. ACh receptor agonists) or an increase of ACh availability.

However, a safe and effective ACh receptor agonist has yet not been developed (24-26). On the other hand, approved cholinesterase inhibitors have been widely used in treatment of inflammatory diseases such as Alzheimer's disease (27-29). Cholinesterase inhibitors reduce the re-uptake and inhibit hydrolysis of acetylcholine, leading to an increase of acetylcholine availability, which results in activation of the cholinergic anti-inflammatory pathway (30).

Therefore, this proof of concept study was designed to test the primary hypothesis that augmenting the availability of ACh by means of the acetylcholinesterase inhibitor pyridostigmine would lead to a reduction of signs and symptoms of CRPS. Secondary hypotheses posed for this study state that patients with autonomic disturbances and patients with a predominant inflammatory symptom profile will have a stronger response to pyridostigmine administration compared to patients without these features.

Methods

Patients

Between July 2009 and December 2010, ten patients with CRPS-1 of one extremity fulfilling both the diagnostic criteria by Veldman et al. (31) and IASP 'Orlando' (32), not responding to treatment with free radical scavengers and physical therapy according to Dutch multidisciplinary guidelines, were included at the outpatient clinic of the VU University Medical Center in Amsterdam. The hospital's medical ethics committee approved the study, and all patients gave written informed consent.

Intervention

All patients were treated with the cholinesterase inhibitor pyridostigmine. The study comprised a three week titration phase (T) in which the highest tolerable dosage of oral pyridostigmine was established for the individual patient (30mg/day to 180 mg/day), two four week treatment phases (A) and two three week control phases (B) (T-A-B-A-B), resulting in a total trial duration of 17 weeks. Most common side effects were monitored using a validated scale for pyridostigmine (33). Other side effects were assessed by means of diaries filled out by the patient.

Study protocol

At baseline, parasympathetic activity was measured using three Ewing tests: expiration/inspiration ratio to hyperventilation, heart rate ratio after Valsalva maneuver/during Valsalva maneuver and heart rate response to standing. Heart rate was monitored either by ECG or by non-invasive finger arterial pulse rate measurements (Nexfin HD, BMEYE, The Netherlands). Based on predefined values, normality was assumed if: expiration/inspiration ratio >1.17, heart rate ratio after Valsalva >1.20 and heart rate response to standing >1.03 (34;35). One abnormal test was interpreted as possible parasympathetic dysfunction; two abnormal tests were interpreted as a definite parasympathetic dysfunction (34). Determination of a clinical profile was based on observation of classical clinical signs of inflammation (i.e. dolor, rubor, calor tumor and functio laesa) at baseline (31). Presence of three clinical signs was interpreted as a possible inflammatory profile, four as probable, and five as definite.

Pain scores were assessed on a BOX-11 scale three times a day, one week before and 17 weeks during titration, treatment and control phases. The patients' global impression of change (PGIC) was evaluated after every phase of the trial for key inflammatory signs associated with CRPS (pain, volume differences, discoloration, temperature increase, decrease in range of motion). The severity of impairment was assessed by means of the Impairment Level Sum Score (ISS), a validated compound score (5-50 scale) to assess the severity of CRPS calculated from measurements on pain (BOX-11, McGill (NWC)), temperature, volume and active range of motion (AROM) (36;37). The ISS was obtained six times during the course of the study phases, whereby changes ≥5 points were considered relevant. Furthermore, functional limitations (Questionnaire on Walking and Rising (QWR) for lower extremity CRPS (38;39), or Radboud Skill Questionnaire (RSQ) for upper extremity CRPS, whereby changes ≥1 point were considered relevant) (40) and health related quality of life (SF-36, and changes >10 points were considered relevant (41)) were measured at baseline and at the end of the trial. All assessments were performed by a single investigator, based on a rigid protocol used as a source document in the TREND research consortium. The investigator followed regular training sessions (3 times a year) for these assessments.

Statistical analysis

Data were stored in a NEN-7511 certified central web-based database (ProMISe ®). Analyses were performed in SPSS version 15.0. Pain scores were evaluated using time-series analysis per individual patient, whereby phases were compared and outcome was adjusted for auto- correlation to account for fluctuations over the day (morning-afternoon-night). Analysis of the ISS was performed per patient, whereby an improvement of ≥5 points during the treatment phases (A and T) compared to either baseline measurements and during the control phases (B) was considered to be clinically relevant. Patients with relevant improvement were subsequently reported as 'responders'.

Disability and quality of life were analyzed using paired sample T-tests in case of a normal distribution, and the Wilcoxon signed ranks tests if a non-normal distribution was observed. For all analyses a two sided $p<0.05$ was used to indicate statistical significance.

Results

Patient characteristics

In this study more female then male patients participated (9 vs 1), as expected considering the higher female prevalence in CRPS. A broad range in age and disease distribution was observed in our study sample. Mean scores for spontaneous pain (BOX-11) at baseline ranged from 2 to 8 points, whereby 8 patients had a score of 5 or higher and during use of the extremity all patients reported a score higher then 5, generally considered to indicate moderate to severe pain (42). For half the patients pyridostigmine could be titrated up to the highest proposed dosage of 180 mg/day (5), patient 1 had to return to the lowest dosage of 30 mg/day due to moderate gastro-intestinal side effects, polyuria, headache and nausea and patient 8 had to lower the dosage to 60 mg/day because of fatigue and dizziness. Precipitating trauma's were diverse, with a large percentage of soft tissue injuries (Table 1).

Table 1. Patient characteristics.

N	10		
Sex			
Female	9		
Male	1		
	Mean (SD)	Median	Range
Age	40.4 (11.1)	39	29-58
Duration of CRPS (months)	33.5 (29.3)	25	9-102
Pain score at baseline (Mean over 1 week)	5.40 (1.74)	5.10	2-8
Pain score during activity	7.00 (1.87)	7.00	5-10
	Number of patients		Patients
Dosage of pyridostigmine			
180 mg/day	5		3,5,6,9,10
90 mg/day	3		2,4,7
60 mg/day	1		8
30 mg/day	1		1
	Number of patients		
Affected extremity	Arm	Leg	
	2	8	
Left	1	2	
Right	1	6	
Temperature affected extremity			
Cold	2	4	
Warm	0	4	
	Number of patients		
Trauma			
Fracture	1		
Operation	2a		
Distortion/contusion	4		
No trauma	1		
Other	2b		

a Dupuytren and ankle tendon
b Achilles tendon rupture and tendonitis

Autonomic and inflammatory profile

Based on the Ewing tests, two patients had a definite abnormal parasympathetic function and five patients had a possible abnormality of parasympathetic nervous system. Three patients showed no autonomic function abnormalities (Table 2).

Based on clinical profile assessing classic signs of inflammation (dolor, rubor calor, tumor, functio laesa), two patients had a probable inflammatory profile and four patients a possible inflammatory profile (Table 3).

Table 2. Parasympathetic functioning measured using Ewing tests.

Ewing tests					
Patient	E/I ratio HV	RRi Valsalva	HR to standing	Abnormal tests	Parasympathetic dysfunction?
1	1.07	1.65	1.04	1	Possible
2	1.35	1.64	1.61	0	No
3	1.08	1.20	0.93	2	Definite
4	1.12	1.28	1.08	1	Possible
5	1.19	1.12	1.05	1	Possible
6	1.16	1.18	1.08	2	Definite
7	1.20	1.19	1.11	1	Possible
8	1.50	2.76	1.48	0	No
9	1.11	1.93	1.21	1	Possible
10	1.69	2.12	1.11	0	No
norm values	> 1.17	> 1.20	> 1.03	1 abnormal test = possible dysfunction 2 abnormal test = definite dysfunction	

E/I ratio HV=expiration/inspiration ratio during hyperventilation, RRi Valsalva= heart rate after valsalva/during valsalva, HR standing = heart rate 30 beats/heart rate 15 beats after standing up. Patients with definite abnormal parasympathetic nervous system functioning are depicted in orange.

Table 3. Inflammatory profile based on clinical observation.

			Inflammatory signs				
Patient	Dolor	Calor	Tumor	Rubor	Functio laesa	Number of signs	Inflammatory profile?
1	1	0	0	1	1	3	Possible
2	1	0	0	0	1	2	No
3	1	0	0	0	1	2	No
4	1	0	0	0	1	2	No
5	1	1	0	0	1	3	Possible
6	1	0	0	?	1	2	No
7	1	0	1	0	1	3	Possible
8	1	1	0	0	1	3	Possible
9	1	1	0	1	1	4	Probable
10	1	1	1	0	1	4	Probable
						3=possible, 4=probable, 5=definite inflammatory profile	

Table 4. Results on pain in points on the NRS scale and in percentages improvement (significance a p<0.05, b p<0.01).

Patient num-ber	NRS scores missing	Number of days for auto-correlation	Ljung Box Q	NRS intercept (baseline) (BL)	Titration phase (T) vs baseline (BL)	Treatment phase 1(A) vs baseline (BL)	Control phase 1 (B) vs baseline (BL)	Treatment phase 2 (A) vs baseline (BL)	Control phase 2 (B) vs baseline (BL)	Treatment (A) vs Control phases (B)	Phase 1 (T1) vs Phase 2(T2)c
1	1 (0.3%)	2	0.27	5.18	1.25b	0.03	0.11	0.02	0.01	0.06	-0.55
2	0 (0.0%)	3	0.07	3.60	-0.16	0.06b	-0.02	-0.04b	0.04	-0.66b	0.09
3	8 (2.1%)	3	0.01	2.35	0.22	0.00	0.01	0.02	0.01	-0.15	0.18
4	1 (0.3%)	1	0.34	5.40	0.07	0.07	0.11	-0.07a	0.17	-1.16b	-0.44
5	1 (0.3%)	4	0.01	8.11	-0.53**	0.04b	-0.01	0.00	0.00	-0.20a	-0.24b
6	0 (0.0%)	1	0.09	7.75	-0.29	-0.12b	0.02	-0.15b	0.00	0.01	0.63a
7	32 (8.5%)	4	0.00	5.06	-0.16	-0.05	0.06	0.01	0.08	0.77	0.31
9	4 (1.1%)	4	0.02	5.66	0.35	0.02	-0.16	0.01	-0.09	1.01b	0.97a
10	3 (0.8%)	3	0.05	4.79	-0.29	-0.01	0.00	-0.04	-0.01	0.16	0.06

in green significant reduction of pain during treatment phase compared to baseline/phase 2 compared to phase 1;

in red significant increase of pain during treatment phase compared to baseline/phase 2 compared to phase 1.

c treatment phase 1 and control phase 1 compared to treatment phase 2 and control phase 2.

For patient 8 analysis was not performed due to too many missing values (138 missing, 36.5%).

Outcome

Pain (BOX-11)

NRS scores were analyzed in nine patients. Data from one patient showed too many missing values to allow analysis. Compared to baseline, one patient showed significant pain reduction during the titration phase (patient 5), two patients showed significant pain reduction in treatment phase 2 (patients 2 and 4), and one patients showed significantly lower pain scores during both treatment phases (patient 6). Compared to the control phases, significantly lower pain scores were found during the treatment phases (A vs. B) for three patients (patients 2, 4 and 5). In two patients, significant pain reduction was achieved over the total course of the trial (patient 6 and 9). Pain reduction ranged from 0.04 to 1.16 points on the NRS scale points (table 4 previous page), i.e. 1.1% to 21.5%.

Impairment level Sum Score

Three patients showed a clinically relevant reduction in ISS over the course of the trial (≥5 points) (patient 6, 7 and 8) (Table 5). Changes in ISS were largely due to reduction in pain scores (NRS and McGill (NWC)), small effects were observed for increase in range of motion in patient 6.

Table 5. ISS scores (based on mean NRS during 1 week prior to measurement).

patient	baseline	Treatment phase 1 (A)		Control Phase (B)	Treatment phase 2 (A)		End of trial (B)
		start	end	3 weeks	start	end	
1	26	27[a]	30		33	32	30
2	19	22	27		22	23	18
3	23	26[a]	32		37	24	25
4	29	26[a]	33		23[a]	32	34
5	22	22	27[a]		24	21	24
6	34	27	22[a]		31	27	29
7	20	19	20		12[a]	14	15
8	27	16[a]	23		20	20	
9	24	25	35		21	20	26
10	25	24	19		20	21	26

In green improvement of ISS ≥ 5,
in red worsening of ISS ≥ 5 compared to baseline.
a ISS score without McGill sub-score, when green improvement of ISS without McGill sub-score
≥ 5 compared to baseline ISS without McGill sub-score.

Patient global impression of change

On the PGIC, a decrease of pain severity was reported by three patients (patient 4,6,7) during the treatment phases, decrease of swelling in four patients (patient 4,7,9), decrease of color abnormalities and an increase of range of motion in three patients (patient 4,6,9), and a decrease of temperature asymmetry in three patients (patient 6,7,9). These observations corresponded with changes observed in NRS-pain measurements for one patient, and for measured active range of motion in two patients

Table 6. Functional status of the affected hand or affected foot.

Patient	Functional limitations baseline				Functional limitations end of the trial				Change			
	RSQ	QWR			RSQ	QWR			RSQ	QWR		
		House	Outside	Stand-ing up		House	Outside	Stand-ing up		House	Outside	Stand-ing up
1		7.1	9.6	10								
2		8.8	8.7	9.5		7.1	9.1	9.5		-1.7	0.4	0
3		5.9	10	5.3		5.9	10	4.2		0	0	-1.1
4	3				3.5				0.5			
5		4.7	7.4	8.9		2.9	7	6.3		-1.8	-1.1	-2.6
6		8.8	7.8	10		8.2	8.7	8.4		-0.6	0.9	-1.6
7		0	1.7	0.5		0	0.9	0		0	-0.8	-0.5
8	3.3				--				--			
9		5.3	7.8	7.9		5.3	8.7	10		0	0.9	2.1
10		2.9	4.8	0		0	4.4	3.7		-2.9	-0.4	-1.1
Mean (SD)		5.2 (3.14)	6.89 (2.77)	6.01 (4.22)		4.20 (3.30)	6.96 (3.25)	6.01 (3.62)		-1.00 (1.15)	0.08 (0.66)	0.00 (2.19)
p										0.60	0.77	1.00

(Hand: Radboud Skills Questionnaire (RSQ) on a 1-5 score resulting from the mean of all scored questions, foot: (Walking Skill Questionnaire (QWR) on a 0-10 scale, lower scores indicate better function, negative change-scores indicate improvement (green), positive change as reduced function (red)). Changes of more than 1 point are considered relevant.

Functional limitations and Health Related Quality of Life

One patient with upper extremity CRPS reported no change in functional limitations as measured by the RASQ. Overall, reductions of functional limitations for patients with lower extremity CRPS were found for walking skills in the house, however these changes were not significant. No improvements were found for other subscales of the QWR (table 6) (38). Health related quality of life was assessed as measured with the

SF-36 and showed improvements in the mental and physical health domains, however these changes were not statistically significant on group level. In three patients (2,6 and 7) this improvement was relevant in both domains, one patient (patient 4) showed severe deterioration in both domains of the SF-36 (table 7).

Table 7. SF-36.

Patient	Baseline Mental Health	Baseline Physical Health	End of trial Mental Health	End of trial Physical Health	Change Mental Health	Change Physical Health
1	41	21				
2	60	27	94	40	34	13
3	47	27	49	26	2	-1
4	49	66	41	52	-8	-15
5	73	39	82	38	9	-1
6	30	12	55	32	25	20
7	72	67	83	79	11	12
8	76	48				
9	70	38	70	40	0	2
10	79	37	66	66	-13	29
Mean (SD)	60 (16.75)	39.13 (19.03)	67.50 (18.37)	46.50 (17.91)	7.5 (15.90)	7.38 (13.89)
					$p = 0.22$	$p = 0.18$

Quality of life measured by the SF-36, considering subjective mental health and physical health (on a 0-100 scale; higher scores indicate better QoL). Changes > 10 points are considered to be relevant.

Subgroup analyses

Based on the response obtained on the ISS, one of the responders showed definite parasympathetic dysfunction (patient 6), and one patient had a possible parasympathetic dysfunction (patient 7). Two responders showed a possible inflammatory profile (patient 7 and 8). One patient with a probable inflammatory profile fulfilled responder criteria at the end of the first treatment phase and the start of the second treatment phase. Overall, disease duration was slightly shorter for responders then for non-responders (median 21 vs. 29 months (p=0.91)) and responders were older (median 51 vs 35 years (p=0.42)), but these differences were not significant. No relationship was observed between dosage of pyridostigmine and response.

Side effects

No patients dropped out of the trial due to side effects. However gastro-intestinal disturbances (bloating, diarrhea, nausea), fatigue, muscle weakness, pain and polyuria were reported (table 8). Side effects mostly disappeared during the titration phase, for two patients after lowering the pyridostigmine dosage.

Table 8. Reported side effects due to pyridostigmine use.

Side effects	Number of patients	Severity	Course
Gastro-intestinal	5	Mild to moderate	Spontaneously relieved, lowering dosage of pyridostigmine, acceptable side effect, start loperamide
Fatigue	4	Mild to moderate	Spontaneous improvement over time, lowering dosage of pyridostigmine
Muscle weakness or pain	2	Mild to severe	Spontaneously relieved
Polyuria	2	Mild to moderate	Spontaneously relieved, lowering dosage of pyridostigmine

(pyridostigmine scale: 1-2 = mild, 3-4 = moderate, 5-6 = severe side effects)

Discussion

In this proof of concept study the effects of increasing acetylcholine availability were studied on characteristics of CRPS, whereby (partial) pain reduction was found in five out of nine evaluated patients, a reduction of impairment in three patients and improvement in functional limitations and quality of life in respectively six and three out of eight evaluated patients. With the exception of one patient, all subjects showed improvement at one or more outcome measures. However, the extent of the observed improvements was limited, with inter-individual differences with regard to the domain in which improvement was obtained.

Looking in detail at the evaluated patients reveals that the patient with the most marked response (pain and impairment reduction and health related quality of life improvement) also exhibited parasympathetic dysfunction. Likewise, for two patients with a possible autonomic dysfunction predominantly positive results were obtained on the evaluated outcome measures were observed. These observations may be suggestive for a possible role of autonomic dysfunction in CRPS, in line with recent reports by Terkelsen et al. (21) who observed increased heart rate and decreased heart rate variability or vagal tone in CRPS patients. Similar findings have been reported

for fibromyalgia patients (43). The combination between observed autonomic disturbances and effects of increasing the acetylcholine availability lends further support for a possible role of a disturbed anti-inflammatory cholinergic pathway in the pathophysiological process in these CRPS-1 patients. Increasing the acetylcholine availability may have led to restoration of the autonomic balance affecting inflammation and a subsequent decrease in signs and symptoms of CRPS (44).

Notwithstanding these findings, for one patient classified as having definite autonomic dysfunction and two patients with possible autonomic dysfunction, effects of treatment with pyridostymine were clearly limited, and even negative on impairment level for two patients. Although a uniform response to an intervention is not to be expected that in a complex disease such as CRPS whereby different pathophysiological mechanisms may be at work simultaneously in a patient (45), the latter results warrant a more thorough evaluation of the autonomic balance in CRPS patients. One way related to the primary hypothesis would be to directly measure acetylcholine in peripheral tissues of CRPS patients, but clinical tests to assess these levels are not available (46). For the purpose of our study, we analyzed plasma acylcholine-acyl-hydrolase or pseudo-cholinesterase as a marker for cholinesterase availability, and assess possible changes in these levels as a consequence of pyridostigmine administration. We found baseline levels to be within the normal range (6270 – 12651 U/l; reference values females <= 39 years: 4300-11500 U/l, males and females > 40 years: 5400-13200 U/l. Furthermore, no significant change in pseudo-cholinesterase levels could be established over the course of the trial. We do acknowledge, however, that these measures provide an indirect indication of cholinesterase availability at best, also taking into consideration that changes in cholinesterase levels might have occurred in neurogenic tissue which were not reflected in blood plasma levels, as has been reported by Anglister et al. (47). An additional explanation for the lack of change in pseudo-cholinersterase following pyridostigmine administration may be related to the dosage of pyridostigmine and duration of administration thereof. Although a titration phase was included in this study up to the highest tolerable dose based on subjective experience by the patient, some patients did not experience side effects at the highest allowed dose in this study, which was chosen conservatively for obvious reasons of patient safety that should be taken into account when testing a new drug. Possibly, a higher dose of pyridostigmine, or longer treatment duration could have resulted in relevant changes in cholinesterase levels and indeed more substantial effects on outcome parameters for the CRPS-1 patients with (possible) autonomic dysfunction.

In line with our hypothesis that patients with a more pronounced inflammatory profile would respond better to pyridostimine treatment, two patients with a possible and one patient with probable inflammatory profile had a predominantly positive response to pyridostigmine intervention. However, for another two patients (one possible and one probable) this was clearly less the case. We also found that three patients without an inflammatory profile showed a predominantly positive response over the course of the study. Therefore, the premise of an inflammatory profile as established by clinical phenotyping would lead us to conclude that our assumption that augmenting the cholinergic anti-inflammatory pathway with the intervention used in this study would reduce inflammatory signs of CRPS could not be maintained. However, some remarks have to be made in this context. For the present study, physiological markers of inflammation, i.e. associated with neurogenic inflammation (48) oxidative stress (7) or immune activation (49) have not been assessed in this patient sample. Although clinical signs of inflammation are reported to be associated with physiological parameters of inflammation (50), this does not appear to be the case for patients with intermediate and long disease durations (51), whereby elevated levels of pro-inflammatory cytokines were found in absence of clinical inflammatory signs. In other words, the process might still be at work in the patient without the presence of clear signs of inflammation, therefore the positive response to pyridostigmine treatment observed in the patients without a clinical profile of inflammation might well be related to reduction of an underlying inflammatory process. In order to ascertain that an inflammatory process is indeed involved in an individual patient, it is therefore advisable for future studies evaluating the influence of this anti-inflammatory pathway to measure biomarkers of inflammation in CRPS patients.

Other predictors for effects of activating the cholinergic anti-inflammatory pathway may be the age of the patient and the disease duration. Al patients had a relatively long disease duration (minimum of 9 months), and did not respond sufficiently to previous treatments, indicating the difficulty of changing the disease course in these patients. In addition, it has been shown that patients with a young onset of CRPS have a more severe phenotype and are less likely to respond positively on treatment (52). Although lacking statistical difference related to sample size, the fact that some association was observed in our sample between both disease duration and age at onset with treatment effect corroborate with these findings. The fact, therefore, that five out of ten patients showed some form of improvement despite their refractory character, may be viewed in favor of the evaluated intervention.

Four patients reported improvement based on personal impression of change, and the majority (eight out of ten patients) wanted to continue the use of pyridostigmine after the trial. However, these patient assessments of change only partially corroborated with for all patients. Although rapid changes in severity of signs and symptoms know to occur in CRPS and can be an alternative explanation for this discrepancy, placebo effects need to be considered as well in this non-controlled trial.

Another limitation that should be taken into account is the small sample size, which is inherent to the single-subject design used in this study leading to qualitative interpretation of the results.

Altogether, positive effects following pyridostigmine administration on clinical features of CRPS were observed, which varied over subjects and was limited in magnitude. In our view the results are insufficient to justify clinical application of this intervention, but they do warrant further research into the possibilities of augmenting the cholinergic anti-inflammatory pathway.

Future research should focus on specified patient populations with CRPS of a shorter duration, established inflammatory pathophysiological mechanism and autonomic deregulation. Extensive assessment of peripheral parasympathetic activity may be helpful in understanding the role of the parasympathetic nervous system and the cholinergic anti-inflammatory pathway in CRPS. Although direct assessment of parasympathetic function by means of recording activity of efferent fibers of the vagus nerve would be most reliable, the invasive nature of these methods needs to be taken into consideration (53).

Furthermore, our findings with regard to the side effects suggest higher dosages would have been tolerated in some patients, possibly resulting in more pronounced effects. In that respect, pharmaco-kinetic/pharmaco-dynamic modeling of this drug in CRPS patients would be the best way to establish optimal dose response relationships. A longer treatment period may be required, allowing for longer targeting of more robust neuroplastic changes in CRPS patients. Other, more central acting medicine that increase the acetylcholine availability such as galantamine, should be considered in this context as well (54), even as direct invasive stimulation of the parasympathetic nervous system (55;56). New pharmaceutics directly mimicking acetylcholine (eg. GTS-21) (57;58) are of interest, but safe acetylcholine agonists are not available for use in patients.

Conclusion

Taken together, positive but limited effects were found for treatment of CRPS patients with pyridostigmine. More research is needed to confirm the involvement of the cholinergic anti-inflammatory pathway in CRPS, and to determine the therapeutic potential of interventions aimed at this pathway.

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Intravenous Magnesium for Chronic Complex Regional Pain Syndrome type 1 (CRPS-1)
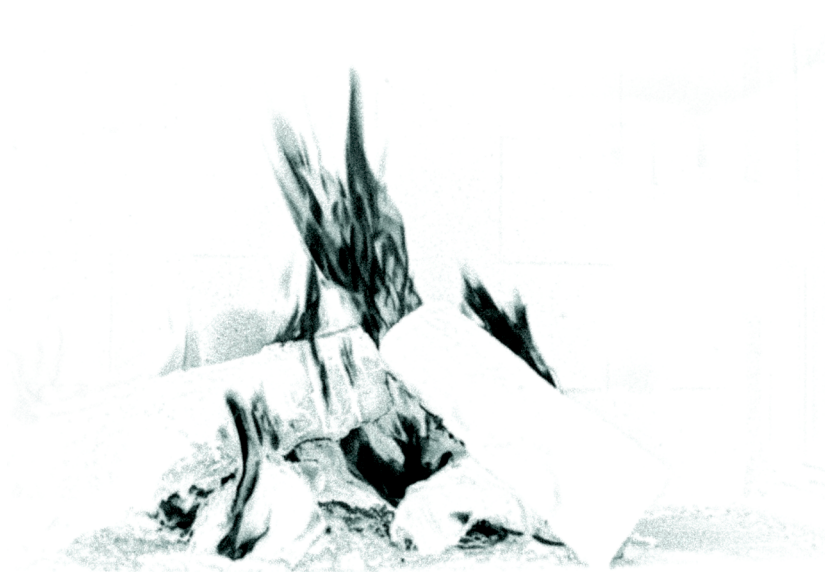
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Abstract

Objective:
To assess the effects of intravenous administration of magnesium on CRPS-1, a randomized double-blind placebo controlled trial was performed.

Methods:
Fifty-six patients with CRPS-1 (IASP-Orlando criteria) received $MgSO_4$ 70 mg/kg or placebo (NaCl 0.9%) in 4 hours over 5 consecutive days. Pain (BOX-11 and McGill), the level of impairment (ISS), functional limitations (RSQ, WSQ/QRSD), participation (IPA) and quality of life (SF-36, EuroQol, IPA) were evaluated at baseline and at 1, 3, 6 and 12 weeks.

Results:
No significant differences were found between $MgSO_4$ and placebo on the BOX-11 and ISS at different time points during the trial on intention-to-treat and per protocol analysis. A significant improvement on the BOX-11 was found after the first week of the trial in both groups (mean 0.7; SD 1.1). For the $MgSO_4$ group, a clinically relevant and statistically significant improvement on the ISS at 1 week (median 5, IQR -1-8), and a significant improvement on the McGill up to 6 weeks (median 2 words, IQR 0-4.5) were found compared to baseline, which were not found in the placebo group. Significant improvement in perceived job participation was found for the $MgSO_4$ group at 12 weeks (median improvement 1.44 to 1.17; p=0.01). ISS improved significantly more in patients with a low HADS score (≤ 10) in the $MgSO_4$ group (mean 4.4 vs. mean -3.1; p=0.02).

Conclusion:
Administration of the physiological competitive NMDA receptor antagonist magnesium in chronic CRPS provides insufficient benefit over placebo. Future research should focus on patients with acute CRPS and early signs and symptoms of central sensitization.

Introduction

CRPS-1 is a pain syndrome of an extremity, which mostly develops after trauma (e.g. distortion, fracture or surgical intervention), and is characterized by disproportional pain, sensory disturbances, swelling, color changes, change in temperature, decreased motor function and trophic changes (1). Aberrant inflammation after trauma and subsequent peripheral and central sensitization are proposed as main mechanisms in the development and maintenance of CRPS-1 (2). In the cascade of sensitization, excessive release of cytokines (e.g. TNFα), substance P and CGRP, can lead to increased glutamate release in the central nervous system. Continued release of glutamate can activate the dormant NMDA receptor antagonist resulting in increased calcium influx into the synaptic cleft, therewith increasing the efficiency of synaptic transmission. The activation of the NMDA receptor is a crucial step in the development of central sensitization, and is associated with spontaneous pain and increased reaction to peripheral stimuli (3). Besides activation of NMDA receptors, local inflammation also are thought to lead to an increase in NMDA receptors density in peripheral tissue and sensory nerves, thereby further contributing to the process of sensitization (4;5).

To counter the process of peripheral and central sensitization and to reduce sensory disturbances, NMDA receptor antagonists have been proposed (6;7). Studies by Collins et al. (8) and Sigtermans et al. (7;9) have shown significant decrease of pain in CRPS patients following intravenous administration of magnesium and ketamine. However, ketamine is associated with a broad spectrum of severe side effects (10), and costs of treatment are high. Magnesium is a physiological substance involved in many cellular processes, and is needed for catalyzation of enzymes and synthesis of DNA. In the nervous system magnesium acts as a competitive NMDA receptor antagonist, stabilizing abnormal nerve excitation. Because of its favorable physiological profile and relatively limited costs, magnesium has been used in treatment of various medical conditions with limited side effects (11). Treatment with magnesium has been shown to significantly reduce pain in acute and chronic pain states (12;13). Significant reduction of pain and sensory disturbances in acute stage CRPS patients were found on intravenously administered magnesium in a randomized, blinded pilot study (6). However, the efficacy of this intervention in CRPS patients with long standing CRPS has not yet been investigated. Consequently, we performed a randomized placebo controlled trial comparing magnesium sulphate IV (MgSO$_4$) with placebo IV (NaCl 0.9%), evaluating effects on pain, aspects of sensitization, level of impairment, activities, participation and quality of life in CRPS-1 patients.

Methods

Patients

CRPS-1 patients diagnosed according to the IASP-Orlando criteria (International Association for the Study of Pain of 1994) were recruited at the outpatient clinic of the VU University Medical Center between June 2006 and December 2011. Inclusion criteria were a pain score higher than 5 on the BOX-11 scale before inclusion, age between 18 and 70 years, CRPS limited to one extremity and patients had to give written informed consent. Exclusion criteria were other (pain)syndromes interfering with outcome or measurements, severe liver or kidney function disturbances, heart or lung diseases, active infection, pregnancy, mental retardation, psychiatric abnormality or active malignant disease. Medication for the treatment of CRPS (e.g. DMSO cream and N-actylcysteine), analgesics with NMDA antagonistic properties and oral magnesium were stopped at least one week before starting the trial. Use of analgesics without anti-oxidative or NMDA antagonist properties were allowed during the trial. The Medical Ethical Committee of the VU University Medical Center approved the study (National Trial Registry number: NTR1873).

Intervention

Patients were randomized to receive either magnesium sulphate ($MgSO_4$) 70 mg/kg or placebo (NaCl 0.9%) via intravenous infusion of 25 ml/hour in 4 hours a day for a period of 5 consecutive days in indistinguishable syringes. These dosages were based on a previous pilot study resulting in positive results and limited side effects (6). This dose is known to give minimal side effects, and is well below the dose given to pre-eclampsia patients (14;15). There is extensive clinical experience with magnesium in a broad range of indications, such as pre-eclampsia/eclampsia (15), acute stroke (16), head trauma (17), postoperative pain (18), acute bronchospasm (19) and heart disease (20;21) (see NTR 1873 for further information).

The randomization was performed in blocks of four such that half the patients receive $MgSO_4$ and the others placebo. The institutional pharmacist performed both blinding and randomization independently. The patient, researcher and physician were blinded for the type of intervention for the duration of the trial. After the 12 week follow up, when all measurements were performed, the code was broken to be able to offer the placebo patient group intravenous $MgSO_4$ in an off label setting. Success of

blinding was assessed at the end of the trial for each patient by asking the researcher and patient which intervention they thought the patient received. Concomitant use of analgesics was allowed and was given according to the Dutch multidisciplinary treatment guideline (22), and was registered in a medication diary. All patients received standard physical therapy according to a standardized treatment protocol (23). As safety measurements prior to the intervention, creatinin levels and cardiac function (using an electrocardiogram (ECG)) were determined for each patient. Plasma levels of magnesium and calcium were recorded daily prior to and after the 4 hour intervention. ECG monitoring was performed continuously during administration of the study medication. Possible systemic and local side effects were recorded during intervention by the researcher and registered by the patient in the pain diary.

Assessments

Assessments were performed using a standardized assessment protocol used within the TREND consortium (www.trendconsortium.nl) using valid and reliable tools. The assessment protocol was based on the International classification of Functioning, Disability and Health (ICF model) (24), in line with the IMMPACT guidelines (25) for evaluation of chronic pain. Primary effect measures were the ISS score evaluating the level of impairment in patients with CRPS, and the 11-point BOX scale, a numerical rating scale on severity of pain at 12 weeks after starting the trial.

Functioning

One week before the intravenous treatment (T0), during the administration of trial medication (T1) and 3 (T2), 6 (T3) and 12 (T4) weeks following the start of the intervention, patients filled out the 11-point BOX scale ranging from 0 (no pain) to 10 (most pain imaginable) for pain severity three times daily during one week (26). The adjectives list of the Dutch version McGill Pain Questionnaire was filled out consecutive to the BOX-11 ratings in order to obtain the total number of words chosen (NWCt) and the pain-rating index (PRI) (27;28).

The sensitivity of the skin (detection threshold) was measured with Semmes Weinstein Monofilaments (SWM) comparing the affected extremity to the contra-lateral extremity. Monofilaments representing different forces (0.0045 to 447.0 gr) were used starting with the smallest filament up to the largest. The testing areas for the hand were the palmar side of the distal phalanx of dig. 1, the distal and proximal

phalanx of dig. 2, the distal and proximal phalanx of dig. 5 and the hypothenar of dig. 5. The feet were tested on the plantar side: distal on phalanx dig. 1, the distal phalanx of dig. 2, the distal phalanx of dig 5, the medial and lateral arcus plantaris. The mean of the 5 tested areas was used to acquire an overall sensibility of the affected and contra-lateral extremity. The difference of skin sensitivity between the affected and non-affected extremity was evaluated over time (29-31).

Impairment was assessed with the Impairment level Sum Score (ISS), a validated score comprising the assessment of pain (Box-11, McGill score (NWCt)), and comparisons between the affected and contra-lateral extremity of temperature by means of an infrared thermometer, volume by means of water displacement volumeters and active range of motion by means of standardized goniometers (32;33). The ISS ranges from 5-50 whereby higher scores indicate higher levels of impairment. The measurements were carried out under environmentally stable conditions by a researcher that attended training sessions 3 times a year within the TREND consortium.

Activities

Functional limitations were assessed with the Radboud Skills Questionnaire (RSQ) (upper extremity) (34) or the Walking Skills Questionnaire (WSQ) and questionnaire rising and sitting down (QRSD) (lower extremity) (35). Changes on the RSQ, WSQ, QRSD were analyzed at all time points.

Participation and health

Participation was evaluated with the Impact on Participation and Autonomy (IPA) questionnaire, comprising the domains autonomy indoors (getting around and family role) and autonomy outdoors (getting around, social life/relationships, work/education) (36) at T0, T3 and T4. Quality of life was assessed with the Short Form-36 (SF-36) (37) and the EuroQol (38) at T0, T3 and T4.

Personal factors

Subjective assessment of signs and symptoms and personal factors were evaluated using the TREND symptom inventory (TSI) at T0, T3 and T4. Psychological assessments were performed at T0 using the Hospital Anxiety and Depression scale (HADS) (39), the Tampa Scale for Kinesiofobia (TSK) (40) and the Pain Coping Inventory (PCI) (41).

Sample size calculation

According to standard power calculation, 33 patients per group would have been required to detect a clinically relevant difference of 2 points on the primary outcome measurement BOX-11 ($\delta=2$), with a significance level of $\alpha=0.05$ and power $\beta=0.1$.

Off label analysis

Patients assigned to the placebo group in double-blinded phase were offered the opportunity to receive intravenous $MgSO_4$ treatment after completing the 12-week follow-up period. Assessments during the off label treatment consisted of pain diaries (BOX-11) and McGill questionnaires over 4 consecutive weeks. Evaluations were performed at 1 and 3 weeks after starting the intervention in order to parallel timing of assessments during the double-blinded phase. The last assessment of the double-blinded phase was used as baseline for the off label trial.

Statistical analysis

Data were stored in a NEN-7511 certified central web-based database (ProMISe ®). Blind analyses were performed using SPSS version 15.0. Comparability of the treatment group and the placebo group on patient characteristics and prognostic measures was assessed at baseline, using Chi square, independent sample *t* tests or Mann Whitney tests. Effects of treatment over time were analyzed using the paired student *t* and Wilcoxon signed ranks test. Differences between groups at the follow up assessments were compared using the independent sample *t* test or the Mann-Whitney-U test. Primary outcome (pain and ISS) was analyzed according to intention to treat as well as by per protocol principles. Subgroup analyses were performed for gender, cold/warm extremity and acute versus chronic CRPS (6 months or less) to evaluate effects of these characteristics on outcome using the independent sample student *t* test or the Mann Whitney test. For all analyses a two-sided p-value lower than 5% was used to indicate statistical significance.

Results

Patient characteristics

From June 2006 to December 2011 56 patients were recruited out of 229 eligible patients with CRPS-1 according to the IASP Orlando criteria (figure 1). The most prominent reasons for non-participation of eligible patients (n=171) were that the study would be too time consuming/interfered with personal circumstances (36%), signs or symptoms resolved before entering the trial (18%), not wanting to postpone standard treatment (13%), fear of intravenous medication or needles (12%), or patients could not be traced (16%). Of the 56 included patients, 52 female and 4 male, 29 were assigned to receive $MgSO_4$ infusion and 27 received placebo infusion with NaCl 0.9%. Seven patients did not complete the intervention week (4 assigned to $MgSO_4$, 3 to placebo) and one patient violated the protocol by starting DMSO in the period of the trial (assigned to placebo).

In the magnesium group significantly more patients reported a colder affected extremity and in the placebo group more patients reported alternating temperature of the affected extremity, however, this did not lead to effect modification. The disease duration differed as well between both groups, however this was not significant due to the large range and uneven distribution of this variable. Other differences in prognostic variables were not found between the patient groups (table 1). Pain and ISS scores did not differ between patients with an upper or lower affected extremity at baseline or during the course of the trial (independent sample *t* tests; p range 0.1-1.0). Differences between the upper or lower affected extremity in sensitivity to touch as measured with SWM were found as expected (table 5) (31) (related to difference in tactile discrimination and thickness of skin between hand and foot), however changes over the trial were similar for upper and lower extremity, therefore results were pooled for all patients. Duration of CRPS at the start of the trial was not related to effects of the intervention on the BOX-11 and ISS, therefore no subgroup analyses were performed concerning disease duration. Effects of treatment in patients with a high score on the HADS (>10) differed from effects on patients with low scores on the HADS (≤10), therefore subgroup analyses for patients with low and high scores on the HADS are presented. Missing data ranged from 0.4% for the ISS to 11% for the McGill Pain Questionnaire.
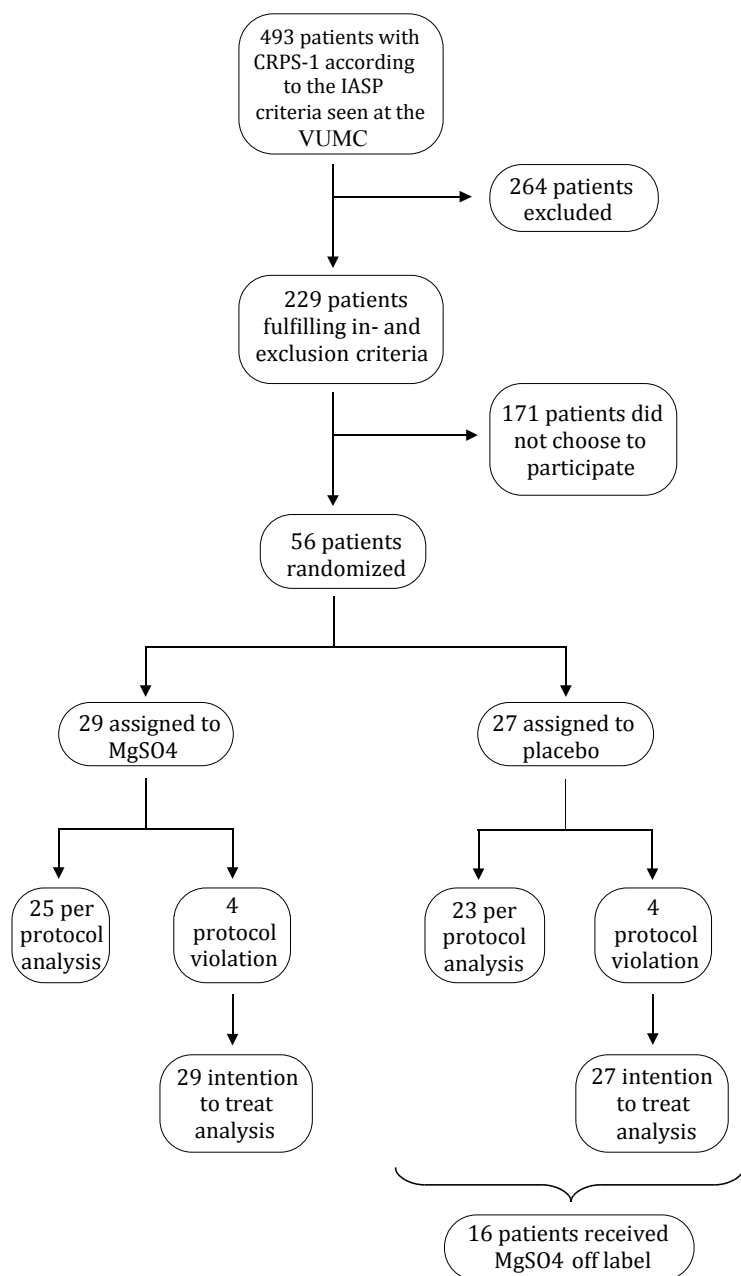
Figure 1: Flow diagram on selection,
Randomisation and follow up of studied patient groups.

Table 1 Patient characteristics.

	Total	Placebo	MgSO4
N	56	27	29
Female/male	52/4	25/2	27/2
Age (years)*	46.7 (11.5)	46.1 (11.0)	47.2 (12.2)
Duration (months)**	16.0 (6.0–41.8)	10.5 (5.0-26.8)	23.0 (8.5-64.8)
Upper/lower extremity	16/40	10/17	6/23
Right/left	25/31	14/13	11/18
Initial trauma			
Fracture	15	7	8
Soft tissue injury	11	5	6
Operation	11	7	4
Nerve related operation	3	2	1
Spontaneous	3	2	1
Wound	2	1	1
Other traumas	11	3	8
Initial temperature			
Warm	12	5	7
Cold	30	11	19
Alternating	13	10	3
Unknown	1	1	0
Mean NRS at baseline*	6.2 (1.7)	6.3 (1.6)	6.1 (1.8)
ISS score at baseline*	30.0 (6.6)	30.7 (6.9)	29.2 (6.2)
CRPS score at baseline*	12.2 (2.3)	12.8 (2.3)	11.6 (2.3)
RSQ at baseline** (n=15)	2.9 (2.5-3.5)	3.0 (2.5-3.5)	2.8 (2.4-4.1)
WSQ at baseline**			
In house	5.6 (2.0-7.1)	5.6 (2.3-7.8)	5.9 (1.7-7.1)
Outside	7.4 (4.7-8.3)	7.2 (4.2-8.1)	7.4 (5.1-8.6)
Sitting and Rising	6.3 (3.3-8.9)	7.1 (4.6-9.1)	5.8 (2.6-9.1)
SF-36 at baseline*			
Vitality	49.2 (20.5)	50.4 (23.1)	48.0 (18.0)
Social functioning	63.6 (25.7)	66.4 (26.3)	61.0 (25.3)
EuroQol at baseline**	0.43 (0.20-0.78)	0.46 (0.18-0.78)	0.42 (0.20-0.75)
IPA at baseline**			
Autonomy inside	1.1 (0.7-2.0)	1.3 (0.6-2.0)	1.2 (0.7-2.0)
Autonomy outside	2.0 (1.6-2.8)	2.0 (1.6-2.8)	2.1 (1.7-3.0)
PCI at baseline*	69.3 (13.7)	67.0 (11.7)	71.3 (15.2)
TSK at baseline*	36.8 (7.3)	35.4 (6.5)	38.1 (7.8)
HADS at baseline**	8.0 (6-13.3)	8.0 (6.0-15.0)	8.0 (5.0-12.0)

* mean (standard deviation (SD))
** median (interquartile range (IQR))

Outcome

No significant differences were found between the MgSO$_4$ treated group and the placebo group on the primary effect measures BOX-11 and ISS at different time points during the trial on intention-to-treat and per protocol analysis. Both groups showed a statistically significant improvement of 1 point compared to baseline on the BOX-11 scale on all time points up to 12 weeks after starting the trial (p 0.00 to 0.02) (table 2, figure 2). A clinically relevant and statistically relevant improvement of the

ISS (5 points; p<0.05) was found in the intervention group at T1 (table 3, figure 2), which was not found in the placebo group.

Pain as assessed with the McGill NWC-total improved up to 6 weeks after baseline in the magnesium group, this differed significantly from the placebo group directly after the week of infusion (p=0.01). This improvement could be attributed to the improvement in the sensory subscale of the McGill questionnaire (NWCs) (p<0.01). The McGill PRI improved in both groups, but no differences were found between the magnesium and placebo group (table 4, figure 3). Sensitivity to touch as measured by SWM showed no significant change over time or significant differences between the groups. Functioning measured by the RSQ and WSQ did not improve over time in either of the groups. Quality of life measured by the SF-36 did not change over the course of the trial, but the EuroQol improved significantly in the magnesium treated group (median 0.43 at T0 to 0.56 at T3, p=0.05) and not in the placebo group, however no differences were found between groups. Participation and autonomy slightly improved in both groups (median 1.42 to 1.22; p=0.02) and for job participation only in the patient group treated with $MgSO_4$ improved (median 1.44 to 1.17; p=0.01) (as measured with the IPA).

Table 2: Pain scores (NRS) at all time points (mean, SD).

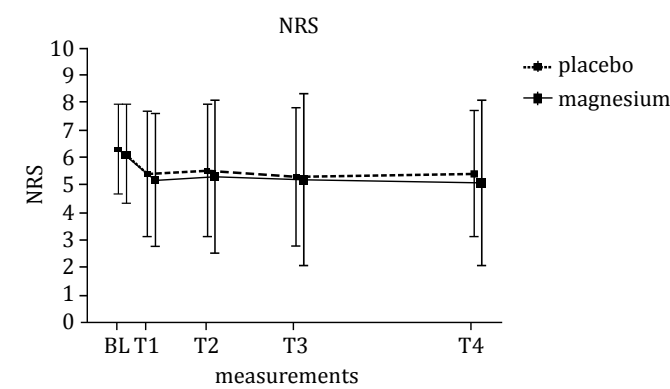|	Baseline (T0)	T1	T2	T3	T4
All	n=56	n=55	n=53	n=54	n=52
	6.2 (1.7)	5.3 (2.3)	5.4 (2.6)	5.3 (2.8)	5.2 (2.7)
Placebo	n=27	n=26	n=26	n=27	n=25
	6.3 (1.6)	5.4 (2.3)	5.5 (2.4)	5.3 (2.5)	5.4 (2.3)
$MgSO^4$	n=29	n=29	n=27	n=27	n=27
	6.1 (1.8)	5.2 (2.4)	5.3 (2.8)	5.2 (3.1)	5.1 (3.0)

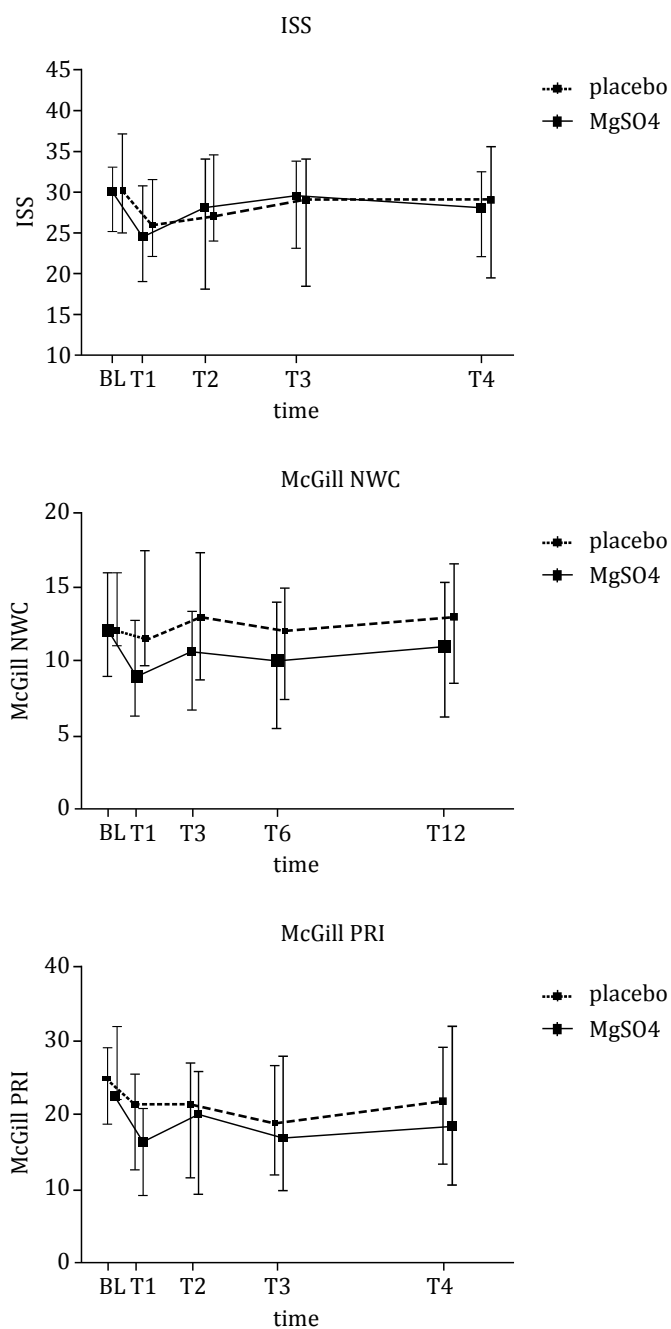**Figure 2:** painscores (NRS) and Impairment level sumscore (ISS) over time (mean and SD).

Figure 3: Changes on McGill: number of words counted (NWC)
and pain rating index (PRI) (median and IQR).

Table 3: Impairment level sumscore (total and subscores).

	Baseline (T0)			T1			T2			T3			T4		
	All	Placebo	MgSO4	All	Placebo	MgSO4	All	Placebo	MgSO4	All	Placebo	MgSO4	All	Placebo	MgSO4
ISStot	30 (25-33) n=55	30 (25-37) n=27	30 (25.25-33) n=28	25** (21-31) n=53	26* (22-31.5) n=25	24.5** (19-30.75) n=25	27* (22.75-34) n=50	27 (24-34.5) n=25	28 (18-34) n=25	29* (22-33.5) n=49	29* (18.5-34) n=25	29.5 (23-33.75) n=25	28* (20-34.25) n=50	29* (19.5-35.5) n=25	28 (22-32.5) n=25
ISSMcGill	6 (6-8) n=55	6 (6-8) n=27	6 (5-8) n=28	5* (4-8) n=53	6 (5-9.5) n=25	5*§ (3.25-6.75) n=28	6* (4-9) n=51	6 (4.75-9) n=26	5.5* (3.5-7) n=25	6* (3-8) n=49	6* (3.5-8) n=25	5* (3.5-7) n=24	6 (4-8) n=50	7 (4.5-8.5) n=25	6 (3.5-7.5) n=25
ISSBOX	8 (7-8) n=56	8 (7-8) n=27	8 (7-8.5) n=29	7* (4-8) n=54	6* (3.75-8) n=26	7* (4.25-8) n=28	7* (6-8) n=54	7* (6-8) n=26	7* (5.25-8.75) n=28	8* (5-9) n=51	8* (5.5-8) n=25	7.5 (4.25-9) n=26	8* (5-9) n=55	7.5* (5-8) n=27	8 (6-9) n=28
ISSTEMP	5 (2-10) n=56	5 (3-10) n=27	5 (2-10) n=29	4 (1-8) n=54	4 (1-9) n=26	3.5* (1.25-5) n=28	5 (1-10) n=54	4.5 (2-10) n=26	5 (1-10) n=28	5 (2-9) n=51	5 (1.5-9) n=25	4.5 (2-10) n=26	5 (2-10) n=55	6 (2-9) n=27	5 (2-10) n=28
ISSVOL	3 (1-6) n=56	3 (1-7) n=27	2 (1-5.5) n=29	2 (1-5) n=54	3 (1-5.25) n=26	1 (1-4.75) n=28	2 (1-4) n=54	2.5 (1-4) n=26	1 (1-3) n=28	3 (1-6) n=51	3 (1-5.5) n=25	2.5 (1-6) n=26	2 (1-4) n=55	2 (1-4) n=27	1 (1-3) n=28
ISSAROM	7 (5-8) n=56	7 (6-8) n=27	6 (5-8) n=29	6* (4-7) n=54	6* (5-7) n=26	5* (4-7.75) n=28	6 (5-8) n=54	6.5 (5.75-7.25) n=26	6 (5-8) n=28	6 (5-8) n=51	6* (5-8) n=25	6.5 (4.75-8) n=26	6* (4-8) n=55	6* (4-8) n=27	5.5 (4-7.75) n=28

ISS Median and IQR, *Significant difference to baseline (p<0.05) ** Significant and relevant improvement (ISStot change > 5) compared to baseline (p<0.05) §Significant difference between intervention and placebo group

Table 4 : Baseline McGill pain questionnaire values and median changes at baseline, T1, T2, T3, T4.

	Baseline (T0)		T1		T2		T3		T4	
	Placebo n=27	MgSO$_4$ n=28	Placebo n=26	MgSO$_4$ n=28	Placebo n=26	MgSO$_4$ n=26	Placebo n=26	MgSO$_4$ n=25	Placebo n=25	MgSO$_4$ n=25
NWCt	12 (11-16)	12 (9-16)	11.5 (9.75-17.50)	9*# (6.25-12.75)	13 (8.75-17.25)	10.5* (6.75-13.25)	12* (7.50-15)	10* (5.5-14)	13 (8.5-16.5)	11 (6.25-15.25)
NWCsen	8 (6-10)	7 (6-10)	7.5 (6-11)	6*# (4-8)	8 (5-11)	6 (4-9)	7 (5.75-9.25)	5* (4-9)	8 (5.5-10.5)	5.5 (5-9.75)
NWCaff	2 (1-4)	2 (1-4.75)	1.5 (1-5)	1* (0.75-3.25)	2 (1-4.25)	1* (0.75-3.25)	1.5* (0.75-3.25)	2 (0.5-3)	2 (1-4)	2 (1-3.75)
NWCev	3 (2-3)	3 (2-3)	3 (2-3)	2*# (1-3)	3 (2-3)	3 (1-3)	3 (2-3)	3* (1-3)	3 (2.5-3)	3 (1-3)
PRIt	25 (19-29)	22.5 (16.25-30.25)	21.5* (12.75-28.25)	16.5* (9.25-21)	21.5* (11.75-27)	20* (9.5-26)	19* (12-26.75)	17* (10-28)	22 (13.5-29)	18.5 (10.5-32)
PRIsen	15 (12-18)	13 (9.25-19.75)	11* (8-17.25)	9* (6-14.25)	13* (7-16.25)	11.5* (6-15.25)	12* (8-17.5)	9 (6-16.5)	13 (8.5-18.5)	11 (8-19)
PRIaff	3 (1-6)	3.5 (1-5)	2 (1-5.25)	2* (0.25-4.75)	2.5 (1-5)	1.5* (0.75-5.25)	2 (0.75-4.25)	2* (0.5-5)	2 (1-5)	2 (1.25-5)
PRIev	6 (5-8)	5 (4-7)	5 (3-6.5)	4* (2-5.75)	5* (3-6.25)	5 (2.75-8)	5* (3-7.25)	4* (2.5-7)	5 (4-7.5)	5 (2-8)

Data in median and interquartile range (Wilcoxon and Mann Whitney analysis)
* significant improvement compared to T0 ($p<0.05$)
# significant difference compared to placebo ($p<0.05$)

Table 5: Sensibility measured by Semmes Weinstein filaments.

	Baseline		T1		T2		T3		T4	
	Affected	Non-aff.	Affected	Non-aff.	Affected	Non-aff.	Affected	Non-aff.	Affected	Non-aff.
All (n= 54)	3.66	4.25	3.61	3.88	3.64	4.16	3.53	3.94	3.79	4.08
Placebo (n=27)	3.98	4.09	3.53	3.56	3.67	3.64	3.51	3.57	3.61	3.72
MgSO4 (n=27)	3.64	4.36	3.67	3.70	3.57	4.36	3.63	4.06	3.81	3.76
Upper extremity (n=16)	3.19	3.22	3.29	3.22	3.35	3.19	3.22	3.22	3.30	3.18
Placebo (n=10)	3.15	3.16	3.22	3.16	3.15	3.16	3.30	3.22	3.29	3.06
MgSO4 (n=6)	3.42	3.29	3.30	3.35	3.35	3.19	3.16	3.22	3.19	3.32
Lower extremity (n=38)	4.40	4.77	4.21	3.89	4.42	4.57	4.49	4.16	4.51	4.23
Placebo (n=17)	4.56	4.94	3.85	3.78	4.21	4.56	3.61	4.04	4.20	4.20
MgSO4 (n=21)	3.98	4.51	4.16	3.94	3.99	4.67	4.47	4.24	4.17	4.25

Median and interquartile range (IQR) of sensed monofilament of the affected and the non affected extremity (used filaments from thinnest to thickest: 1.65, 2.83, 3.22, 3.61, 3.84, 4.08, 4.31, 4.56, 6.65) (measures are related from the mean felt monofilament of the 5 tested locations).

Subgroup analysis

Patients with low HADS scores (≤10) improved significantly more on the ISS than patients with higher HADS scores (median 3.9 vs. 0.7; p=0.05). When analyzing this for both intervention groups separately, this change of ISS was statistically significant for the patients treated with magnesium (mean improvement of 4.4 vs. deterioration of 3.1; p=0.02) and not for the placebo group.

Off label

Of the 27 patients who received the placebo in the double blinded phase, sixteen patients chose to receive $MgSO_4$ treatment after completion of the trial and were evaluated in the off label phase (table 6). Evaluation on the BOX-11 showed a significant mean improvement of 0.7 (SD 1.0) after the treatment week (p=0.02) Improvement on the McGill scale was found 1 week after starting the intervention (PRI total and sensory subscale) and after 3 weeks (NWC and PRI total).

Table 6: Patient characteristics off label study.

	Total
N	16
Female/male	15/1
Age (years)*	46.1 (12.7)
Duration (months)**	9.5 (4.25-19.25)
Upper/lower	5/11
Right/left	7/9
Initial trauma	
Fracture	4
Soft tissue injury	4
Operation	4
Nerve related operation	1
Spontaneous	1
Wound	0
Other traumas	2
Initial temperature	
Warm	3
Cold	5
Alternating	3
Unknown	4
Mean NRS baseline*	6.6 (1.5)

* mean (standard deviation (SD))
** median (interquartile range (IQR))

Side effects

Common side effects in the magnesium group were flushing and dizziness during and shortly after the 4-hour infusion. One patient experienced a vasovagal reaction and one patient reported palpitations. Two patients who received placebo reported palpitations. In both the placebo and the intervention group, pain in the vicinity of the insertion site of the intravenous cannula was reported, one patient receiving $MgSO_4$ developed phlebitis and one patient receiving placebo developed spreading pain around the cannula. During the off label period one patient experienced bradycardia leading to vasovagal collapse, and was subsequently shortly admitted to the hospital for observation.

Blinding

Blinding was evaluated in all 56 patients by asking researcher and patients what treatment they thought they received. In 17 cases patients did not know what treatment they received (30%), 10 patients who received placebo were correct in their assumptions (37%) and 16 patients who had received $MgSO_4$ were correct (55%). Researchers were correct for patients that received placebo treatment in 17 cases (63%) and in 23 patients that received $MgSO_4$ (79%). Evaluations on assumption of either the patient or the researcher showed no significant difference for correct or incorrect assessment (Sign Test: $P=0.58$ and $P=0.45$, respectively).

Discussion

The results of this trial show a decrease of pain in the magnesium group as well as the placebo group, comparable to the changes observed in the off label phase of the study. Although this change is statistically significant, 1 point improvement should not be considered clinically relevant. A statistically significant and clinically relevant improvement was observed for the ISS at the end of the intervention period and the McGill Pain scale (NWCt) up to 6 weeks follow up in the magnesium group, which differed significantly from placebo at one week, mostly related to differences in the sensory subscale. This outcome suggests that sensory aspects of CRPS are influenced by magnesium. Although this measurement is clinically not relevant, it may strengthen the hypothesis about the effects of magnesium on sensory aspects in neuropathic pain. Job participation and EuroQol improved in the patient group treated with $MgSO_4$. Patients with low HADS in the magnesium group improved significantly more than patients with high scores on the HADS in this group.

The results of this study parallel those of the pilot study performed by our group (6), but the magnitude of the effects are substantially smaller. The difference in the studied population between the present trial and the pilot study concerning disease duration and inflammatory profile may have resulted in differences in effect sizes. Patients with a shorter disease duration as included in the pilot study are generally more likely to respond positively to interventions (42) and the prognosis of spontaneous improvement is better in CRPS-1 patients with shorter disease duration. Differential effects of magnesium for patients with a shorter disease duration compared to chronic patients may be expected as a consequence of the activity of the NMDA receptor during the cascade leading to central sensitization. Reduction of the activation of NMDA receptors in an early stage can possibly prevent or counter the still relatively limited process of central sensitization and therefore prevent more substantial changes found in the dorsal horn and the cortex in later phases of CRPS (3;43;44). As exaggerated inflammation has been proposed to play a role in the initial stages of CRPS-1 development. The direct properties of magnesium as an anti-inflammatory agent may play a role in differences of outcome between the pilot study and the present trial. Treatment with $MgSO_4$ has been shown to inhibit the release of inflammatory molecules (45;46) including TNFα and NF-κβ, which are related to the development of CRPS (47;48). In addition, for both the pilot and the present study, the effects of standardized adjuvant physical therapy may have contributed to improvement in pain and impairment (23), however, a placebo effect cannot be ruled out.

Studies focusing on the NMDA receptor antagonist ketamine for CRPS show much more pronounced effects on pain compared to those found in the present study. Ketamine as a non-competitive NMDA receptor antagonist inhibits NMDA signalling by interacting with phencyclidine (PCP) binding sites and receptors on membrane associated sites, whereas magnesium, a competitive NMDA receptor antagonist, blocks the calcium channel to reduce calcium influx (49). The difference in pharmacotherapeutic profile of both substances relates to the higher potency of ketamine as an NMDA receptor antagonist. Effects observed in the present study appear to be time limited, which was also observed for ketamine in the study by Sigtermans et al (7). The limited duration of these effect suggests that permanently reversing the process of central sensitization and maladaptive neural plasticity is not achieved and that the effect may be related to short term analgesic effects of the NMDA receptor antagonists (2). The strong analgesic potency of ketamine is known as it is used in surgical settings.

We hypothesize that on one hand the severity and the duration of the inflammation relates to the probability of sustained maladaptive neuroplastic changes, while continuous activation may lead to irreversible changes in peripheral and central NMDA receptors. On the other hand, differences in the intra-synaptic environment of the NMDA receptor between individuals concerning availability of calcium, magnesium or inflammatory mediators influencing NMDA receptor phosphorylisation and activation should be considered. In this context, measurement of the biological availability of magnesium to assess the predictive value of possible deficiencies in the development of central sensitization in CRPS-1 may be warranted.

In order to understand the mechanism of central sensitization, changes of the NMDA receptors and associated neuroplastic changes in CRPS-1 fundamental research is needed. Studies focusing on histological changes or spreading of the NMDA receptors may also lead to a better understanding of the role of central sensitization and the NMDA receptor in CRPS (4).

Some limitation with regard to the present study have to be addressed. The heterogeneity of patients with CRPS in general and consequently in our trial, is a challenging aspect in CRPS research. The primary focus of this study was to target aspects of central sensitization in CRPS-1. However, the patients included in this study differed with regard to spectrum and severity of features of central sensitization, therewith contributing to between subject variance. Furthermore the long disease duration and a predominantly cold affected extremity may have contributed to the lack of efficacy found in this study. The fact that the placebo group had a shorter disease duration at baseline may have contributed to the lack of difference found between interventions in this study. Inclusion of patients with a clinical profile more favorable to respond to magnesium may have led to other results than found in this study. Heterogeneity of the patient population may be limited by using the current Budapest criteria (clinical or reseach), which have been validated in 2010 and have a higher specificity and sensitivity for diagnosing CRPS. Objective outcome measurements in studies on CRPS are challenging. In this study it was decided to use the validated ISS as primary outcome measure, however, limitations of this measurement tool are the standardized measurements on temperature (limited to 5 locations) and range of motion (limited to chosen joints). This may have under- or overestimated the disease severity in individual patients. Improvement of objective measurement tools for impairment in CRPS can be considered in future research. Furthermore, inclusion of patients for this study proved difficult, whereby only 25% of the eligible patients agreed to participate. As a

consequence, the number of included patients fell short of the number required in the power analysis (i.e. 56 as opposed to 66). However, reaching a significant difference between magnesium and placebo would have been highly unlikely considering the very small difference between both interventions for the evaluated patients (i.e. 0.3 point on the BOX -11 at 12 weeks).

Conclusions

Intravenous administration of magnesium as used in our study has no additional benefit over placebo in treatment of CRPS-1 in chronic CRPS-1. Studies involving selected groups of CRPS-1 patients with shorter disease duration, a florid inflammatory profile or severe signs and symptoms of sensitization are required in order to assess magnesium for its additional value to available treatment methods for CRPS-1.

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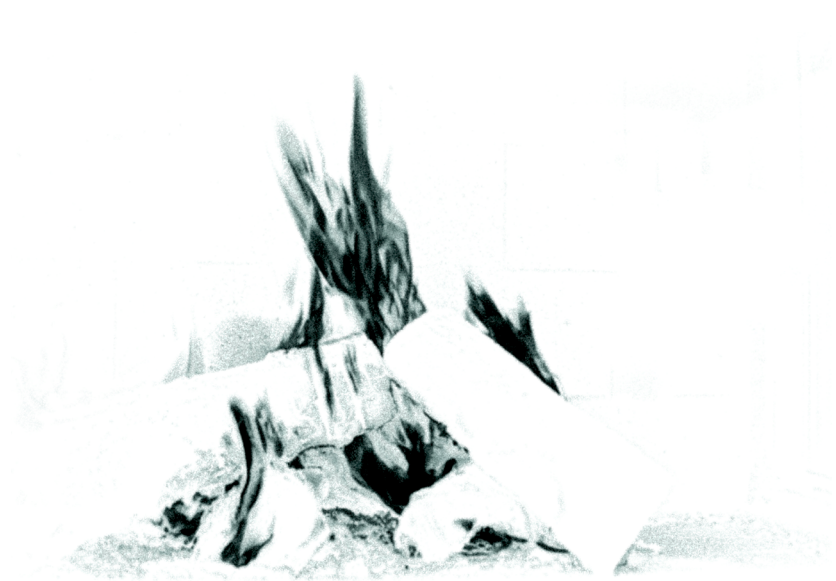# Further validation of the CRPS Severity Score

8

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Abstract

Background:

Heterogeneity and fluctuation of disease severity of patients with Complex Regional Pain Syndrome (CRPS) can complicate adequate clinical communication and follow up. The goal of our study is to evaluate the validity and responsiveness of the evolving CRPS Severity Score (CSS) in expressing disease severity as compared to the Impairment level Sum Score (ISS).

Methods:

Patients with CRPS-1 according to the Budapest criteria participating in a clinical trial were included in this study. The ISS and the CSS were determined at the start of the trial (BL), and at 4 (T4), 6 (T6), 9 (T9) weeks and 6 months (T26) after starting treatment. Correlations between ISS, CSS, patients' impression of change on pain and changes in ISS and CSS between time points were calculated using the Pearson correlation coefficient. Changes in CSS were compared between responders and non-responders.

Results:

Fair to excellent correlations were found between the ISS, the CSS and the patients' impression of change on pain (r=0.34 to r=0.88). Change in CSS correlated strongly with change in ISS, and both were correlated to the patients' impression of change on pain (up to r=0.65). Patients classified as responders showed significantly higher improvement in CSS (improvement of 7 versus 3 points on the CSS).

Conclusions:

This study confirms that the CSS is a promising clinical and research tool for the assessment of the severity of CRPS. Adequate responsiveness of the CSS over time was found, suggesting that this tool is useful for the follow up of patients with CRPS.

Keywords: CRPS, CRPS severity score, CSS, ISS, validation

Introduction

Complex Regional Pain Syndrome (CRPS) is a clinical condition characterized by sensory, autonomic, motor, vasomotor, sudomotor and trophic disturbances of an extremity, and mostly develops after trauma (table 1, 2). Diagnosis is performed using validated criteria ('Budapest' criteria, now the 'new' IASP criteria) based on clinical observation and assessment of signs and symptoms (Harden et al 10b;Harden et al 07). The IASP/Budapest criteria have a good specificity and sensitivity, however the dichotomous outcome provides no information on the dynamic nature of the severity, signs and symptoms of CRPS in individual patients.

The pilot CSS, a continuous quantitative index of the signs and symptoms of CRPS, is proposed (Harden et al 10b) as a practical 'bedside' tool consisting of 17 reported and observed diagnostic features based on the Budapest criteria. The 17 aspects are coded as present (=1) or absent (=0) and summed to create the overall CSS score (table 1). Advantages of this multi-component instrument are the simple and objective measurements of prominent impairment associated with CRPS. It is capable of detecting observed signs as well as patient reported symptoms are, which is relevant considering possible disparate changes that can occur in CRPS features over time. The CSS may ultimately function as a practical assessment tool that provides simple means of communication about severity of CRPS, as a follow up instrument for clinical practice and as a complementary research tool.

However, the CSS has not been compared to a validated comprehensive disease severity scale specific for CRPS, and its responsiveness as a follow up tool has not been determined.

Table 1: CRPS Severity score.

	Variable	Scores
	Anamnestic / self reported	
Sensory	Allodynia, hyperpathia	0/1
Vasomotor	Temperature asymmetry	0/1
	Skin color asymmetry	0/1
Trophic	Sweating asymmetry	0/1
	Edema	0/1
	Skin, nail or hair changes	0/1
Motor	Movement disorders	0/1
	Decreased range of motion	0/1
	max. anamnestic score:	8

Table 1: continued.

	Variable		Scores
	Observed	*Objectified*	
Sensory	Hyperpathia to pinprick		0/1
	Allodynia		0/1
Vasomotor	Temperature asymmetry	>0.4 ˚C	0/1
	Skin color asymmetry		0/1
Trophic	Sweating asymmetry		0/1
	Edema	> 3.5%	0/1
	Skin, nail or hair changes		0/1
Motor	Movement disorders		0/1
	Decreased range of motion	> 5%	0/1
		max. observed/ objectified score:	9
		max. total score:	17

Table 2: Impairment level Sum Score (ISS).

	Variable	Scores
Sensory	McGill (NWC)	1-10
	NRS	1-10
Vasomotor	Temperature asymmetry	1-10
Trophic	Edema	1-10
Motor	Decreased range of motion	1-10
	score:	5-50

Table 3: Conversion of measurements and pain scales into ISS scores.

NRS - during use[a]	0	1	2	3	4	5	6	7	8	9-10
NRS[a] - Mean	0-0.9	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	9-10
McGill - NWC[b]	0-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
AROM[c]	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-25
Temperature difference[d]	0-0.3	0.4-0.5	0.6-0.7	0.8-0.9	1.0-1.1	1.2-1.3	1.4-1.5	1.6-1.7	1.8-1.9	≥2.0
Volume difference[e]	3.5%	5%	6.5%	8%	9.5%	11%	12.5%	14%	15.5%	>15.5%
ISS score	1	2	3	4	5	6	7	8	9	10

a NRS, numeric rating scale, pain scale presented as BOX-11, assessed during use or as a mean over 1 week prior to measurements b NWC: number of words chosen of the McGill pain questionnaire, c AROM: active range of motion, related to amount of decreased motion compared to the unaffected limb (1 for < 5%, 2 for 6-15%, 3 for 16-35%, 4 for 36-74% and 5 for >75% per group of affected joints d difference of average temperature measured on five standardized places of the hand or foot, e volume difference compared to unaffected contralateral limb measured by volumetry in percentages.

The Impairment level Sum Score (ISS) is a validated disease specific score to assess the severity of CRPS (Oerlemans et al 98;Perez et al 03). The ISS is a compound score comprised of measurements of clinical features of CRPS: pain (NRS and McGill), edema or atrophy (measured by water displacement volumetry), temperature asymmetry (measured by infrared thermography) and reduction of active range of motion (AROM) (measured by goniometers). Raw scores of these five components are transformed into 1-10 scores resulting in the total ISS score ranging from 5-50 (table 2, 3). Advantages of this multi-component instrument are the simple and objective measurements of prominent impairments associated with CRPS, and it is capable of detecting change in the disease over time (Collins et al 09;Perez et al 08) and its use has been advocated in Dutch multidisciplinary CRPS guidelines (Perez et al 10). A clinically relevant difference on the ISS is reflected in a change of 5 points or more (Oerlemans et al 98;Oerlemans et al 00).

The primary goal of the current study is to assess associations between the CSS and the ISS. Furthermore, the responsiveness of the CSS over time will be evaluated and compared to improvement of ISS and patients' impression of change in pain.

Methods

Patients with CRPS-1 according to the Budapest criteria participating in a clinical trial (NTR 2713) were recruited at the VU University Medical Center in Amsterdam. Assessments were performed at the start of the trial (BL) and 4 (T4), 6 (T6), 9 (T9) weeks and 6 months (T26) after starting treatment. At all time points measurements of volume, temperature and active range of motion were performed and the pain score (BOX-11) during use of the affected extremity was obtained (Oerlemans et al 98;Perez et al 03). One week prior to a scheduled visit, 3 times daily pain scores (BOX-11) were registered by the patient for a period of one week, alongside the McGill Pain Questionnaire (NWC) in a pain diary (Kloot et al 95).

In order to calculate the CSS and ISS scores, signs and symptoms present at the scheduled time points were recorded. Signs pertaining to both scores were measured using a standardized assessment protocol used within the TREND consortium (www.trendconsortium.nl) using valid and reliable tools. Comparisons between the affected and contra-lateral extremity of temperature were measured by means of an infrared thermometer on 5 standardized locations of the hand or foot. Volume was compared by means of water displacement volumeters of the hand and wrist up to 20 cm distal to the elbow or the foot and ankle up to 15 cm proximal to the lateral malleolus. Active

range of motion was measured by means of standardized goniometers of respectively wrist and finger movements, or knee, ankle and toe. The measurements were carried out under environmentally stable conditions by a researcher that had attended training session 3 times a year within the TREND consortium.

The 'clinical' CSS (CSS (clinical)) is a sum of the features as reported by the patient and clinical observation of the physician. The CSS consists of 5 domains representing sensory, vasomotor, sudomotor and motor/trophic aspects of signs and symptoms of CRPS patients. The 'measured' CSS (CSS (plus measured)) is a sum of the features as reported by the patient, observed by the physician plus measured differences for temperature, volume (water displacement volumeters) and range of motion (hand held goniometers). Temperature differences > 0.4 degrees C, >3.5% difference in volume and >5% reduction in range of motion of the affected wrist/elbow or ankle/knee were interpreted as impaired (coded as 1) (Harden et al 10b). Allodynia, hyperesthesia and hyperalgesia were tested using pinpricks are cotton buds (table 1).

The ISS ranges from 5-50 and each sub-score ranges from 1 to 10. Higher scores indicate higher levels of impairment. Two total ISS scores were calculated (ISStot) using either mean pain scores over one week (ISS (mean)) or pain during/after use of the extremity (ISS (during movement)) and five sub-scores were assessed representing the 5 domains the ISS consists of (volume, temperature, active range of motion, pain severity and McGill pain questionnaire) (Oerlemans et al 98;Perez et al 03) (table 2,3).

The patients' impression of change in pain consisting of a 7 point scale (strong deterioration to strong amelioration) were filled out by patients.

Statistical analysis

Data were stored and analysed with SPSS 20. Validity of the CSS was evaluated by calculating Pearson's correlations at all times between the CSS and ISS total and sub-scores. Associations between the CSS 'clinical' and 'measured' scores were calculated using Pearson's correlation to assess the accuracy of clinical assessment of the researcher. To evaluate responsiveness of the CSS associations between change scores of CSS and ISS related to baseline, and between change in CSS and patients' impression

of change in pain were calculated with Pearson's correlations. Furthermore, patients were classified as responders based on improvement on the ISS (i.e. clinically relevant decrease in ISS score (> 5 points) (Oerlemans et al 98;Oerlemans et al 00). Differences between improvement of CSS in responders and non-responders were analysed with the independent student *t*-test or the Mann Whitney test.

Changes of the CSS and ISS over time were represented graphically using the absolute values of the CSS and ISS and the CSS and ISS transformed into Z-scores. The Z-scores were calculated using the percentage of the maximum score per assessment (ISS Z score = $ISStot-5/45x100$; CSS Z-scores = $CSStot/17x100$). The Z-scores are calculated to compare the ISS and CSS on the same scale of 0-100.

Correlation was considered to be fair for correlation coefficients between 0.25 and 0.50, moderate to good between 0.50 and 0.75 and good to excellent when higher then 0.75 (Portney 00), and a two sided p-value ≤ 0.05 was used to indicate statistical significance.

Results

Patient characteristics

Between January 2011 and April 2012 34 patients participated in the study; 27 patients were analysed at four different time points (4, 6 and 9 weeks after baseline) and 17 patients were evaluated after 6 months. Three patients did not finish the 9 week follow up at time of analysis and 4 patients were lost to follow up due to withdrawal from the trial.

The majority of the patients were female (29 female, 5 male), with a mean age of 45.1 years (SD 14.6), median duration of CRPS of 3 months (IQR 2-5). Baseline assessments revealed a mean pain score (BOX-11) of 5.2 (SD 2.2), mean total ISS respectively 25.2 (SD 7.0) and 26.4 (SD 7.1) (table 4), CSS (clinical) 10.3 (SD 2.4) and CSS (plus measured) 10.0 (SD 2.4) (table 4-6).

Table 4: Patient characteristics.

	Baseline Single measure	Baseline Follow up	T4	T6	T9	T26
n*	34	27	27	27	27	17
Female/male*	29/5	23/4	23/4	23/4	23/4	14/3
Age (years)**	45.1 (14.6)	46.3 (15.3)	46.3 (15.3)	46.3 (15.3)	46.3 (15.3)	47.2 (15.9)
Duration of CRPS (months)	3 (2-5)	3 (2-3)	3 (2-3)	3 (2-3)	3 (2-3)	3 (2-4)
Upper/lower* extremity	18/16	14/13	14/13	14/13	14/13	9/8
Right/left extremity*	20/14	15/12	15/12	15/12	15/12	10/7
Mean NRS**	5.2 (2.2)	3.7 (2.6)	3.7 (2.6)	3.8 (2.7)	3.5 (2.7)	2.9 (2.6)
Median NRS***	5.6 (4.0-6.7)	3.9 (1.4-5.9)	3.9 (1.4-5.9)	4.0 (1.2-5.7)	3.7 (0.8-6.1)	3.5 (0-5.1)
Mean ISS score** (mean NRS)	25.2 (7.0)	21.9 (7.8)	21.9 (7.8)	21.3 (8.0)	20.7 (8.5)	17.1 (7.0)
Mean ISS score** (NRS move)	26.4 (7.1)	22.9 (8.2)	22.9 (8.2)	21.4 (8.0) (n=26)	21.7 (8.7)	18.8 (7.9) (n=16)
Mean CRPS** score ('clinical')	10.3 (2.4)	9.3 (3.4)	9.3 (3.4)	8.1 (3.0)	8.2 (3.4)	5.9 (3.8)
Mean CRPS** score ('measured')	10.0 (2.4)	9.0 (3.3)	9.0 (3.3)	7.9 (2.8)	8.2 (3.2)	5.9 (3.5)

*number of patients, **mean and SD, ***median and IQR
ISS ranges from 5-50, CRPS scores (CSS) ranges from 0-17, whereby low scores indicate low levels of impairment.

Table 5: ISS total scores and sub-scores.

	Baseline (n=34)	T4 (n=27)	T6 (n=27)	T9 (n=27)	T26 (n=17)
ISS total (mean NRS)	25.2 (7.0)	21.9 (7.8)	21.3 (8.0)	20.7 (8.5)	17.6 (7.1)
ISS (NRS move)	26.4 (7.1)	22.9 (8.2)	21.4 (n=26) (8.0)	21.7 (8.7)	18.7 (n=16) (7.8)
ISS NRS (mean)	5.7 (2.2)	4.2 (2.5)	4.4 (2.6)	4.0 (2.6)	3.7 (2.4)
ISS NRS (move)	6.9 (2.6)	5.2 (2.8)	4.7 (n=26) (2.5)	5.0 (2.8)	4.6 (2.8)
ISS McGill	6.5 (2.6)	5.4 (3.0)	5.3 (3.2)	5.3 (3.3)	4.7 (3.2)
ISS temperature	4.6 (3.4)	4.6 (3.1)	4.2 (3.4)	4.1 (3.3)	3.4 (2.9)
ISS Volume	2.5 (2.6)	2.5 (2.5)	2.4 (2.2)	2.5 (2.2)	1.2 (0.6)
ISS AROM	5.9 (1.8)	5.2 (2.0)	5.0 (2.0)	4.8 (1.8)	4.7 (1.4)

Results expressed as mean and SD.
ISS totaal ranges from 5-50, ISS subscores from 1-10, whereby low scores indicate low levels of impairment.

Table 6: CSS total scores and sub-scores.

	Baseline (n=34)	T4 (n=27)	T6 (n=27)	T9 (n=27)	T26 (n=17)
CSS 'anamnestic + clinical'	10.3 (2.4)	9.3 (3.4)	8.1 (3.0)	8.2 (3.4)	5.9 (3.8)
CSS 'anamnestic + measured'	10.0 (2.4)	9.0 (3.3)	7.9 (2.8)	8.2 (3.2)	5.9 (3.5)
CSS 'anamnestic'	5.7 (1.3)	5.0 (2.0)	4.2 (1.8)	4.2 (1.9)	3.1 (2.3)
CSS 'clinical'	4.7 (1.4)	4.3 (1.8)	3.9 (1.4)	4.0 (1.9)	2.9 (1.8)
CSS 'measured'	4.4 (1.4)	3.9 (1.7)	3.7 (1.3)	4.0 (1.8)	2.8 (1.4)
CSS 'sensoric'	1.2 (1.1)	1.1 (1.2)	0.9 (1.2)	0.9 (1.3)	0.6 (1.1)
CSS 'vasomotor (clinical)'	3.5 (0.7)	3.0 (1.0)	2.8 (1.1)	2.8 (1.3)	2.0 (1.5)
CSS 'vasomotor (measured)'	3.4 (0.7)	2.9 (1.1)	2.7 (1.0)	2.7 (1.1)	2.1 (1.3)
CSS 'sudomotor (clinical)'	2.5 (0.9)	2.2 (1.2)	1.9 (1.3)	1.9 (1.1)	1.3 (1.1)
CSS 'sudomotor (measured)'	2.2 (1.0)	2.0 (1.2)	1.8 (1.2)	1.9 (1.2)	1.0 (1.1)
CSS 'motortrophic (clinical)'	3.1 (1.2)	2.9 (1.4)	2.6 (1.2)	2.5 (1.9)	2.1 (1.1)
CSS 'motortrophic (measured)'	3.2 (1.2)	3.0 (1.3)	2.6 (1.1)	2.6 (1.1)	2.2 (1.1)

CSS scores expressed as mean and SD.
CRPS scores (CSS) ranges from 0-17, CSS sensoric ranges from 0-3 , CSS vasomotor ranges from 0-4, CSS sudomotor ranges from 0-6, CSS motortrophic ranges from 0-4, whereby low scores indicate low levels of impairment.

Validity of CSS

Association between the total scores of the CSS, the ISS Scores and the patients' impression of change in pain

At baseline, fair correlations were found between ISS (movement) and CSS (clinical) (r =0.34). Both ISS scores (movement and mean) and the CSS (plus measured) showed a fair correlation as well (r=0.44 and r=0.42) (table 7). Fair to good correlations were found between the patients' impression of change in pain and the ISS (movement and mean) (r=0.44, r=0.51).

Correlations at all follow up points were fair to excellent between ISS (movement and mean) and CSS (clinical and measured) (r=0.49 to r=0.84) (table 7). Fair to good correlations were found at time points T4 to T9 between the patients' impression of change in pain and the ISS and the CSS (r=0.47 to r=0.61). At T26 correlations were excellent ranging from r=0.79 to r=0.88.

Table 7: Correlation coefficients between ISS and CSS.

	Baseline CSS (n=34)		Baseline CSS (n=27)		T4 CSS (n=27)		T6 CSS (n=27)		T9 CSS (n=27)		T26 CSS (n=17)	
	'clinical'	'measured'	'clinical'	'measured'	'clinical'	'measured'	'clinical'	'measured'	'clinical'	'measured'	'clinical'	'measured'
ISS total (mean BOX-11)	0.34	0.44*	0.42*	0.53*	0.66*	0.68*	0.65*	0.75*	0.50*	0.58*	0.82*	0.75*
ISS total (BOX-11 move)	0.34*	0.42*	0.40*	0.48*	0.65*	0.67*	0.49*	0.55*	0.51*	0.59*	0.84*	0.77*
ISS NRS (mean)	0.17	0.37*	0.26	0.46*	0.50*	0.43*	0.55*	0.48*	0.39*	0.32	0.67*	0.52*
ISS NRS (move)	0.10	0.16	0.10	0.17	0.47*	0.42*	0.56*	0.52*	0.36	0.39	0.73*	0.44
ISS McGill	0.02	0.13	0.19	0.30	0.46*	0.46*	0.55*	0.61*	0.33	0.36	0.70*	0.60*
ISS Temperature	-0.02	0.11	-0.05	0.12	0.01	0.14	-0.11	0.01	-0.06	0.03	0.51*	0.36
ISS Volume	0.51*	0.56*	0.54*	0.59*	0.46*	0.55*	0.20	0.44*	0.24	0.43*	0.16	0.07
ISS AROM	0.36*	0.34*	0.39*	0.34	0.49*	0.38	0.44*	0.31	0.18	0.25	0.31	-0.07

* significant correlation (p<0.05)

Associations between total CSS scores and sub-scores of the domains of the ISS

Mean ISS sub-scores at baseline ranged from 2.5 points (ISS-volume) to 6.9 points (ISS-pain during movement) (SD 1.8 to 3.4 points). Fair to good correlations were found between the total CSS score and sub-scores of ISS (r=0.37 for the ISS pain score at baseline to r=0.70 for the ISS McGill at T26) (table 7).

Associations between total ISS scores, clinical and measured CSS sub-scores.

Mean CSS sub-scores at baseline ranged from 2.2 (sudomotor) to 3.5 (vasomotor) (SD 0.9 to 1.2). Fair to good correlations were found between the CSS sub-scores and the total ISS (r=0.39 for all CSS sub-scores at T4 to r=0.73 for all CSS sub-scores at T26) (table 7).

Associations between 'clinical' and 'measured' CSS

CSS clinical scores and measured scores were similar, ranging from a mean of 5.9 (respectively SD 3.5 and 3.8) of the measured and clinical CSS at T26 to a mean score of 10.3 (SD 2.4) for the clinical CSS at baseline. Correlations between the clinical and measured CSS were excellent ranging from r=0.94 at T6 to r=0.98 at T26.

Responsiveness

Associations between change of ISS and change of CSS.

Fair to good correlations were found between change scores of the CSS and the ISS at T9 and T26 ranging from r=0.40 at T9 for ISS (pain during movement) and CSS (measured) to r=0.72 at T26 for ISS (movement) and CSS (clinical) (table 8).

Table 8: Correlations of change scores.

		CSS	
		clin	meas
T4	ISS		
	Mean	0.18	0.14
	Move	0.19	0.10
T6	ISS		
	Mean	0.00	0.16
	Move	0.26	0.28
T9	ISS		
	Mean	0.24	0.41*
	Move	0.25	0.40*
T26	ISS		
	Mean	0.63*	0.53*
	Move	0.72*	0.57*

* significant correlation (p<0.05)

Associations between change of CSS and patients' impression of change in pain

At T6 to T26 fair to good correlations were found between the CSS and the patients' impression of change in pain. These correlations ranged from *r*=0.39 for patients' impression of change in pain and CSS (clinical) at T6 to *r*=0.55 for patients' impression of change in pain and CSS (clinical) at T26.

Responders versus non-responders

Significantly higher median changes of CSS were found between responders and non-responders at all time points (T4: 3.0 vs. 1.0 point, p=0.04; T9: 7.0 vs. 3.0, p=0.01; T26: 6.0 vs. 2.0, p=0.02) (table 9).

The ISS and the CSS showed a comparable course of change over time (figure 1, 2). The graphical analysis on the Z-scores represented a higher outcome of the CSS, however this difference narrows at T26.

Table 9: Responders/non responders (improvement on ISS ≥5 points).

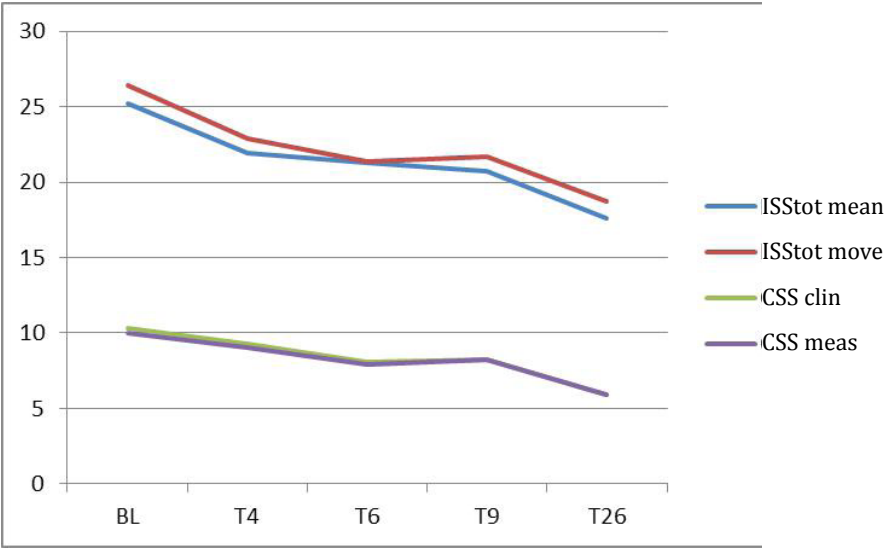| | | Responder ISS | |
		Yes	no
T4	CSS	(n=8)	(n=19)
	'clinical'	3.0 (2-4.75)	1.0 (-1.0-2.0)
	'measured'	2.5 (1.25-3.75)	1.0 (0-2)
T6	CSS	(n=8)	(n=19)
	'clinical'	4.0 (1-5)	3.0 (1-5)
	'measured'	3.5 (1.25-4.75)	2.0 (0-4)
T9	CSS	(n=9)	(n=18)
	'clinical'	3.0 (0-4.25)	3.0 (2.5-4.5)
	'measured'	2.0 (-0.25-3)	4.0 (2.5-4.5)
T26	CSS	(n=7)	(n=8)
	'clinical'	6.5 (5.25-8)	3.0 (1-5)
	'measured'	6.0 (5.25-7)	2.0 (1-4)

Scores expressed as median and IQR

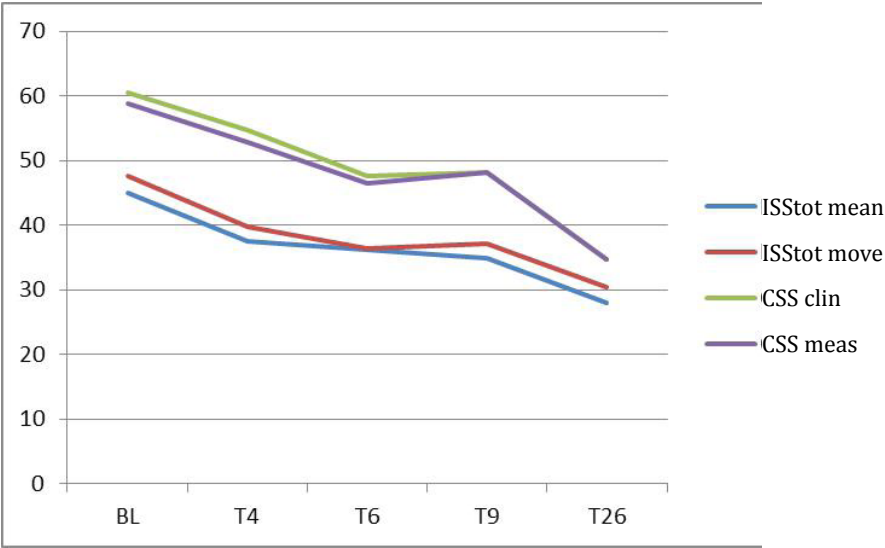**Figure 1:** Change of mean absolute scores of CSS and ISS.

Figure 2: Change of mean Z-scores of ISS and CSS.

Discussion

Valid and responsive tools to assess severity of CRPS are of importance in communication between health care workers and follow up of patients where direct and objective validated measurements ('gold standard tests') are lacking. The CSS as clinical assessment tool can help to obtain a quantifiable point classification of disease severity, which may help in the evaluation of treatments over time. The results of this study show fair to excellent positive correlations between the CSS and ISS. These findings indicate that higher degrees of impairment measured with the ISS correspond with higher measured severity of CRPS by the CSS. In less severe disease the scores of the CSS as well as the ISS are low and are both high with more extensive and chronic disease. These findings are in line with previous research showing positive correlations between the CSS and the indices measuring quality of Life (Rand-36), temperature abnormalities and limitations in range of motion (Harden et al 10b).

The present study shows that the CSS correlates with a validated disease specific tool to express disease severity and impairment level for CRPS. Furthermore, changes in the ISS over time and subjective expression of change in disease course as measured with the patients' impression of change in pain correlate in the same direction. This suggests that the CSS correlates with observer based instruments (ISS) as well as perceived change experienced by the patient in pain and provides an indication for convergent validity of the CSS. Compared to the ISS, the CSS is measured in a dichotomous fashion based on clinical assessment, requires less elaborate measurement, and can therefore serve as a practical bed side tool in clinical practice for the follow up of patients. The limited contribution to the CSS based on measured indices as used in the present study, opposed to the CSS based on clinical assessment provides further support for this observation. However, we previously showed that interrater agreement for clinical observation of the severity of specific features of CRPS (pain, volume, temperature and range of motion differences) is poor (Perez et al 05). Therefore, if grading of the severity of these features is required, use of the ISS is preferred over the CSS.

A challenge in expressing disease severity of CRPS and other pain syndromes is the lack of a comprehensive tool incorporating the full spectrum of the condition. The CSS is based on a comprehensive set of signs and symptoms of CRPS, which makes it a broad tool to address the severity of CRPS. Furthermore, because the CSS is based on the same statistically derived factor structure as the Budapest diagnostic criteria

(Harden et al 10a;Harden et al 07), this severity score provides information that may show a stronger relationship with presence or absence of the disease.

In this study, fair correlations between CSS and the ISS were found at the start of the trial and excellent correlations after a follow up of six months. These differences in correlation strength found between different time points may be related to the differences of the methods of measurements of both scores. Where the ISS quantifies the signs in CRPS using a Likert score, the CSS has a dichotomous scoring system identifying a sign or symptom as present or absent and in the initial stages of the disease, where a more florid phenotype of CRPS can be expected, a non-dichotomous scale may capture more detail than a dichotomous scale. In the present study, this may be shown by the fact that in the initial stages of the study correlations between pain severity (BOX-11 and McGill), and temperature as measured with the ISS and the CSS were low. Therefore it may be considered that for example, large differences in temperature which are clinically related to more severe disease, are not fully captured in the CSS.

In order to further add to the validity of the CSS, changes in relative weighting of different items incorporated in the CSS might be considered. For instance, grading of the different CRPS features (eg. 0=absent, 1=moderate, 2=severe) in order to incorporate the severity of signs and symptoms, might add to the responsiveness of the CSS without considerable change in the practical applicability of the instrument. Another notable aspect is the uneven distribution of symptoms and signs over the factors of the CSS, and the difference in relative contribution of specific features between the CSS and the ISS. For instance, pain related features in the sensory domain of the CSS give only a small contribution to the total score (max 3 out of 17 points) compared to the contribution pain indices have in the ISS (max 20 out of 50 points). Pain related signs and symptoms have a high impact on the patients' experience of CRPS: and therefore, hypothetically on their perception of disease severity. A more even distribution of features within the factors constituting the different domains of CRPS might improve the correlation between the ISS and the CSS, and an international effort is underway to assess a more balanced weighting of the factors in a definitive validation.

Limitations of the study are the fact that a golden standard for assessment of severity is not available for CRPS. Furthermore, assessments were performed by one researcher, therefore we cannot conclude that these results in daily practice will be transferable to other observers. Interrater reliability of the CSS should be assessed in future studies.

Test-retest reliability of the CSS should be established to allow for the calculation of standard error of measurement (SEM). The SEM is an expression of the within subject error, and can serve as a basis for determining relevant change of the CSS. In addition, although concurrence with changes reported by the patient for the CSS were found, the patient perspective with regard to what they considered the most severe or impairing aspect of their disease was not taken into account. Future studies should assess whether changes perceived in the most important feature of CRPS for the patient concur with changes in the severity observed with the CSS.

Our study population consisted of CRPS patients with a relatively short duration of CRPS, which may limit the validity for the chronic population of patients with CRPS. Studies involving patients with a longer duration of CRPS should be considered. The value of the CSS as a responsive research tool may be discussed because of the small changes of severity in our patient sample.

Taken together, these findings are suggestive for validity of the CSS to assess disease severity and to monitor clinical changes of CRPS. With further validation the instrument may prove useful to establish point/visit relative severity, to monitor clinical progress and responsiveness to interventions, and as a research outcome.

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GENERAL DISCUSSION 9

Various pathophysiological perspectives (i.e. neurogenic and immune-mediated inflammation, disproportional oxidative stress, autonomic dysfunction, vasomotor dysfunction, increased neuronal excitation, central sensitization, cortical reorganisation and psychological predisposition) have been proposed to provide an explanation for the development and disease course of CRPS (1). Different signs and symptoms appear in subgroups of patients with CRPS (2) related to separate pathological mechanisms. Several studies provided evidence that a cascade beginning with exaggerated inflammation and excessive oxidative stress followed by peripheral and central sensitization, autonomic disturbances and cortical neuroplastic changes may underlie the heterogeneity of phenotypes of CRPS (3;4). Aberrant inflammation in reaction to prior trauma is most pronounced in early stages of CRPS, revealing clear clinical inflammatory signs and symptoms (5). However, inflammation may also persevere in later stages of CRPS (6-11). In later stages secondary signs and symptoms such as hyperalgesia and vasomotor disturbances can be more pronounced (5;12).

Despite increased understanding about the disease mechanisms of CRPS, for a considerable group of patients CRPS can still develop into a disabling and chronic disease. Effective treatment options for CRPS are still limited, and provide sufficient improvement in only a part of the patient population. This is partly explained by the broad range of clinical phenotypes and differences in disease course exhibited by patients. A multidisciplinary approach with pharmacologic treatment, physiotherapy, and if needed psychological support is advised (13;14). Diagnostic accuracy of CRPS has improved over the past decade due to the development of a validated set of diagnostic criteria, with adequate sensitivity and specificity. However, uniform assessment of disease severity is still in a developing stage.

The studies presented in this thesis focused on exaggerated inflammation and subsequent sensitization as key aspects of CRPS. The prevalence of comorbidities was studied in CRPS patients, as these comorbidities may relate to or be suggestive for possible disease mechanisms for CRPS (**Chapter 3**). The underlying mechanisms of inflammation and oxidative stress, as well as anti-inflammatory interventions for treatment were studied in patients with CRPS **(Chapter 4 and 5)**. Studies directed towards mechanisms later in the cascade, autonomic disturbances and central sensitization in CRPS were presented **(Chapter 6 and 7)**. Furthermore, a study was performed to improve clinical assessment of severity of CRPS-1, which may help in research and clinical communication **(Chapter 8)**.

Comorbidities

Epidemiological studies on comorbidities of CRPS revealed associations between CRPS related mechanisms and pathological mechanisms underlying these comorbidities. Prior research revealed a high prevalence of neurological disorders (e.g. migraine), asthma and menstrual cycle related disorders co-occurring with CRPS, and patients with musculoskeletal disorders such as rheumatoid arthritis were found to be more susceptible to develop CRPS (4;15). In line with these finding, we performed a study on comorbidities in a large group of CRPS patients and compared these to non CRPS pain patients **(Chapter 3)**. The main findings in our study were a high prevalence for gastro-intestinal disorders and muscle, bone and skin disorders. Furthermore, a relatively high prevalence (up to 10%) for headache (mainly migraine), asthma, menstrual cycle related disorders and allergies was found, although these prevalences were considerably lower than previously described (4).

Asthma, allergies and migraine have been related to the same pathological mechanisms as CRPS, whereby neurogenic inflammation, increased activity of mast cells and nuclear factor kappa B activity have been proposed (6;9;16-18). An association between gastrointestinal disorders and CRPS has previously been described, whereby this was hypothesized to be related to exaggerated inflammation from intestinal immune systems leading to systemic disease (19). Another proposed overlapping mechanism between gastro-intestinal disturbances and CRPS is related to disturbances of the autonomic system (20). Besides locally appearing signs related to autonomic disturbances (such as increase of hair growth, extensive sweating and vascular deregulation (20-22)), systemic disturbances attributable to the autonomic nervous system activity have also been proposed. Increase of systemic catecholamines (23), hyper-responsivity to noradrenalin (24;25), generalized osteoporosis (15) and the fact that CRPS can spread to other limbs (26) suggests that CRPS is not a purely localized disease, but especially in later stages appears to show a systemic spread. This systemic component may be related to autonomic disturbances as found in a recent study related to sympathetic-parasympathetic imbalance measured by cardiac responses to vagal stimulation (27). Muscle, bone and skin disorders were also well represented in the group of non CRPS pain patients, suggesting that this comorbidity is related to chronic pain states in general, rather than being related to CRPS specifically (28). In addition, disturbances of the autonomic system are also thought to relate to disturbances in bone muscle-bone metabolism. The sympathetic nervous system is believed to influence bone resorption and osteoblast function through the catecholaminergic pathway (29).

With regard to the relation between CRPS and migraine, overlapping inflammatory pathology and central sensitization have been suggested to play a role in both diseases. Recent studies focusing on calcitonine gene-related peptide (CGRP) in migraine emphasize the importance of CGRP in peripheral sensitization of the trigeminal nerve, but also central sensitization associated with photophobia in migraine patients (16;30). A cascade in which CGRP activates degranulation of mast cells in the dura was also proposed (31). The cascade that leads from neurogenic inflammation to headache and photophobia in migraine patients is suggested to be similar to the mechanisms that are believed to be involved in the development of sensory symptoms and central sensitization in CRPS (32;33).

Taken together, these co-occurrences of diseases with similar pathophysiological pathways and CRPS provide further support for inflammation and sensitization as key mechanism in CRPS. Furthermore, these comorbidities may also lead to the identification of prognostic factors related to the risk of developing and maintenance of CRPS (4). A prognostic profile can help to identify individual patients with a higher risk of the development of CRPS and can lead to prevention or early detection of CRPS, which can lead to a better prognosis.

Anti-inflammatory therapy for CRPS

As a result of views concerning involvement of inflammation and oxidative stress, a considerable amount of research has been performed evaluating the effects of anti-inflammatory treatment in CRPS-1. We present a systematic review summarizing the effects of anti-inflammatory therapy on prevention, pain reduction, and improving range of motion and overall clinical improvement in CRPS-1. Our results suggest that anti-inflammatory therapies with free radical scavengers or glucocorticoids are potentially beneficial on these outcomes in patients with CRPS-1. Preventive effects were found for the use of vitamin C. Although glucocorticosteroids and free radical scavengers differ considerably in anti-inflammatory mode of action, both interventions revealed positive effects. Free radical scavengers appeared to be most effective in reducing pain, whereas corticosteroids exhibited the largest effect on combinations of symptoms. Besides effects on different modalities of CRPS, the mode of administration (iv, topical, oral) may also influence the efficacy of the intervention. As most studies presented effects in heterogeneous samples of CRPS-1 patients without accounting for possible differences related to prevailing pathophysiological mechanisms in individual patients, studies with a phenotype or mechanism based approach should

be considered. Furthermore, other forms of anti-inflammatory therapy, for instance anti-TNF-a and immunoglobulins should be studied more extensively.

Therapeutic options in this early stage are of importance to inhibit development of possibly irreversible disabilities. However, in clinical practice the disease course and progression of CRPS are poorly predictable and early diagnosis is not always easy. Therefore, research into new therapeutic options for chronic CRPS remains relevant because of the high levels of disability and subsequent problems for patients fulfilling their societal role associated with chronic CRPS.

Oxidative stress

Excessive oxidative stress, resulting in continued regional derangement is increasingly being considered as a possible step in the cascade leading to inflammatory features in CRPS (34). However, biochemical studies on markers of oxidative stress are limited, and precise detection of free radicals is difficult (35;36). One of the main limitations is that increased levels of free radicals can result from several physiological processes such as aging or exposures such as cigarette smoke. Furthermore, direct detection of free radicals is unfeasible due to their highly volatile nature, therefore indirect methods are used. Products of lipid peroxidation or DNA damage are proposed as reliable markers for oxidative stress. A prior study showed systemically elevated markers for oxidative stress (MDA and anti-oxidant status) in patients with CRPS (34). In this thesis we presented a study that compared levels of markers of lipid peroxidation and DNA damage in a concise sample of female CRPS patients with a short duration of CRPS to age and gender matched healthy volunteers. No systemically elevated levels of these markers in plasma and in urine were found. This was an unexpected finding, and in contrast with previous findings of Eisenberg et al. The differences with our study may be related to patient selection and the laboratory methods chosen. In accordance with our findings is the fact that several studies evaluating systemic markers did not find elevated levels of markers for inflammation and oxidative stress, (11;37) suggesting that inflammation in CRPS in early stages is not systemic. This was further supported by studies that analyzed markers for local inflammation and oxidative stress. In these studies elevated levels of cytokines and increased levels of mast cell degranulation were found in blister fluid of the affected extremity (7;8). The small study in this thesis does not justify abandoning the hypothesis of increased oxidative stress in CRPS, but the stages of CRPS in which oxidative stress is most prominent may be subject to further analysis.

Cholinergic anti-inflammatory pathway

An alternative target to influence inflammation in CRPS is the cholinergic anti-inflammatory pathway. Inflammatory mechanisms involved in CRPS have been suggested to be related to hyperactivity of the sympathetic nervous system (27). The cholinergic anti-inflammatory pathway as an autonomic endogenous regulatory mechanism has been proposed to play a key role in regulation of inflammation (38). Following this line of thought, disturbances in the autonomic nervous system as described in CRPS may lead to disturbances of the inflammatory/anti-inflammatory balance and may therewith have a role in sustaining inflammation in CRPS. In this thesis we present a proof of concept study in which the effects of increasing acetylcholine availability on characteristics of CRPS were studied by treating ten patients with the cholinesterase inhibitor pyridostigmine, using a single subject design. Although all but one patient showed improvement on one or more outcome measures, the magnitude of the observed improvements was limited. However, the patient with the most marked response did exhibit parasympathetic dysfunction. Likewise, for two patients with a possible autonomic dysfunction, predominantly positive results on the evaluated outcome measures were observed. Two patients with a more pronounced inflammatory profile also displayed a predominantly positive response to treatment with pyridostigmine. These observations may be suggestive for a role of autonomic dysfunction in CRPS, in line with recent reports (27). The combination between observed autonomic disturbances and effects of increasing the acetylcholine availability on inflammatory signs and symptoms in CRPS lends support for a possible role of a disturbed anti-inflammatory cholinergic pathway.

Basic research into the role of changes in the cholinergic pathway in CRPS can be of interest to better understand the involvement of autonomic disturbances in CRPS. Recently, several studies have been performed for inflammatory bowel diseases, myocardial ischemia and sepsis targeting this pathway, the findings of which are promising (39-42). In these studies acetylcholine receptors agonists such as GTS-21, and mechanical stimulation of the vagal nerve have been suggested (43-45).

Central sensitization

In CRPS, features of central sensitization such as allodynia or hyperesthesia are often present (12;46). Central sensitization is triggered by the release of SP, CGRP and glutamate after tissue damage, the latter of which activates the normally dormant

N-methyl-D-aspartic acid (NMDA) receptor. The activation of the NMDA receptor and increase in NMDA receptor density in peripheral tissue is a crucial step in the development of central sensitization and is associated with spontaneous pain and increased reaction to peripheral stimuli (47). To counter the process of peripheral and central sensitization and to reduce sensory disturbances, NMDA receptor antagonists have been proposed (33;49). We presented a randomized placebo controlled trial on the effects of the NMDA receptor antagonist magnesium sulphate on pain, aspects of sensitization, level of impairment, activities, participation and quality of life in CRPS-1 patients. In this study, no additional benefit of treatment with this NMDA receptor antagonist was found in more chronic CRPS-1. The difference in results found in the present study compared to a previous pilot study (32) may be related to the difference in included patient population. Patients with a shorter disease duration as included in the pilot study are generally more likely to respond positively to interventions, and are more likely to show spontaneous non-therapy related improvement. Furthermore, reduction of the activation of NMDA receptors in an early stage can possibly prevent or counter the still relatively limited process of central sensitization and interrupt the cascade from inflammation to neurological changes. Further basic research is needed for a better understanding of changes of the NMDA receptor and associated neuroplastic changes in CRPS.

Assessment tools

The last aspect discussed in this thesis is the challenge of the lack of objective assessment tools in CRPS. Considerable progression was made in recent years to improve diagnosis to distinguish CRPS from other conditions (50). The comparability of studies of CRPS and agreement between clinicians involved in diagnosing and treating CRPS has been improved by an internationally accepted and validated criteria set; the Budapest criteria. A severity score for CRPS, the CSS, is developed based on the same factor structure as these Budapest diagnostic criteria (50;51). This severity score provides information that shows a strong relationship with presence or absence of the disease and is proposed as a practical 'bedside' tool to assess the severity of CRPS. In this thesis, a study is presented to further validate the CSS. The presented study shows that the CSS correlates with validated assessment tools for CRPS, the ISS, and subjective improvement rated by patients. These findings are in line with previous research showing positive correlations between the CSS and indices measuring quality of Life (Rand-36), temperature abnormalities and limitations in range of motion (51).

Furthermore, excellent correlations were found during the follow up period of the trial between changes of ISS and CSS. However, differences in correlation strength were found between different time points, which may be related to the differences between the CSS and the ISS, where the ISS uses an ordinal scale to quantify severity of signs in CRPS and the CSS uses a dichotomous scoring system. Although some improvements with regard to scaling the severity of symptoms and more even weight distribution of symptoms in the CSS are warranted, the CSS showed to be a valid assessment tool. Advantages of this tool are its simplicity in a broad range of signs and symptoms associated with CRPS. It may function as a practical assessment tool that provides simple means of communication about the severity of CRPS.

Limitations

Generally challenging aspects in research on CRPS are the relatively low incidence and heterogeneity between patients, which make inclusion of a well-selected population difficult, resulting in small numbers of studied CRPS patients in clinical trials. Although sufficient numbers were reached to ensure adequate power, the heterogeneity of CRPS and need for subgroup analyses limits the scope of the studies presented in this thesis. Selection of patients with specific signs and symptoms related to the mechanism of treatment in a clinical trial (e.g. inflammatory disease profile of included patients in studies on anti-inflammatory therapy) can improve research. A large database and collaboration between medical centers is of value, as shown in the study on comorbidities in CRPS. Besides differences in patient selection, objective outcome measurements in studies on CRPS are limited, and research is performed using different subjective scales. This provided limitations for the presented systematic review, whereby a limited amount of high quality trials could be included, with large heterogeneity of used clinical criteria and assessment tools for CRPS. Studies on biochemical markers can be of importance such as the presented study on CRPS and oxidative stress to help objectify the diagnosis of CRPS. However, establishing the diagnostic properties of biochemical markers is limited due to the lack of reference values for products of lipid peroxidation and DNA damage in healthy subjects.

Future perspectives

In recent years, a large amount of research has been performed on CRPS, directed at unravelling the underlying mechanisms, and improving strategies for its prevention and treatment, alongside the unification of diagnostic procedures. The broad range of available treatment options suggests that the optimal therapy for CRPS has not yet been identified. The heterogeneous phenotypes of CRPS may suggest that a mechanism-directed approach to treatment of CRPS appears preferable.

Evidence based treatment, such as targeting inflammation and physical therapy have been widely used. However, head to head comparisons of established interventions should be performed and evaluated in terms of clinical efficacy and cost effectiveness in relevant subgroups. As proposed in chapter 3, direct comparison of corticosteroid therapy and DMSO in patients with an inflammatory profile appears warranted at this point given the scientific base for these interventions.

Although activation of the cholinergic anti-inflammatory pathway did not result in relevant effects on CRPS severity in this study, different approaches influencing this pathway should be followed. For example the development of cholinergic receptor agonists (e.g. GTS-21 (52), CNI-1493 (53)), that have been evaluated for the treatment of sepsis, may be of interest for a selected group of CRPS patients.

In theory, prevention and early recognition of CRPS and treatment appears to be most promising to prevent CRPS to occur or persist. The identification of prognostic factors for CRPS needs to be pursued further, in order to develop a clinical decision rule for prevention, early identification and reduction of chronification of CRPS. Prospective cohort studies on the development of CRPS are necessary to gain a better understanding of prognostic factors related to disease onset and disease course.

Outside the scope of this thesis we would like to propose additional recommendations for further research. CRPS research would benefit from uniform use of diagnostic criteria, and uniform assessment of prognostic factors and assessment tools for outcome. To that matter, a core dataset for CRPS research, comparable to the one used in rheumatologic diseases (54;55) would be helpful. In addition, what defines a favorable or unfavorable outcome in CRPS needs to be defined. To that respect, the patient perspective with regard to her or his definition of recovery should be evaluated, giving proper attention to perspectives of both cured and sick patients.

Attention should be given to the assessment of biochemical markers for disease, whereby research on markers of inflammation and oxidative stress (such as cy-

tokines, tryptase, products of lipid peroxidation or DNA damage) should be limited to well selected subgroups of CRPS patients, with the clinical phenotype and the disease duration taken into account. Another biochemical approach may be to study deficiencies that may be related to the development of CRPS. For instance, research on vitamins related to inflammatory disturbances or neurogenic impairments, for example the status of vitamin B, folic acid and homocystein could be considered. Histological assessments of affected tissue of CRPS patients related to local changes of the somatosensory system (e.g. status of NMDA receptors) or autonomic changes (e.g. cholinergic receptors) may increase basic knowledge about local changes in CRPS patients. Aside from studies related to the inflammatory cascade, research should be directed towards the role of cortical changes. Promising results have already been shown regarding detection of functional (56;57) as well as morphological (58) maladaptive cortical changes, and therapeutic interventions directed at this (59;60). Preliminary studies on improving awareness and knowledge about pain in CRPS in combination with physical modalities (graded exposure in vivo and pain exposure physical therapy) have yielded promising results and should be evaluated further (61;62). Cooperation of different research centers may help to find links between the proposed mechanisms in CRPS and can help to further understand this complex phenomenon called CRPS.

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SUMMARY

SAMENVATTING
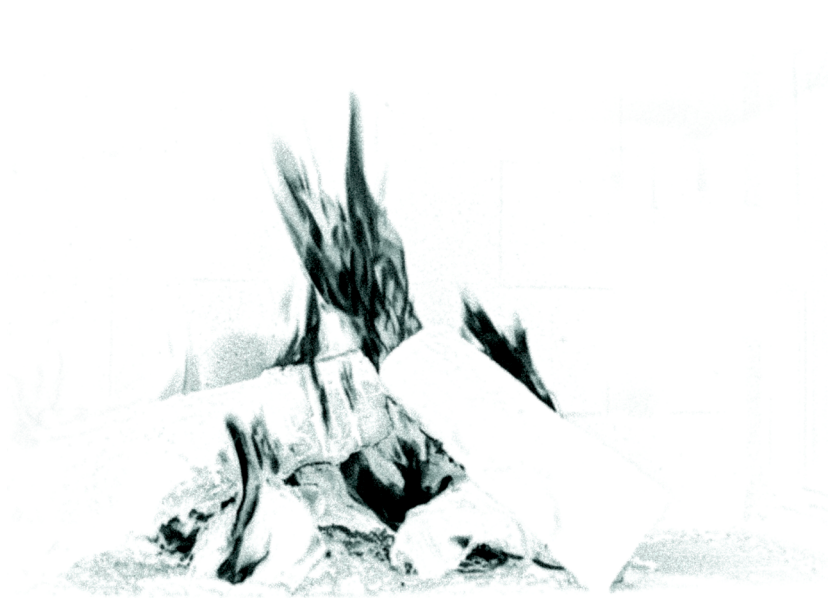
This thesis comprises studies on underlying pathological mechanisms, treatment and assessment tools in Complex Regional Pain Syndrome (CRPS). We hereby focused on the role of inflammation, oxidative stress and subsequent sensitization in patients with CRPS.

In **Chapter 1** the aim of the thesis was outlined and the performed studies are introduced.

In **Chapter 2** a general introduction is presented about the current state of research performed on CRPS-1. The development of clinical diagnostic criteria resulting in the internationally accepted IASP Budapest criteria is presented, which may help to uniform diagnosis and maximize comparability between studies on CRPS. Several pathological mechanisms for CRPS have been suggested in recent years, such as inflammation, oxidative stress, but also autonomic disturbances, vascular pathology, central sensitization, cortical deregulation and psychological factors. In line with these proposed mechanisms a wide range of therapies are described, related to pain management, and mechanism related approaches (e.g. anti-inflammatory therapy, vasodilatory medication). Invasive therapy, such as spinal cord stimulation, is still in an experimental stage. Furthermore, physical and psychological modalities have been developed to improve clinical conditions of patients with CRPS.

Recently, several epidemiological, genetic and clinical studies have been performed in an attempt to identify factors which may be involved in developing CRPS. This information may help in understanding disease mechanisms and identify patient profiles to prevent development of CRPS.

Chapter 3 presents a study on co-morbidities concurring with CRPS. Questionnaires on demographic characteristics, symptoms, general health status, medication use and history of surgery were collected in a sample containing 669 CRPS-1 patients and 180 non-CRPS pain patients from four University Medical Centers. The main findings were a high prevalence of gastro-intestinal disorders and muscle, bone and skin disorders. This may be related to disturbed inflammatory balance, but can also relate to autonomic disorders as proposed for CRPS patients. However, muscle, bone and skin disorders were also described in other chronic pain syndromes which is suggestive for mechanisms occurring in pain conditions in general. Future case-control studies where both patient and medical assessed co-morbidities are systematically evaluated should be conducted to confirm our findings and help to recognize patient profiles with higher chances to develop CRPS.

In **Chapter 4** we present a systematic review on effects of anti-inflammatory therapy for CRPS-1. Twenty-two independent studies were analysed in this review investigating effects of corticosteroid treatment, free radical scavengers and the combination of both substances. Pain reduction, improvement of range of motion and improvement of clinical outcome were found after treatment with free radical scavengers and with corticosteroids. In addition, the free radical scavenger vitamin C showed substantial preventive effects. More research on anti-inflammatory therapy in patients with CRPS-1 is indicated, since most included studies exhibited methodological deficiencies. Research targeted at well-defined subgroups of CRPS-1 patients with a clear inflammatory profile may add to a more mechanism based approach.

In **Chapter 5** a study was presented investigating levels of markers for oxidative stress in patients with CRPS-1. In nine female patients with a short duration of CRPS and nine age matched healthy female volunteers samples of blood and urine were analyzed. In this study, levels of markers of lipid peroxidation (MDA and F2 isoprostanes) and DNA damage (8OHdG) were not found to be elevated in CRPS patients. This was in contrast with previous studies whereby elevated levels of MDA were found in serum and saliva. This result may be related to the systemic measurements performed in bodily fluids while CRPS is, especially in early stages, thought to be a regional disease..

In **Chapters 6 and 7** clinical trials were presented evaluating therapeutic targets for patients with CRPS. **Chapter 6** describes a proof of concept study evaluating the effects of increasing acetylcholine availability. Autonomic endogenous subsystems such as the cholinergic anti-inflammatory pathway have been proposed to play a key role in regulation of inflammation. Whereas autonomic disturbances as well as inflammatory deregulation are proposed pathologic mechanisms in CRPS, influencing the cholinergic anti-inflammatory pathway in CRPS patients may provide a new therapeutic pathway for CRPS. In this study ten patients with CRPS-1 were treated with the cholinesterase inhibitor pyridostigmine in a cross-over design comprising two four week treatment phases and two three weeks control phases. Patients were screened for autonomic disturbances and the inflammatory profile was registered. All but one patient showed improvement at one or more outcome measurements, however these improvements were limited. In a small subgroup analysis patients with either autonomic disturbances or an inflammatory profile tended to have a better outcome after treatment. Although these findings may lend some support for a role for activating the cholinergic anti-inflammatory pathway in CRPS, the effects are too limited to for current clinical application.

In **Chapter 7** a randomized controlled trial on the effects of intravenous administration of magnesium sulphate was presented. Inflammation following trauma can lead to increased peripheral and central sensitization by activating dormant NMDA receptors and local increase in density of NMDA receptors. In CRPS patients this can present as spontaneous pain and increased reaction to stimuli (e.g. allodynia). To counter the process of peripheral and central sensitization and to reduce sensory disturbances, NMDA receptor antagonists (e.g. magnesium, ketamine) have been proposed. In this study 56 patients were included and randomized to receive magnesium sulphate IV ($MgSO_4$) or placebo IV (NaCl 0.9%), during 5 consecutive days. Intravenous administration of magnesium as used in our study showed no improvement on pain or disability in patients with chronic CRPS-1 compared to placebo. .

Comprehensive assessment tools for the severity of CRPS which are in line with current views on diagnosis for CRPS have been lacking. Recently the CRPS severity score (CSS) has been proposed, in which clinical as well as anamnestic features of CRPS are incorporated. In **Chapter 8** we present a validation study of the assessment tool. In this study correlations between the CSS, the ISS and subjective change were assessed in 34 CRPS patients during a clinical trial. The results of this study show fair to excellent correlations between the CSS and ISS and the CSS and subjective change. These findings are in line with previous research showing positive correlations between the CSS and the indices measuring quality of life (Rand-36), temperature abnormalities and limitations in range of motion. Therefore, we may conclude that the CSS can be regarded as a valid assessment tool for disease severity in CRPS-1. Changes of the CSS and the Impairment level Sum Score over the course of a trial correlated well, suggesting that the CSS can be used as a follow up tool on disease severity. To improve the CSS, a more even distribution of signs and symptoms in the total score is proposed, as well application of weighting of individual signs and symptoms according to their severity.

Chapter 9 includes a general discussion about the research presented in this thesis.

Dit proefschrift bevat studies met betrekking tot pathologische mechanismes, behandeling en meet instrumenten bij Complex Regionaal Pijn Syndroom (CRPS). Hierbij ligt de focus op de rol van inflammatie, oxidatieve stress en centrale sensitisatie bij patiënten met CRPS.

In **Hoofdstuk 1** wordt het doel van dit proefschrift beschreven en worden de verrichte studies geïntroduceerd.

Hoofdstuk 2 bevat de algemene introductie, waarin de huidige stand van zaken met betrekking tot de kennis omtrent CRPS-1 wordt samengevat. De Boedapest criteria worden gepresenteerd als internationaal geaccepteerde klinische diagnostische criteria met als doel de uniformiteit van diagnostiek te vergroten en de vergelijkbaarheid tussen studies te verbeteren. Verschillende pathologische mechanismes geassocieerd met CRPS die in de afgelopen jaren zijn bestudeerd worden beschreven. De nadruk ligt hierbij op inflammatie en oxidatieve stress, maar ook autonome stoornissen, vasculaire pathologie, centrale sensitisatie, corticale verstoringen en psychologische factoren worden behandeld. Hieruit volgend wordt een breed scala aan therapieën beschreven gericht op pijnbestrijding en meer mechanisme gerelateerde behandelingen (bijv. anti-inflammatoire therapie, vasodilatatoire medicatie). Invasieve therapieën, zoals ruggenmerg stimulatie, worden aangestipt, maar dienen verder te worden ontwikkeld.

Recent zijn er verschillende epidemiologische, genetische en klinische studies verricht om factoren te identificeren, die betrokken zijn bij het ontstaan van CRPS-1. Dergelijk onderzoek is van belang om ziektemechanismes beter te begrijpen en patiënt profielen te identificeren om preventie van CRPS te verbeteren. In **Hoofdstuk 3** wordt een studie gepresenteerd naar het voorkomen van co-morbiditeit bij CRPS patiënten. Hiervoor werden 669 CRPS-1 patiënten en 180 pijnpatiënten zonder CRPS geïncludeerd in vier universitaire medische centra waarbij vragenlijsten werden verzameld over demografische kenmerken, symptomen, algehele gezondheid, medicatiegebruik en operaties in de voorgeschiedenis. De belangrijkste bevindingen waren een hoge prevalentie van gastro-intestinale stoornissen en spier, bot en huidafwijkingen. Dit kan gerelateerd zijn aan een verstoorde inflammatoire balans, maar kan ook samenhangen met autonome stoornissen. Spier, bot en huidafwijkingen werden even vaak beschreven door patiënten met andere vormen van chronische pijn, waaruit mag worden opgemaakt dat het hierbij gaat om algemene pijn gerelateerde mechanismen. Toekomstige case-control studies, waarbij zowel de patiënt als de controle persoon op gelijke wijze worden onderzocht, zijn nodig om onze bevindingen te bevestigen.

In **Hoofdstuk 4** wordt een systematische review over de effecten van anti-inflammatoire therapie op CRPS-1 weergegeven. Tweeëntwintig onafhankelijke studies werden geanalyseerd waarin het effect van corticosteroïden, vrije radicalenremmers en een combinatie van corticosteroïden en vrije radicalen remmers werden onderzocht. Pijnvermindering, verbetering van bewegingsuitslag en klinische verbetering werden zowel gevonden bij behandeling met corticosteroïden als met vrije radicalenremmers. Daarnaast blijkt de vrije radicalenremmer vitamine C bewezen positieve effecten met betrekking tot de preventie van CRPS te hebben. Toekomstig onderzoek naar het effect van anti-inflammatoire therapie bij patiënten met CRPS-1 is gewenst, omdat de meeste geïncludeerde studies methodologische tekortkomingen vertoonden en de uitkomsten op punten tegenstrijdig waren. Daarnaast kan onderzoek gericht op specifieke subgroepen van CRPS-1, waarbij een duidelijk inflammatoir profiel op de voorgrond staat, een toegevoegde waarde hebben bij het bestuderen van meer mechanisme gebaseerde benaderingen.

In **Hoofdstuk 5** wordt een studie gepresenteerd waarin markers voor oxidatieve stress bij CRPS patiënten worden onderzocht. Bij negen vrouwelijke patiënten met kortdurend CRPS en negen gezonde leeftijd gematchte vrijwilligers werden bloed en urine samples verzameld. In deze studie waren markers voor lipiden peroxidatie (MDA en F2 isoprostanen) en DNA schade (8OHdG) niet verhoogd in CRPS patiënten ten opzichte van de gezonde vrijwilligers. Deze uitkomst was tegengesteld aan eerdere studies, waarbij verhoogde hoeveelheden MDA werden gevonden in serum en speeksel. De uitkomst kan gerelateerd zijn aan het feit dat het in onze studie ging om metingen van systemische lichaamsvloeistoffen, alhoewel CRPS, met name in de vroege inflammatoire fase met name regionaal tot uitdrukking komt. Toekomstig onderzoek zou zich moeten richten op lokale inflammatoire markers en markers voor oxidatieve stress in geselecteerde CRPS patiënten met een inflammatoir profiel.

In **Hoofdstuk 6 en 7** worden klinische studies beschreven, waarbij effecten van anti-inflammatoire therapie worden geanalyseerd. **Hoofdstuk 6** beschrijft een proof-of-concept studie over de effecten van het verhogen van beschikbaarheid van acetylcholine. Autonome endogene subsystemen, zoals het cholinerge anti-inflammatoire pad, zouden een belangrijke rol kunnen spelen in het temperen van inflammatie. Autonome stoornissen en inflammatoire ontregeling zijn beide genoemd als pathologische mechanismes bij CRPS. Beïnvloeding van dit pad kan mogelijk een nieuwe therapeutische optie bieden voor patiënten met CRPS. In deze studie werden tien

patiënten met CRPS-1 behandeld met de cholinesteraseremmer pyridostigmine in een cross-over opzet bestaand uit twee maal een vier weken durende behandelfase en twee maal een drie weken durende controlefase. Patiënten werden gescreend op de aanwezigheid van autonome stoornissen en het inflammatoire profiel werd geregistreerd. Negen van de tien patiënten lieten verbetering zien op één of meer uitkomstmaten, maar deze verbeteringen waren beperkt. In een kleine subgroepanalyse werd bij patiënten met autonome stoornissen of een inflammatoir profiel een betere uitkomst gezien na behandeling. Deze bevinding kan wijzen op een verstoord cholinerg anti-inflammatoir pad bij CRPS.

In **Hoofdstuk 7** wordt een gerandomiseerde gecontroleerde studie beschreven over de effecten van intraveneus magnesium sulfaat. Inflammatie na een trauma kan lijden tot perifere en centrale sensitisatie door activatie van inactieve NMDA receptoren en lokale toename van dichtheid van NMDA receptoren. Bij CRPS patiënten kan zich dit uiten in spontane pijn en toename van reactie op stimuli (bijv. allodynie). Eerdere studies tonen aan dat het proces van perifere en centrale sensitisatie en het verminderen van sensorische stoornissen kan worden tegen gegaan door het gebruik van NMDA receptor antagonisten (bijv. magnesium of ketamine). In deze studie werden 56 patiënten geïncludeerd en gerandomiseerd, waarbij de helft magnesium sulfaat ($MgSO_4$) en de andere patiënten placebo IV (NaCl 0.9%) kregen gedurende vijf aansluitende dagen. In deze studie toonde behandeling met intraveneus magnesium sulfaat geen verbetering van pijn of beperkingen bij patiënten met CRPS in vergelijking met placebo.

Het aantal studies naar de diagnostiek en wijze waarop de ernst van CRPS kan worden bepaald is in de afgelopen jaren sterk toegenomen. Valide meetinstrumenten, die de ernst van CRPS in overeenstemming met de huidige diagnostiek uitdrukken en betrouwbaar kunnen worden gebruikt voor het klinisch vervolgen van patiënten zijn beperkt. Recent is de CRPS severity score (CSS) geïntroduceerd die klinische en anamnestische symptomen gevat in de huidige diagnostische criteria combineert. In **Hoofdstuk 8** wordt een studie beschreven waarin de CSS nader wordt gevalideerd. In deze studie worden correlaties tussen de CSS, de Somscore op Stoornis Niveau (SSN) en subjectieve veranderingen geëvalueerd bij 34 CRPS patiënten, die gevolgd worden tijdens een klinische studie. Er wordt een matige tot excellente correlatie gevonden tussen de CSS en de SSN en tussen de CSS en subjectief ervaren veranderingen. Deze

bevindingen zijn vergelijkbaar met eerder onderzoek, waarin positieve correlaties tussen de CSS en kwaliteit van leven (Rand-36), temperatuurverschillen en verminderde bewegingsuitslag werden gevonden. Hieruit kan geconcludeerd worden dat de CSS een valide meetinstrument is om de ernst van CRPS uit te drukken. Veranderingen van de CSS en ISS over de tijd van de studie correleerden ook sterk, waaruit kan worden opgemaakt dat de CSS kan worden gebruikt als meetinstrument voor het vervolgen van de ernst van CRPS over de tijd. Ter verbetering van de CSS werd een gelijkwaardiger verdeling van symptomen op de gehele score voorgesteld en er zou een weging kunnen worden toegepast op basis van de ernst van een symptoom.

Hoofdstuk 9 bevat een algemene discussie met betrekking tot het gepresenteerde onderzoek in dit proefschrift.

DANKWOORD
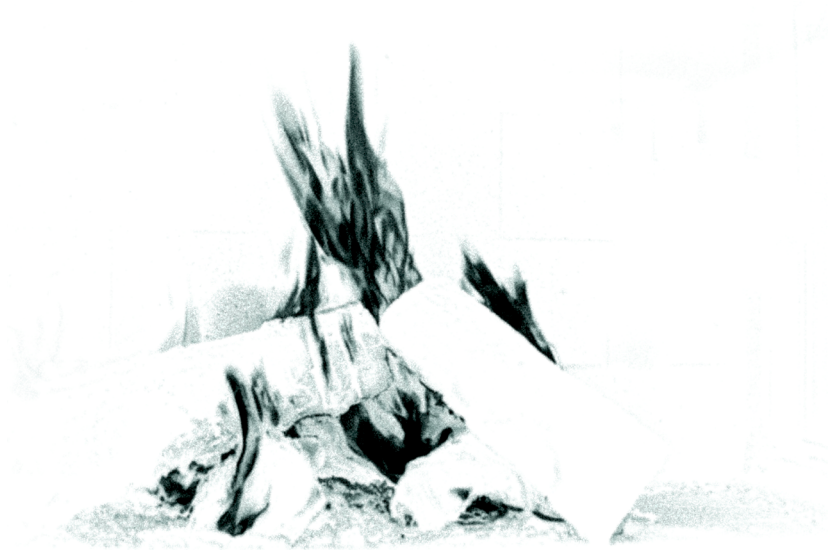
Op deze plek wil ik een aantal mensen bedanken die mijn bijzondere tijd als promovendus met mij hebben meebeleefd en zonder wie ik dit proefschrift niet had kunnen schrijven. In de vier jaar op de VU in ons knusse kamertje heb ik een hoop geleerd over de wetenschap, patiënten bejegening, time management, hoe te werken op twee vierkante meter, hoe te communiceren door muren heen, maar vooral over mezelf.

Beste Roberto, als eerste wil ik jou bedanken voor de directe begeleiding en continue betrokkenheid bij mijn onderzoek. Ik heb erg genoten van de discussies en brainstorm sessies, waarbij alles bedacht kon worden, maar jij gelukkig de realiteit niet uit het oog verloor. Alhoewel jouw kritische opmerkingen en taalkundig perfectionisme me af en toe wel gestolen konden worden, heeft dit er wel toe geleid dat er een proefschrift ligt waar ik trots op ben. Daarnaast was er ook regelmatig tijd voor een praatje, drankje of lunch, vakantie verhalen of verjaardagstaartje inclusief knuffel (waar ik wel even aan moest wennen). Er is nog veel meer te zeggen, maar ik hou het erbij dat ik veel van je heb geleerd en altijd met een bijzonder gevoel aan deze samenwerking zal terug denken.

Beste prof. Zuurmond, ook u was altijd erg betrokken bij alles wat er op ons kleine kamertje gebeurde. Met uw persoonlijkheid, humor en optimisme heeft u me weten te winnen voor het lastige onderzoek op het gebied van CRPS. Uw passie voor patiënten en de overtuiging het vak van arts op uw manier uit te oefenen zal ik me nog vaak voor de geest halen bij mijn verdere carrière. Daarnaast waren de discussies over te bewandelen pathways en nieuwe, misschien vergezochte hypothesen verrijkend en heb ik het idee dat deze gesprekken de essentie van wetenschap zijn en tot mooie nieuwe ideeën hebben geleid.

Lieve kamergenootjes, Susan, Sabine, Tijn, Mariëtte en Stefania. Al heb ik niet met jullie allemaal even lang of intensief samen gewerkt, de sfeer was altijd fijn en ik had altijd het gevoel thuis te komen, zelfs nu ik al langere tijd niet meer in de VU werk. Het oefenen van praatjes of posters, het mopperen op onderzoek wat te traag gaat, weer terug gekregen manuscripten met veel te veel rood, ik zal er nog vaak aan terug denken. Af en toe werd de kamer nog extra gevuld door studenten, waarvan ik Stefanie en Cindy wil noemen, omdat die heel veel werk voor mij hebben gedaan.

Beste prof. Loer, hartelijk dank voor uw kritische kijk op mijn onderzoek. Van een afstandje kon u vaak andere vragen stellen dan in onze kleine groepje van de pijnpoli omhoog kwamen en dit was zeker constructief voor de artikelen in mijn proefschrift.

Verder wil ik alle collega's van de afdeling anesthesiologie bedanken waar ik mee heb samengewerkt. De AIOS die stage liepen op de pijnpoli en hun best deden om patiënten voor mij te includeren. Mijn lunchmaatjes Carolien, Charissa en Florence. Alyson en Inez bedankt voor het regelen van alle bestellingen en de dagelijkse praatjes en roddels. Collega's wat verder af, waarmee ik samenwerkte binnen TREND, bedankt voor de leuke symposia, meetmiddagen en hulp bij het vinden van patiënten.

Naast collega's van de afdeling anesthesiologie heb ik met veel mensen mogen samenwerken, die niet direct binnen ons vakgebied vielen. Peter Scheffer, waarvan ik heb geleerd dat een laboratorium maar een doel is om al je ideeën uit te werken. Gelukkig had ik daarbij Jan Nouta die me begeleidde met alle praktische zaken in het lab en me behoede voor bevroren handjes door de -80 vriezer. Dr. Strijers die me de basis vaardigheden van de neurofysiologie heeft laten zien. De statistiek was me nooit gelukt zonder Dirk Knol, die me van heel basaal, naar toch wel ingewikkelde statistiek door mijn artikelen heeft geholpen en er daarbij altijd op bleef hameren dat ik het zelf ook wel echt moest begrijpen (wat bijna is gelukt).

Helaas zijn er in zo'n periode ook altijd projecten die het proefschrift niet halen, maar waarvan ik wel veel geleerd heb. Met veel plezier heb ik met Piet Hoogland en Evelien samengewerkt bij de neuroanatomie. Hier heb ik een hoop geleerd over de mogelijkheden van histologisch onderzoek en kon ik altijd terecht met vragen. Het project met Henk Blom, waarbij met name tijdnood de remmende factor bleek, over de rol van een verstoorde vitamine status bij CRPS blijft wellicht iets om in de toekomst verder uit te diepen. Bedankt voor alle inspirerende gesprekken.

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Lieve Laura, fijn om jou in de buurt te hebben, met een bakkie koffie of een appje kan je me altijd weer een zetje in de goede richting geven. Lieve Orgie-Ders, allemaal bijzondere mensen waar ik bijzondere gevoelens bij heb, het is heel fijn bij jullie zo mezelf te kunnen zijn, waarbij ik weer een beetje student kan zijn en nooit op mijn woorden hoef te letten

Lief cordial, een fijne thuisbasis op de maandag avond, waarbij ik mijn dagelijkse beslommeringen kwijt kan, maar ook gewoon lekker kan komen eten. De weekendjes weg geven echt een vakantie gevoel, op naar het 3e lustrum.

Lieve teamgenootjes/ex-teamgenootjes, sporten tijdens je promotie is van levensbelang. Maar in dit team voelde het als meer dan een avondje sporten. Al wordt al dat beppen niet door iedereen gewaardeerd, we blijven een vrouwenteam en het is fijn je ei kwijt te kunnen in woorden maar lekker meppen helpt ook echt. Ik hoop dat we nog lang met elkaar doorspelen.

Lieve Anke en Melanie, bedankt dat jullie deze dag mijn paranymfen willen zijn en naast me willen staan. Anke hopelijk gaat de wetenschap jou veel brengen en blijf je er veel plezier aan beleven. Mel, als studiegenoot, oud-collega en cordialgenoot, heb je me van veel verschillende kanten gezien en hopelijk komen we elkaar in het vak nog af en toe tegen, ook al zit daar helaas momenteel een zee tussen.

Lieve broers en zus, Karen en Michiel: ja ik ga ook promoveren, al had ik zo gezegd dat ik dat nooit zou doen, ik kon niet achterblijven. Dank voor alle discussies, maar vooral voor de warmte en de oprechte interesse in werk en persoon.

Lieve Ria, Gerrit, Lennert en Gerrit Jan. Al moest ik even mijn plekje opeisen en wat meer volume kweken, het is fijn om in jullie mannenhuishouden wat vrouwkracht (x2) te kunnen toevoegen en me zo thuis te voelen op de Zwenkgras.

Lieve Aleid, ja nummer 4 gaat ook promoveren, wie had dat gedacht. De basis die ik van jou heb meegekregen heeft ervoor gezorgd dat ik mezelf durf te zijn en mezelf niet snel uit het veld laat slaan. Thuis thuis is altijd een fijne plek, ook al heerst er vaak een gezellige chaos.

Lieve Gijs, jij bent er gewoon. Dat is eigenlijk wat ik vaak het hardst nodig had. Al begreep je vaak de helft niet van wat ik je vertelde, je probeerde het wel en jouw samenvattingen van wat ik nou precies deed waren vaak ontwapenend en relativerend. Ik zal nog veel gaan genieten van alle mooie dingen die wij samen gaan meemaken.

Lieve Sofie, natuurlijk de liefste en vrolijkste dochter van de wereld. Het is een feestje om je elke dag weer wat meer te zien kunnen en om je moeder te mogen zijn.

Curriculum Vitae

Sigrid Fischer was born on the 16th of January 1980 in Rotterdam, The Netherlands.

1992-1998	Secondary education (VWO), Montessory Lyceum, Rotterdam
1998-1999	Tarrant County Junior College, Fort Worth, Texas
1999-2006	Medicine, University of Leiden (LUMC)
2007	Resident Cardiac surgery, Leyenburg hospital, The Hague
2007-2008	Resident Internal medicine, 't Lange Land Ziekenhuis, Zoetermeer
2008-2012	PhD student at the Department of Anesthesiology, VU University Medical Center, Amsterdam
2012-2013	Resident Neurology, Kennemer Gasthuis, Haarlem.

Sigrid is married to Gijsbert de Bock and living in Leiden with their daughter Sofie.