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COMPLEX REGIONAL
PAIN SYNDROME:
THE SEARCH FOR
INFLAMMATORY
BIOMARKERS

**Complex Regional Pain Syndrome:
the search for inflammatory biomarkers**

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**Complex Regional Pain Syndrome:
the search for inflammatory biomarkers**

Complex Regionaal Pijn Syndroom:
de zoektocht naar inflammatoire biomarkers

Thesis

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To my parents,

Ku pasenshi, bo ta gana gloria

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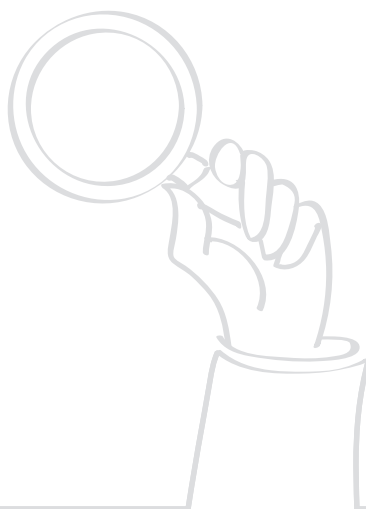
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Part 1



Chapter 1

General introduction

Complex Regional Pain Syndrome (CRPS) is defined by the International Association for the Study Pain (IASP) as 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. CRPS type I develops after any type of trauma, especially fracture, soft tissue lesion. CRPS type II occurs after major nerve damage” (1). CRPS is considered to be a rare syndrome with an estimated incidence ranging from 5.5-26.2 per 100.000 person-years (2, 3). Despite the rareness of this syndrome, research into this syndrome is important as, if left untreated, this syndrome can lead to a debilitating loss of function of the affected limb and can also have a significant social impact on the life of patients (4).

CRPS is arguably one of the most controversial diagnoses of our time. This is due to two main factors: 1) a common denominator in the pathophysiology has not yet been identified, and 2) there are no diagnostic tests yet to objectively diagnose this syndrome. The history of CRPS has therefore been turbulent and is characterized by numerous changes to the name of this syndrome, the clinical criteria to diagnose this syndrome (5), and the treatment of this syndrome.

Further, the lack of a common pathophysiological denominator and the lack of objective diagnostic tests lead to skepticism among physicians on the existence of this syndrome (6, 7), despite extensive empirical evidence proving otherwise. The consequence is a negative impact on the patient, starting with a delay in diagnosis, a delay in initiation of appropriate therapy, and a general lack of acknowledgement and awareness of the patient's illness. Thus, it is important that research on CRPS focusses on further advancing our understanding on the pathophysiology of this syndrome and with this, exploring possible objective tests that may aid in the diagnosis and management of this syndrome. To this end, clinical and biochemical biomarkers are an interesting topic of research.

At present, it is widely accepted that CRPS has a multi-mechanism pathophysiology and that treatment of this syndrome should target the multiple mechanisms that may play a role in each CRPS case (8). Due to this multi-mechanism pathophysiology, it is likely that there will never be one diagnostic test or biomarker specific for CRPS, rather there may be a panel or combination of tests or biomarkers that will be used, with each test and biomarker reflecting a different pathophysiological mechanism.

In this thesis, we have chosen to focus on one of the most important pathophysiological mechanisms in CRPS, i.e. inflammation, and the biomarkers related to this inflammatory process. Inflammation plays a role both in the initiation and maintenance of CRPS. Three main sources of inflammation have been identified in CRPS: neurogenic inflammation, neuroinflammation and dysregulation of the immune system. Each of these sources can be identified using different clinical and biochemical biomarkers and can be targeted by specific

therapies. In this thesis, we focus on biomarkers that reflect dysregulation of the immune system in CRPS.

A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (9). Building on this definition, a biomarker can also be used in the diagnosis, prognosis and monitoring of activity and/or severity of a disease (9). For CRPS, various potential biomarkers of inflammation have been identified, however, none of these markers have yet been validated in terms of use in diagnosis, monitoring of disease activity and/or severity, and effect of therapy.

Inflammatory biomarkers in CRPS can be both clinical and biochemical in nature. Clinical biomarkers of inflammation are, for example, pain, redness, swelling, warmth and loss of function of the affected limb, i.e., the classical signs of inflammation. Biochemical biomarkers of inflammation can be measured in various fluids and can range from cytokines to microRNAs (miRNAs) (10, 11). The crux in CRPS is, however, that while increased levels of various potential biochemical biomarkers have been identified, there is often a discrepancy between clinical findings and expected biochemical findings and vice versa. A prominent example is that levels of classic inflammatory markers such as C-reactive protein (CRP) and white blood cell count (WBC) are not increased in patients with acute CRPS (12), a phase in which classic signs of inflammation are often seen (2, 13-15). Another example is the finding that in patients with chronic (cold) CRPS in which inflammation is clinically not present, there still may be biochemical evidence of an inflammatory process (16).

Our group have previously studied various local (i.e., in the affected limb) and systemic (i.e., in venous blood) markers of inflammation in CRPS. Locally, in skin blister fluid, our group previously found significantly higher levels of the pro-inflammatory cytokines tumor necrosis factor(TNF)- α and Interleukin(IL)-6, however, these cytokines were not associated with clinical symptoms and signs of impairment (17). In a follow-up study in the same patient sample, our group assessed the levels of these pro-inflammatory cytokines in the CRPS patients who could then be considered to have intermediate stage CRPS (1-2 years after the initial event), hypothesizing that local inflammation would only be present during the initial, acute phase of disease, and that the production of pro-inflammatory mediators would decrease during the course of disease (18). We found that although there was improvement in clinical symptoms and signs, this improvement was not reflected biochemically in a reduction of TNF- α and IL-6 in blister fluid of the affected and contralateral limb. The levels of these pro-inflammatory cytokines remained higher in the affected limb than in the contralateral limb and there was no difference between levels of these cytokines at follow-up versus baseline (18). In a further follow-up study in 12 CRPS patients from whom data was available at a median disease duration of 4 months, 3 years and 6 years, our group found that the level of these pro-inflammatory cytokines were significantly higher in the affected limb than contralateral limb at a disease duration of 4 months and 3 years but that

this difference had diminished at 6 years follow-up. Importantly, no correlation was found between the pro-inflammatory cytokines and clinical characteristics such as pain and differences between affected and contralateral limb for temperature, volume and mobility (19). Although these findings show that blister fluid TNF- α and IL-6 may not be appropriate biomarkers for monitoring disease severity, there may still be a role for these markers in, for example, monitoring effects of therapy with TNF- α inhibitors at a biochemical level (20, 21). These biomarkers have yet to be validated for this use.

Systemically, our group previously assessed the role of autoantibodies in CRPS (22). We found that the prevalence of anti-nuclear antibodies (ANAs) was significantly higher in CRPS patients (30%) than in the healthy population (4%) (22). Again, no difference in prevalence of these autoantibodies could be found for available clinical characteristics such as warm versus cold CRPS, nor was there a difference in disease duration in CRPS patients with a positive ANA test versus patients with a negative ANA test. In addition, our group noted that the prevalence of ANAs in CRPS patients was closer to the prevalence of an autoimmune disease such as rheumatoid arthritis (RA, 25%), which is thought to have auto-inflammatory subtypes, than the prevalence of ANAs in a classic systemic autoimmune disease such as systemic lupus erythematosus (SLE, >99%) (23-26). Apart from concluding that there may be a role for autoantibodies in the pathophysiology of CRPS (22, 27, 28), studies on autoantibodies still cannot conclude whether these autoantibodies are pathogenic or a result of the inflammatory process in CRPS. Furthermore, treatment with a 6-week low-dose Intravenous Immunoglobulin (IVIG) infusion did not result in significant pain relief in patients with moderate to severe CRPS (29). This made us question the role of autoantibodies in CRPS and our focus shifted from autoantibodies to T-cells and monocytes and macrophages in CRPS. T-cells because these cells, together with B-cells, are the most crucial cells of the adaptive immune system, and monocytes and macrophages, because findings such as higher blister fluid levels of TNF- α and IL-6, which are primarily produced by pro-inflammatory M1 macrophages, point towards a dysregulated innate immune response in CRPS. In addition, therapies such as prednisolone and thalidomide (TNF- α inhibitor) which exert their effects mainly on T-cells and monocytes, respectively, seem to be effective in certain subgroups of CRPS patients (30-34).

We therefore chose to study the activation of T-cells and the monocyte-macrophage system in CRPS using two biomarkers: the soluble interleukin-2 receptor (sIL-2R) which is a marker for T-cell activation (35) and soluble CD163 (sCD163) which is a marker indicating activation of local tissue-resident macrophages, and thus the monocyte-macrophage system (36). Until now, both markers had not been measured or applied as potential diagnostic/therapeutic markers in CRPS. The sIL-2R is already clinically applied to monitor disease activity and/or severity in diseases where T-cell activation is centrally involved, such as rheumatoid arthritis and sarcoidosis (35, 37-40). In addition, a recent retrospective cohort study showed that the sIL-2R has a good diagnostic value in the diagnosis of sarcoidosis

with a high sensitivity (88%) and specificity (85%) (41). Soluble CD163 is a relatively new marker (42). Its use as a biomarker, be it diagnostic or prognostic, has been established for certain inflammatory diseases such as haemophagocytic syndrome (diagnostic and prognostic), sepsis (prognostic), systemic sclerosis (prognostic) and HIV (prognostic) (43). Further, sCD163 is increased in the serum of patients with chronic inflammatory disorders such as rheumatic diseases, psoriasis and obesity (43-45).

A big advantage is that both markers can easily be measured in venous blood using a validated ELISA system. Therefore, if these markers prove to be valuable in the diagnosis and/or prognosis and/or monitoring of effect of therapies in CRPS, they could be easily implemented in clinical practice.

RELEVANCE OF THIS RESEARCH

This thesis further builds on previously established hypotheses of inflammation in CRPS and expands our current knowledge on the (inflammatory) pathophysiology of CRPS. Furthermore, the application of two new potential biomarkers for the diagnosis and/or management of CRPS is explored.

Ultimately, the goal of this thesis is to emphasize the importance of biomarker research in CRPS by exploring the role of biomarkers of inflammatory pathogenesis in CRPS, thereby introducing more objectivity to the clinical diagnostic process and reducing subjectivity, and skepticism, surrounding the diagnosis CRPS.

AIM AND OUTLINE OF THIS THESIS

The aim of this thesis was threefold: 1) to explore the need for diagnostic and therapeutic biomarkers in CRPS; 2) to study the role of the T-cell-specific sIL-2R and macrophage-specific sCD163 as potential biomarkers in CRPS; and 3) to address (recent) concerns that CRPS is not a distinct diagnostic entity.

To this end, we divided this thesis into three parts. Part 1 kicks off this thesis with the introduction (Chapter 1) followed by a concise article on the pathophysiology, diagnosis and management of CRPS (Chapter 2). This is followed by a review article on the importance of biomarkers, especially biomarkers of inflammation, in the diagnosis and management of CRPS (Chapter 3). Part 2 further elaborates on this need for biomarkers in CRPS through hands-on investigation of two potential biomarkers. The first study in part 2 investigates whether sIL-2R levels are increased in CRPS (Chapter 4). This is followed by a study that investigates whether sIL-2R can be used as a diagnostic biomarker in CRPS (Chapter 5). The chapter ends with a study investigating whether the tissue-resident macrophage-specific

activation marker sCD163 is increased in CRPS patients (Chapter 6). Finally, in Part 3, arguments presented in the literature that CRPS is not a distinct diagnostic entity are addressed and refuted in a review article using the extensive empirical literature on CRPS (Chapter 7). Part 3 then ends with the discussion in which findings from the articles in this thesis are summarized and discussed and recommendations for future research are presented (Chapter 8).

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Chapter 2

Complex regional pain syndrome: diagnosis and treatment

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KEY POINTS

Complex regional pain syndrome (CRPS) is a post-traumatic disorder characterized by a non-dermatomal distributed, severe, continuous pain in the affected limb and is associated with sensory, motor, vasomotor, sudomotor and trophic disturbances.

CRPS is a clinical diagnosis and is diagnosed using the new International Association for the Study of Pain (IASP) clinical diagnostic criteria. There is no diagnostic test specific for CRPS.

The pathophysiology of CRPS is multifactorial, with recent studies pointing towards CRPS being an exaggerated inflammatory response as a result of trauma or surgery.

CRPS should be treated in a multidisciplinary fashion with treatment consisting of adequate pain management, physiotherapy and psychological evaluation and intervention.

In the future, we expect a shift from a symptomatic to a more mechanism-based treatment of CRPS.

INTRODUCTION TO CRPS

Complex regional pain syndrome (CRPS) is a clinical disorder that is characterized by severe, continuous pain in the affected extremity, which is accompanied by sensory, vasomotor, sudomotor/edema and motor/trophic changes (1). The pain is regionally restricted (i.e. cannot be related to a specific dermatome) and disproportionate to the inciting event (1, 2).

CRPS is usually precipitated by trauma (mostly fractures) or surgery (2, 3). The upper extremity is affected more often than the lower extremity (2-4). CRPS is usually limited to one extremity, however cases of CRPS in multiple extremities have been described (2).

The incidence of CRPS has been reported to range from 5,5 to 26,2 per 100.000 person years (3, 5). Women are more frequently affected than men with studies reporting a three to fourfold higher incidence in women (3, 5). The highest incidence was found in women aged 61-70 (3).

Two distinctive forms of CRPS are currently described in the literature. CRPS type I where there is no demonstrable nerve lesion and CRPS type II where there is demonstrable nerve lesion (1, 4, 6). CRPS type I and II do not differ in clinical presentation and choice of treatment (7). Consequently, CRPS will be used as a general term in this article referring to both CRPS type I and CRPS type II.

CRPS can have a severe impact on the quality of life of patients and can lead to substantial physical as well as social disability (8, 9). It is therefore important for clinicians to recognize and diagnose this disorder in order to provide appropriate care and guidance to patients suffering from this debilitating disease.

The purpose of this educational article is to provide clinicians with concise information regarding the pathophysiology, diagnosis and treatment of CRPS.

CLINICAL PRESENTATION

Patients generally present themselves with severe, continuous pain that typically takes on a glove- or stocking like distribution (2, 3). Injury or surgery usually precede the symptoms (2, 3).

The pain is often accompanied by sensory, vasomotor, sudomotor/edema and motor/trophic symptoms. These signs and symptoms can vary during the course of the disease.

Patients may report (hyper) sensitivity to painful as well as non-painful stimuli (hyperaesthesia and/or allodynia). Differences in skin temperature between the affected and contralateral limb may be reported.

Sweating patterns between the affected and contralateral limb may be altered. Swelling of the affected limb can be reported. Symptoms of motor dysfunction such as loss of range of motion, tremor and dystonia can be described. Patients may also report changes in hair

and nail growth of the affected limb (2, 3). Patients may further report increase of symptoms after exercise(2).

Findings during physical examination include, but are not limited to, allodynia and/or hyperalgesia, differences in color and skin temperature between the affected and contralateral limb and edema of the affected limb (2, 3). Functional tests may reveal a reduction in the range of motion of the affected limb in comparison with the contralateral limb (3). Tremor, dystonia, and altered nail and hair growth of the affected limb can also be observed(2, 3).

CRPS patients are often described as having warm, intermediate or cold CRPS based on reported and measured skin temperature differences between the affected and contralateral limb(2, 10).

Current research suggests the existence of different phenotypes of CRPS based on the signs and symptoms deemed most prominent during history taking and physical examination (11). These signs and symptoms could reflect the underlying pathophysiological mechanism (i.e. inflammation, pain/sensory disturbances, vasomotor disturbances, motor disturbances and psychological disturbances). When assessing signs and symptoms of CRPS patients, it is important for physicians to recognize which pathophysiological mechanism is most prominent. By determining the most prominent mechanism, physicians can use specific therapies to target these mechanisms (Figure 1).

It is our hypothesis that in the majority of patients, especially patients with warm (acute) CRPS, inflammation is the most prominent mechanism. All the other mechanisms are a result of the ongoing inflammation. During the course of the disease, inflammation disappears in a part of the patients, resulting in different forms of rest damage.

DIAGNOSIS

There is currently no gold standard for the diagnosis and treatment of CRPS. History and physical examination are the cornerstones for appropriate diagnosis and management (1).

Various criteria exist for the diagnosis of CRPS (1, 2). Currently, the most commonly used criteria for the diagnosis of CRPS are the new International Association for the Study of Pain (IASP) clinical diagnostic criteria (1) (Table 1). These criteria are based on observed and patient-reported signs and symptoms (1).

CRPS has an extensive differential diagnosis, which can be summed up into the following categories: neuropathic pain-like syndromes, myofascial pain syndromes, inflammation, vascular diseases and psychological disorders (Table 2) (4). Most of these disorders have similar presentations, occasionally making the diagnosis of CRPS a challenge.

As the pathophysiology of CRPS is still not completely understood, there is limited use for additional clinical and laboratory tests in the diagnosis of CRPS (4). Diagnostic tests can, however, be used to exclude other disorders that could explain the observed signs

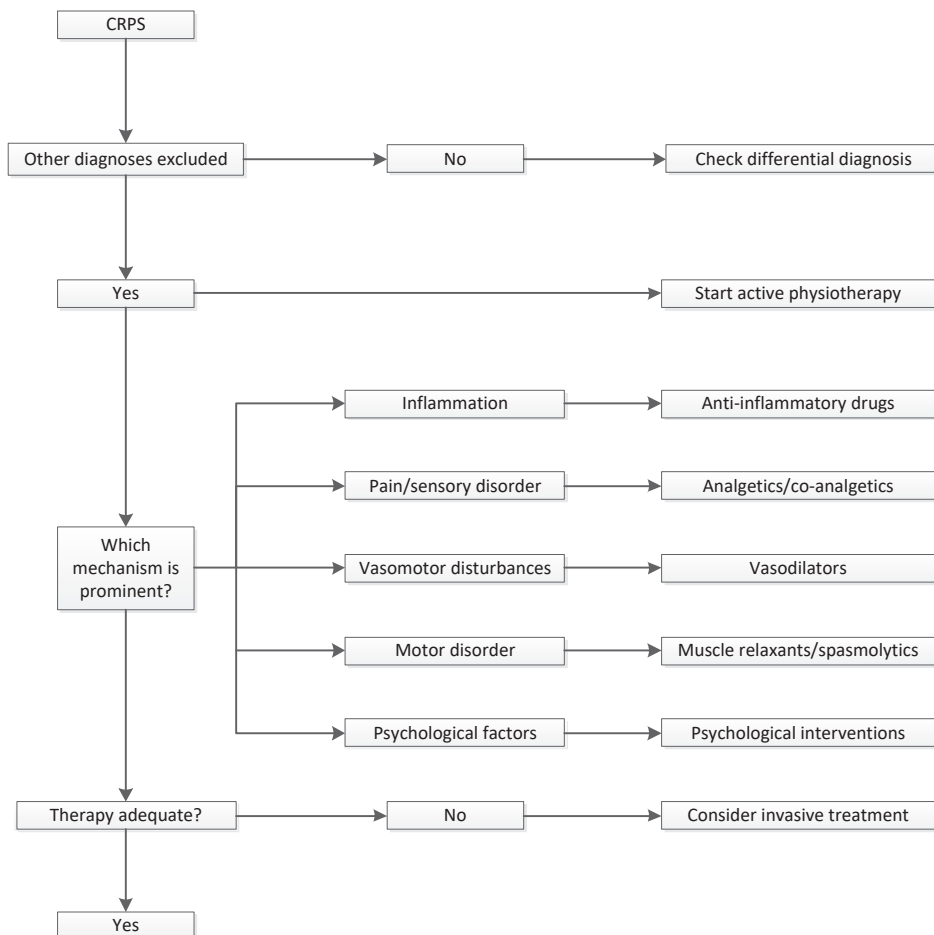


Figure 1 Treatment algorithm for CRPS.

and symptoms or to monitor the signs and symptoms of CRPS. An example of the latter is quantitative sensory testing, which is mostly used in research settings to quantify sensory disturbances found during physical examination.

PATHOPHYSIOLOGY OF CRPS

The exact pathophysiology of CRPS is still unknown (12). Both peripheral and central mechanisms are thought to play a role in the initiation and maintenance of CRPS (12).

Table 1 New International Association for the Study of Pain (IASP) clinical diagnostic criteria for CRPS (1)

1. Continuing pain, which is disproportionate to any inciting event.
2. Must report at least <i>one symptom</i> in <i>three of the four</i> of the following categories:
- <i>Sensory</i> : reports of hyperaesthesia and/or allodynia
- <i>Vasomotor</i> : reports of temperature asymmetry and/or skin color changes and/or skin color asymmetry
- <i>Sudomotor/oedema</i> : reports of oedema and/or sweating changes and/or sweating asymmetry
- <i>Motor/trophic</i> : 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 <i>one sign</i> at time of evaluation in <i>two or more</i> of the following categories:
- <i>Sensory</i> : evidence of hyperalgesia (to pinprick) and or allodynia (to light touch and/or deep somatic pressure and/or joint movement)
- <i>Vasomotor</i> : evidence of temperature asymmetry and/or skin color changes and/or asymmetry
- <i>Sudomotor/oedema</i> : evidence of oedema and/or sweating changes and/or sweating asymmetry
- <i>Motor/trophic</i> : 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.

Inflammation in CRPS

Various studies point towards CRPS being an exaggerated inflammatory response as a result of trauma or surgery (2, 13). This inflammatory response has long been a topic of debate, as general markers of inflammation such as C-reactive protein (CRP), white blood cell count, interleukin-6 (IL-6) and erythrocyte sedimentation rate (ESR) are usually not elevated in plasma of CRPS patients (2, 14). However, when considering the symptoms of (acute) CRPS, ‘classic signs of inflammation’ such as pain, redness, increase in temperature, swelling, and loss of function, are often displayed(8).

Recent studies focusing on inflammatory processes in CRPS have found higher levels of pro-inflammatory cytokines in blister fluid (IL-6, tumor necrosis factor- α (TNF- α)) of the affected extremity compared with the unaffected extremity. This suggests a role for local inflammatory processes in CRPS (13). Elevated levels of pro-inflammatory cytokines have further been found in serum, plasma, and cerebrospinal fluid of patients with CRPS (14-16).

Pro-inflammatory cytokines have been suggested to be involved in peripheral nociceptor activation and sensitization, which in turn could cause symptoms such as pain and hyperalgesia that are experienced in CRPS(17).

Neurogenic inflammation in CRPS

Apart from the ‘classic’ form of inflammation, studies have proposed neurogenic inflammation as an underlying mechanism for symptoms such as edema, vasodilation, and increased

sweating that are observed in CRPS (18). Studies have found increased levels of calcitonin-gene-related peptide (CGRP) and substance P (SP) in serum of patients with CRPS versus healthy controls (14, 18). These neuropeptides have been shown to lead to neurogenic dilatation of arterioles (CGRP) and plasma protein extravasation (SP)(19). This in turn could explain the redness and swelling that are observed in CRPS (18, 19).

Table 2 Differential diagnosis of complex regional pain syndrome. Taken from van Eijs et al., Evidence-based Interventional Pain Practice: According to Clinical Diagnosis. 16. Complex Regional Pain Syndrome(4). Published by John Wiley & Sons, Ltd. Copyright © 2012 John Wiley & Sons, Ltd.

Neuropathic pain syndromes
o Peripheral (poly) neuropathy
o Nerve entrapment
o Radiculopathy
o Post-herpetic neuralgia
o De-afferentation pain post-cerebrovascular accident
o Plexopathy
o Motor neuron disease
Vascular diseases
o Thrombosis
o Acrocyanosis
o Atherosclerosis
o Raynaud's phenomenon
o Erythromelalgia
Inflammation
o Erysipelas
o Inflammation-not otherwise specified
o Bursitis
o Seronegative arthritis
o Rheumatologic diseases
Myofascial pain syndromes
o Overuse
o Disuse
o Tennis elbow
o Repetitive strain injury
o Fibromyalgia
Psychiatric problems
o Somatoform pain disorders
o Munchhausen syndrome

CRPS as an autoimmune disease

CRPS has previously been described as an autoantibody-mediated autoimmune disease (20, 21). Passive transfer of CRPS patient serum-immunoglobulin G has been shown to induce behavioral changes in mice, and serum from CRPS patients has been shown to stain rodent sympathetic ganglia (20, 22). Furthermore, a small group of CRPS patients experienced pain relief after treatment with low-dose intravenous immunoglobulin (21). A study conducted by Dirckx et al. showed a significantly higher proportion of CRPS patients with positive anti-nuclear antibody test results as compared to a population of healthy blood bank donors (23). There are thus many findings supporting this theory of auto-immunity(20, 23).

However, to define a disease as an autoimmune disorder certain criteria (Witebsky's criteria) must be met (24). These criteria have not yet been fulfilled in the case of CRPS which gives rise to the question whether CRPS is more an auto-inflammatory than an autoimmune disease(23).

Deep-tissue microvascular ischemia-reperfusion injury in CRPS

Another hypothesis on the pathophysiology of CRPS is that of deep-tissue microvascular ischemia-reperfusion injury (25). This hypothesis, which was tested in a chronic post-ischaemia pain animal model, proposes a state of deep-tissue ischemia and inflammation caused by a microvascular ischaemia-reperfusion injury as the cause for abnormal pain sensations such as allodynia in CRPS (25, 26).

Genetics and CRPS

Genetics seem to play a role in the predisposition to CRPS. A Dutch cohort study showed the frequency of human leukocyte antigen (HLA)-DQ1 to be significantly higher in CRPS patients than in the controls (27). There is evidence that HLA-B62 and HLA-DQ8 are associated with CRPS with fixed dystonia (28). Another study showed HLA-DR13 to be associated with multifocal or generalized tonic dystonia of CRPS (29). These findings indicate that certain HLA loci may be involved in the susceptibility to certain phenotypes of CRPS (28, 29).

Cortical reorganization in CRPS

Central processes, such as cortical reorganization and changes in pain processing, may also play a role in CRPS (30-33). Cortical reorganization has been shown to take place in both the primary somatosensory cortex (S1) and the motor cortex (30, 32). Maihofner et al. showed changes in S1 to be correlated with the intensity of pain and mechanical hyperalgesia in CRPS (30). In a later study, this group showed reversal of cortical reorganization in S1 to be correlated with pain reduction in CRPS (31).

Cortical reorganization could thus explain some sensory features in CRPS. An example is the distribution of pain and hyperalgesia. The spread of these symptoms typically takes

on a more glove- or stocking-like distribution instead of being limited to the innervation territories of peripheral nerves (2, 31).

CRPS as a small-fibre neuropathy

CRPS has further been proposed to be a small-fibre neuropathy because of its similarity to generalized small-fibre-predominant polyneuropathies (34). Studies have found a decrease in epidermal nerve fibres and a decrease in sweat gland and vascular innervation in patients with CRPS (35). This could explain not only the (neuropathic) pain experienced in CRPS but also the trophic and vasomotor dysfunctions that are observed (34). The latter could be caused by antidromic release of neuropeptides, such as CGRP and SP, by these small fibres in response to trauma and inflammation (36). It is still not completely understood whether this small-fibre loss is a result of CRPS rather than a cause of this disease.

Psychological factors in CRPS

Physicians often consider CRPS patients to be psychologically different from other groups of patients. This is mostly due to the complexity and the poorly understood pathophysiology of this disease.

However, most studies show no association between the onset of CRPS and psychological factors such as depression, anxiety, paranoia and hostility/anger (37-39). There is some evidence for the influence of stressful life events before the onset of the disease (40). Although these factors may not play a role in the onset of CRPS, the probability still remains that these factors play a role in the maintenance of this disease (39, 41).

Taking the above into account, CRPS seems to be a multifactorial disease with a multi-mechanism pathophysiology requiring a multimodal workup and treatment.

TREATMENT

Effective treatment options in CRPS are limited and consist of non-invasive and invasive therapies.

Physical rehabilitation and physiotherapy have been shown to reduce pain and improve function in patients with CRPS. Physicians are therefore advised to start with active physical therapy in the treatment of CRPS (42).

Medication can be started in addition to physiotherapy. The choice of medication should be based on the mechanism deemed most prominent in a specific CRPS case (figure 1).

Anti-inflammatory drugs

In the Netherlands, free-radical scavengers (dimethyl sulphoxide or acetylcysteine) are advised for inflammatory symptoms (42). However, these drugs have not gained general international acceptance.

Immunomodulating medication reduces the manifestation of inflammation by influencing mediators of inflammation such as cytokines, neuropeptides, eicosanoids and amino acids. Standard use of immunomodulating medication in CRPS is still not common, although there is strong evidence for the use of bisphosphonates (43). For other immunomodulating medications, i.e. glucocorticoids, TNF- α antagonists, thalidomide and immunoglobulin, evidence is often conflicting and not sufficient to advise standard use (43).

Analgetics/co-analgetics

Although there is insufficient evidence available on the treatment of nociceptive pain in CRPS, it seems wise to treat nociceptive pain according to the World Health Organization analgesic ladder, bar strong opioids (42).

The little evidence available on the treatment of neuropathic pain in CRPS supports the use of co-analgetics in the management of this disease (42, 44, 45). Gabapentin has been shown to lead to a reduction in pain symptoms in CRPS and can be used in the treatment of neuropathic pain (44).

If intractable pain persists, treatment with low-dose i.v. ketamine in long-standing CRPS can be considered. However, which dose and the length of treatment is still unclear (42). Liver function should be monitored frequently during treatment with i.v. ketamine. If liver enzymes increase, i.v. ketamine should be stopped immediately.

Vasodilators

If vasomotor disturbance, leading to 'cold' CRPS, is the most prominent mechanism, a short-term treatment with a calcium channel blocker, an alpha-sympathetic blocker (46) or phosphodiesterase-5 inhibitor (47) can be considered. The medication should be stopped if no effect is achieved.

Muscle relaxants/spasmolytics

With regard to the use of muscle relaxants in CRPS, research has mainly been focused on the intrathecal use of these drugs.

Intrathecal baclofen is likely to have a positive effect on dystonia in CRPS patients (48). However, given the side effects associated with intrathecal baclofen and the invasiveness of this treatment, it seems justified to try oral muscle relaxants first.

Psychological intervention

When there are indications for psychological problems, signs of chronic pain behavior, or inability to cope with the disease, referral to a multidisciplinary team including a psychologist should be considered.

Invasive treatments

Invasive treatments can be considered if the aforementioned therapies are insufficient, despite adequate treatment of the underlying pathophysiological mechanism.

‘Evidence-based Guidelines Development (EBGD) Guidelines on Complex Regional Pain Syndrome type I’ (updated in 2014) give a negative recommendation on the use of sympathetic blocks, such as stellate ganglion blocks, thoracic sympathetic nerve blocks and lumbar sympathetic nerve blocks, in the treatment of CRPS (42).

Spinal cord stimulation (SCS) may be considered if patients do not respond to pharmacological treatments or rehabilitation therapies (4). The effect of this treatment on (neuropathic) pain and health-related quality of life in CRPS has been demonstrated in a randomized controlled trial (49). SCS is currently the only therapy with a multi-mechanism mechanism of action in CRPS. It has been shown to have a positive effect on both the somatosensory system and vasomotor disturbances (50).

PREVENTION

As treatment options for CRPS are limited, prevention of the disease would be the best medicine. Studies have shown supplementation with vitamin C (>500 mg day⁻¹), initiated immediately after injury or surgery and continued for 45-50 days, helped to reduce the risk of developing CRPS (51-53).

PROGNOSIS

The prognosis and outcome of CRPS is still difficult to predict. Resolution rates range between 74% in the 1st year to 36% after 6 years (5, 54).

The social impact of CRPS is significant (9, 54). Return-to-work rates vary, with one CRPS population study describing a permanent inability-to-work rate of 31% and a partial inability-to-work rate (i.e. work adaptations) of 28% in patients (54).

FUTURE PERSPECTIVES

The current treatment of CRPS is based on the observed and reported signs and symptoms.

The present thinking is that these signs and symptoms reflect the underlying pathophysiological mechanism leading to the different CRPS phenotypes (11). Consequently, it can be derived that patients with a warm, edematous extremity suffer from inflammation, while in patients with a cold, atrophic extremity the role of inflammation diminishes and vasomotor disturbance becomes the predominant process(8).

However, it has recently been shown that (a subgroup of) cold CRPS patients can still suffer from inflammation (55). Therefore, the question arises whether the current diagnostic methods are sufficient. Perhaps the presence or absence of inflammation might be a better distinction for choosing the appropriate therapy.

It is now possible to determine if there is an ongoing inflammation in CRPS-affected extremities by determining the levels of pro-inflammatory cytokines in fluid from artificially induced skin blisters (13). However, this is a time-consuming procedure that limits its use to the field of research and is therefore not easily available for use in daily clinical practice.

It is likely that multiple mechanisms simultaneously can play a role in the pathophysiology of CRPS in an individual patient. As research continues to reveal more about the mechanisms involved in CRPS, future treatment will presumably shift from a symptomatic approach to a more mechanism-based treatment approach.

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Chapter 3

Highlighting the Role of Biomarkers of Inflammation in the Diagnosis and Management of Complex Regional Pain Syndrome

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ABSTRACT

Complex Regional Pain Syndrome (CRPS) is characterized by continuous pain that is often accompanied by sensory, motor, vasomotor, sudomotor and trophic disturbances. If left untreated, it can have a significant impact on the quality of life of patients.

The diagnosis of CRPS is currently based on a set of relatively subjective clinical criteria: the New International Association for the Study of Pain clinical diagnostic criteria for CRPS. There are still no objective laboratory tests to diagnose CRPS and there is a great need for simple, objective and easily measurable biomarkers in the diagnosis and management of this disease.

In this review, we discuss the role of inflammation in the multi-mechanism pathophysiology of CRPS and highlight the application of potential biomarkers of inflammation in the diagnosis and management of this disease.

KEY POINTS

Neurogenic inflammation, neuroinflammation and immune dysregulation contribute to inflammation in complex regional pain syndrome (CRPS).

Biomarkers reflecting these inflammatory mechanisms could aid in both the diagnosis and management of CRPS

Further research is needed to validate these biomarkers of inflammation in CRPS

INTRODUCTION

Complex Regional Pain Syndrome (CRPS) is a painful disease of the extremities that is usually initiated by tissue damage, e.g., following fracture or surgery (1, 2). It is characterized by continuous pain that is disproportionate to the inciting event, and which can be accompanied by sensory, motor, vasomotor, sudomotor and trophic disturbances (3). The incidence of CRPS has been reported to vary between 5.5 and 26.2 per 100.000 person-years and women are reported to be affected more often than men (1, 2).

Currently, the disease is diagnosed using a set of clinical criteria: the new International Association for the Study of Pain (IASP) clinical diagnostic criteria for CRPS (3). There is still no objective test available for diagnosis and/or management of this disease. Additional testing, such as blood tests and radiography, are only used to exclude other diseases, such as rheumatic diseases, in the differential diagnosis (4). Once CRPS is diagnosed, treatment is preferably conducted by a multidisciplinary team consisting of pain physicians, physiatrists, physiotherapists and psychologists. Because CRPS is considered to have a multi-mechanism pathophysiology, it is advised that the treatment be conducted in a mechanism-based manner: it should target the underlying pathophysiological mechanisms of disease in each unique CRPS case (5, 6).

If left untreated, CRPS can lead to a debilitating loss of function of the affected extremity and can have a significant social impact on the life of patients (7). It is therefore important that this disease is diagnosed early and treated with appropriate mechanism-based therapies. However, early diagnosis and therapy selection are often hampered due to the aforementioned lack of objective tests. Currently, physicians have to rely on subjective symptoms reported by patients and relatively subjective signs observed during physical examination for diagnosis and management of CRPS. This subjectivity of symptoms and signs, which is often accompanied by a discrepancy between the symptoms and signs, leads to various diagnostic and therapeutic challenges for clinicians, such as delayed diagnosis and inappropriate selection of therapies. To make these matters more complicated, CRPS is a disease with a heterogeneous clinical presentation and there may be various disease subtypes with their own specific phenotype (8-10). These matters therefore not only complicate diagnosis of this disease but also the selection of therapies based on the underlying pathophysiological mechanisms as, at present, these underlying mechanisms are also deduced from the relatively subjective, and often discrepant, symptoms and signs.

These diagnostic and therapeutic challenges highlight the need for simple, objective, and easily measurable biomarkers in the diagnosis and management of CRPS. In this review, we aim to highlight the application of potential biomarkers, specifically biomarkers of inflammation, in the diagnosis and management of CRPS. For reasons of clarity, we have mostly limited ourselves to biomarkers that can be measured in blood and skin.

PATHOPHYSIOLOGY OF COMPLEX REGIONAL PAIN SYNDROME (CRPS)

It has been generally accepted that multiple pathophysiological mechanisms contribute to CRPS. The following mechanisms have been implicated in the onset and maintenance of CRPS: inflammation, peripheral and central sensitization, altered sympathetic nervous system function, changes in circulating catecholamine levels, endothelial dysfunction, cortical reorganization, and immune-acquired, genetic and psychological factors (11, 12). However, it is as yet unclear how and to what extent each of these mechanisms cause and maintain this disease.

In this article, we focus on the role of biomarkers of inflammation in the diagnosis and management of CRPS. We summarize the current knowledge on inflammation in CRPS as well as the related symptoms and signs. For further information on the role of other mechanisms in CRPS, we refer the reader to more extensive reviews (11, 13-15).

In CRPS, neurogenic inflammation, neuroinflammation and dysregulation of the immune system have all been implicated as a source of inflammation. Peripheral neurogenic inflammation has long been implicated in the pathophysiology of CRPS (16). In peripheral neurogenic inflammation, primary afferent sensory neurons release neuropeptides that cause cutaneous vasodilation (mainly through calcitonin gene-related peptide [CGRP]), changes in vascular permeability (mainly through substance P [SP]), increased protein extravasation, and increased leukocyte recruitment (17, 18). Weber et al. conducted a study using transcutaneous electrical stimulation via intradermal microdialysis capillaries and found significantly increased axon reflex vasodilation in the affected extremity of CRPS patients compared with healthy controls. This study further found increased protein extravasation in the affected extremity of these CRPS patients (19). These findings suggest an increased release of neuropeptides such as CGRP and SP by activated sensory neurons in CRPS patients and thus point towards facilitated neurogenic inflammation in CRPS (19). This increased neuropeptide release may also account for symptoms such as allodynia, hyperalgesia, edema, vasodilation, and trophic abnormalities that are seen in CRPS patients (19, 20).

Besides neurogenic inflammation, recent studies have provided evidence supporting a role for neuroinflammation in CRPS (21, 22). Neuroinflammation refers to inflammation occurring within the nervous system (central nervous system and/or peripheral nervous system) that is characterized by glial cell activation leading to an increased production of pro-inflammatory cytokines and chemokines (23). Neuroinflammation can be initiated by various forms of trauma and surgery, and it has also been suggested that it can be caused by increased neuronal activity of primary afferent nerve fibers and/or higher-order neurons (23, 24). This latter phenomenon has been coined 'neurogenic neuroinflammation' and has also been implicated in CRPS (20, 24). Neuroinflammation can lead to various adverse effects, such as a transition from acute to chronic pain and maintenance of chronic pain (23). This

chronic pain is established via central sensitization, which is induced and maintained by central cytokines, chemokines, and glia-produced mediators (23). Central sensitization is characterized by pain hypersensitivity and manifests clinically as dynamic tactile allodynia, secondary punctuate and/or pressure hyperalgesia, temporal summation and sensory after sensations (25). Symptoms of central sensitization have also been described in CRPS (25-28) and have been attributed to a sensitization of the nociceptive system due to ongoing pain and, therefore, to continuous nociceptive input (11, 29). As neuroinflammation seems to drive central sensitization, and studies now suggest neuroinflammation may play a role in CRPS, it is possible that part of the symptoms of CRPS which are attributed to central sensitization can be caused not only by continuous nociceptive input, but also by neuroinflammation (21-23). Neuroinflammatory findings in CRPS are new and need to be studied in further detail, yet neuroinflammation represents an interesting therapeutic target in patients with symptoms and signs of central sensitization.

Although peripheral neurogenic inflammation has long been implicated in CRPS, it is only in recent years that evidence to support involvement of the immune system in CRPS has grown. Until recently, the involvement of the immune system in CRPS was a topic of intense debate: though classic signs of inflammation such as calor, dolor, rubor, tumor and functio laesa were often seen in CRPS patients, classic systemic markers of inflammation such as C-reactive protein and white blood cells were mostly within normal range in patients (30-32). Because of a lack of objective evidence for immune system involvement, a dysregulation of the immune system was disregarded for years as a possible pathophysiological mechanism in CRPS. In recent years, however, due to a better understanding of the disease and improved research techniques, it has been possible to identify a role of the immune system in CRPS. Several lines of evidence now support a role for dysregulated immune activation and subsequent inflammation in CRPS: [1] increased levels of the pro-inflammatory cytokines tumor necrosis factor (TNF)- α and interleukin (IL)-6 have been found in blister fluid of CRPS-affected extremities compared with clinically uninvolved contralateral extremities (33); [2] a higher prevalence of various autoantibodies has been identified in CRPS patients (34-37); and [3] indications of increased T lymphocyte activity have been found in CRPS patients (38).

These different sources of inflammation have provided us with a few promising biomarkers of inflammation in CRPS. Before we discuss these potential biomarkers of inflammation, however, we discuss the role of subtypes and phenotypical characterization in the diagnosis and management of CRPS.

CRPS: SUBTYPES AND PHENOTYPES

CRPS has been known a multitude of names in the past due to its varied presentation and ideas on its etiology. In 1993, a consensus meeting was held by the IASP Task Force on Taxonomy to develop a more general and neutral term for the symptoms and signs that make up this condition (39, 40). During this meeting, two types of CRPS were recognized: CRPS type I, previously known as reflex sympathetic dystrophy (RSD), in which there is no demonstrable nerve lesion; and CRPS type II, previously known as causalgia, in which there is demonstrable nerve lesion during physical examination and/or on electrodiagnostic testing (39-42). However, these subtypes do not differ in clinical symptoms and signs nor in their response to therapy (11). For the most part, the pathophysiological mechanisms also seem to be shared by both these subtypes (11). Therefore, in our practice, this subtype distinction is no longer used when diagnosing CRPS, and we will use the term CRPS to encompass both these subtypes throughout this article.

Bruehl et al. have conducted two cluster analysis studies in which they found that CRPS patients can be clustered into different subtypes based on symptoms and signs (8, 10). The first study was conducted in 2002; the authors performed a K-means cluster analysis on a group of 113 CRPS patients and were able to cluster patients into three distinct subgroups (8). Based on their findings, the authors proposed the following three subtypes: “1) a relatively limited syndrome with vasomotor signs predominating, 2) a relatively limited syndrome with neuropathic pain/sensory abnormalities predominating, and 3) a florid CRPS syndrome similar to descriptions of Classic RSD” as described by Gibbons and Wilson in 1992 (8, 43). The second study was conducted in 2016; in this study, a two-step cluster analysis in a group of 152 CRPS patients provided evidence for a warm and cold CRPS subtype (10). The warm CRPS subtype associated with a more inflammatory phenotype with a warm, erythematous, swollen and sweaty extremity. By contrast, the phenotype of the affected extremity in the case of cold CRPS was characterized by a colder temperature, blue or pale skin, and also edema, although this latter characteristic was less common than in the warm cluster. The authors further showed that differences between these CRPS subtypes was based on multiple symptoms and signs that showed a consistent covariation across patients indicating the possibility of common underlying pathophysiological mechanisms which could be targeted by specific therapies (10). For example, the symptoms and signs (i.e., phenotype) typically associated with the warm CRPS subtype may reflect an underlying inflammatory mechanism that could be targeted with anti-inflammatory therapies.

The hypothesis that phenotypical characterization in CRPS can be used to assess the underlying pathophysiological mechanisms of disease, and consequently to select targeted therapies, was presented in an article by Birklein and Schlereth in 2015 (9). The authors described two phenotypes of CRPS: the “peripheral inflammatory phenotype” reflecting clinical symptoms and signs that are generated by inflammation, and the “central neuroplasticity

phenotype” reflecting clinical symptoms and signs generated by the central nervous system (e.g. mechanical allodynia) (9). The problem with phenotypical characterization is, however, that clinicians are still dependent on relatively subjective symptoms and signs to characterize the subtype of CRPS that a patient may have. Another issue to take into consideration is the considerable amount of clinical overlap between the various phenotypes and subtypes, which could create confusion when determining underlying mechanisms of disease (10). In addition, phenotypical characterization can also misguide clinicians to a certain degree. For example, a clinician may disregard inflammation as an underlying pathophysiological mechanism in a patient with cold-type CRPS; however, we now know from a study in blister fluid that a subgroup of cold CRPS patients also suffers from inflammation (44). These issues highlight the need for simple, objective, and easily measurable biomarkers in the diagnosis and management of CRPS.

In the following sections we discuss potential biomarkers of inflammation in CRPS. We start by giving a general definition of a biomarker and the criteria that a biomarker for CRPS would need to meet. We then discuss potential biomarkers of inflammation that have been identified in CRPS and their possible role in the diagnosis and/or management of this disease. Lastly, we will provide suggestions for future research.

DEFINITION OF BIOMARKER AND CRITERIA FOR CRPS

A biomarker is defined as: “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (45). Not only can a biomarker be used in regard to the response to a therapeutic intervention, it can also be used in the diagnosis, prognosis, and monitoring of diseases (45). In the case of CRPS, biomarkers could aid in various aspects of diagnosis and management: first, they could be used to aid the (early) diagnosis of this disease; second, they could be used, together with phenotypical characterization, to identify the underlying mechanisms of disease for selection of therapies; and third, they could be used to monitor disease activity and/or effects of therapy. We consider a biomarker that can be used in the first two situations as applicable in the diagnosis of CRPS, while a biomarker that can be used in the third situation is applicable in the management of this disease.

Most CRPS patients are treated by pain physicians in an outpatient clinic setting with limited access to extensive laboratory testing. Taking this into consideration, an ideal biomarker for CRPS would need to meet the following criteria: 1) the tissue or fluid that is required to determine the biomarker needs to be obtained in a simple manner in routine practice; 2) the measurement of the biomarker needs to be simple and reproducible using routine laboratory testing; and 3) the procedure to obtain the biomarker needs to be at a

minimal risk to the patient, for example, a low-risk venipuncture versus a medium-risk skin biopsy.

In the following sections we will highlight a few potential biomarkers of inflammation in CRPS. In the future, these biomarkers could, for example, be used in patients with the “warm subtype” as described by Bruehl et al. or the “peripheral inflammatory subtype” as described by Birklein and Schlereth, to objectively identify inflammation and to start anti-inflammatory therapies (9, 10).

For the purpose of simplicity, we have chosen to divide the biomarkers into two groups: ‘potential biomarkers of neurogenic inflammation and neuroinflammation’ and ‘potential biomarkers of immune dysregulation’ (Tables 1 and Table 2). We have further divided these biomarkers into local and systemic biomarkers: local biomarkers are markers that are typically measured in the affected tissues while systemic biomarkers are measured in blood. Both local and systemic biomarkers will be further subdivided into soluble and cellular markers where possible.

We acknowledge that there is a complex interplay between these inflammatory mechanisms and that some, if not most, biomarkers can be applied to identify all mechanisms.

Table 1 Potential biomarkers of neurogenic inflammation and neuroinflammation in complex regional pain syndrome

Biomarkers	Cellular or Soluble	Acquisition	Type of inflammation	Use in diagnosis of CRPS ^a	Use in management of CRPS ^b
<i>Local markers</i>					
Central microglial activation	Not applicable	[¹¹ C]-(R)-PK11195-PET	Neuroinflammation	?	?
<i>Systemic markers</i>					
CGRP	Soluble	Venous blood	Neurogenic	+	?
SP	Soluble	Venous blood	Neurogenic	?	?

CGRP calcitonin gene-related peptide, *CRPS* complex regional pain syndrome, *PET* positron emission tomography, *SP* substance P, + indicates a possible role as a biomarker in the diagnosis and/or management of CRPS, ? indicates that insufficient information is currently available to determine a possible role as a biomarker in the diagnosis and/or management of CRPS.

^aThe biomarker aids the (early) diagnosis of this disease and/or it could be used, together with phenotypical characterization, to identify the underlying mechanisms of disease for selection of therapies.

^bThe biomarker could be used to monitor disease activity and/or effects of therapy.

Table 2 Potential biomarkers of immune dysregulation in complex regional pain syndrome

Biomarkers	Cellular or Soluble	Acquisition	Use in diagnosis of CRPS ^a	Use in management of CRPS ^b
<i>Local markers</i>				
TNF-α	Soluble	Blister fluid	+	+
IL-6	Soluble	Blister fluid	+	+
Tryptase	Soluble	Blister fluid	+	+
Mast cell numbers	Cellular	Skin biopsies	+	+
<i>Systemic markers</i>				
CD14⁺CD16⁺ monocytes	Cellular	Venous blood	?	?
CD8⁺T lymphocytes	Cellular	Venous blood	?	?
sIL-2R	Soluble	Venous blood	+	?
Autoantibodies^c	Soluble	Venous blood	?	?
miRNA	Soluble	Venous blood	+	+

CRPS complex regional pain syndrome, IL-6 interleukin-6, miRNA microRNA, sIL-2R soluble interleukin-2 receptor, TNF- α tumor necrosis factor- α , + indicates a possible role as a biomarker in the diagnosis and/or management of CRPS, ? indicates that insufficient information is currently available to determine a possible role as a biomarker in the diagnosis and/or management of CRPS

^aThe biomarker aids the (early) diagnosis of this disease and/or it could be used, together with phenotypical characterization, to identify the underlying mechanisms of disease for selection of therapies.

^bThe biomarker could be used to monitor disease activity and/or effects of therapy.

^cAutoantibodies studied in CRPS: autoantibodies against autonomic nervous system structures(34), autoantibodies against autonomic nervous system autoantigens (e.g. β_2 -adrenergic receptor and/or muscarinic-2 receptor) (35, 36) and anti-nuclear antibodies (37).

POTENTIAL BIOMARKERS OF NEUROGENIC INFLAMMATION AND NEUROINFLAMMATION

Local biomarkers: Although this article mainly discusses biochemical markers of inflammation that can be measured in clinical laboratories, we would like to highlight the findings by Jeon et al. and Jung et al. regarding neuroinflammation (21, 22). Jeon et al. conducted a study in which they used [¹¹C]-(R)-PK11195 positron emission tomography (PET) to observe microglial activation in CRPS patients and to identify whether there was an association with symptom severity (21). The authors found a significantly higher distribution volume ratio (DVR) of [¹¹C]-(R)-PK11195 in the caudate nucleus, putamen, nucleus accumbens and thalamus of CRPS patients than in the healthy controls. They further found a statistically significant, positive correlation between [¹¹C]-(R)-PK11195 DVR and pain severity (21).

These findings point towards microglial activation and neuroinflammation in CRPS with a possible association between degree of neuroinflammation and pain severity.

Jung et al. conducted an explorative study in which they studied the correlation between peripheral metabolites in blood and urine, central neurometabolites using proton magnetic resonance spectroscopy (^1H -MRS) and neuroinflammation using [^{11}C]-(R)-PK11195 PET. The authors found statistically significant positive correlations between the levels of lipid 13a and lipid 09 relative to total creatine and neuroinflammation in certain brain regions (22). They further found that peripheral pH, glucose, CO_2 , basophil and creatinine levels were associated with an increase or decrease of the level of neuroinflammation in the brain of CRPS patients (22). The authors suggest that characterization of peripheral and central metabolites may help to understand the role of neuroinflammation in the pathophysiology of CRPS. They further suggest that central lipid levels may be used as a biomarker for neuroinflammation.

The results from these two studies pave the way for a new field of research on the role of neuroinflammation in the pathophysiology of CRPS. These results, however, need to be further analyzed in a clinical context and we cannot yet determine the role of these techniques and markers in the diagnosis and management of CRPS.

Systemic biomarkers: four studies measured venous blood levels of the soluble neuropeptide CGRP in CRPS patients (32, 46-48). Blair et al. conducted a study in which they measured CGRP levels in the blood of CRPS patients and healthy controls: blood CGRP levels were higher in the patients than in the controls (46). Birklein et al. conducted a study in which they measured serum CGRP levels in CRPS patients and age- and gender-matched healthy controls. The authors had some interesting findings: first, serum CGRP levels were significantly higher in CRPS patients than in controls; second, serum CGRP levels did not differ between the affected and contralateral limb; third, serum CGRP normalized in the group of patients who agreed to a follow-up visit at 9 months after the initial assessment, this normalization was accompanied by a clinical improvement of local inflammatory symptoms, but not a reduction in pain; and fourth, higher serum CGRP levels correlated significantly with the incidence of nerve lesions and hyperhidrosis, but not with pain, CRPS duration, or other clinical symptoms (47). Schinkel et al. conducted two studies in which they assessed blood CGRP levels in CRPS patients. In the first study, no difference was found in blood CGRP levels between patients with mostly acute CRPS and healthy age and gender matched controls (32). In the second study, they found significantly lower levels of CGRP in the blood of patients with chronic CRPS than healthy controls, although the authors state that these differences were marginal (48). Though the results from these studies contradict each other, there may possibly be a role for serum CGRP in the diagnosis of an underlying neurogenic mechanism in CRPS, especially in patients with sudomotor (sweating) symptoms. Based on these findings, we cannot conclude whether this marker can be used in the management of this disease. Although studies seem to indicate a role for CGRP in CRPS (19, 46, 47),

CGRP antagonists have, to our knowledge, not yet been tested in CRPS patients. However, it is not unthinkable that serum CGRP levels could be used in the future to select CRPS patients who would benefit from this therapy, if these therapies are ever proven effective in this disease.

The two studies by Schinkel et al. also examined SP levels in the venous blood of CRPS patients (32, 48). In the first study, the authors found significantly higher SP levels in the blood of patients with mostly acute CRPS than in the blood of healthy controls (32). In the second study, the authors found higher blood SP levels in CRPS patients than in healthy controls, although this difference was not statistically significant. There was, however, a significant difference between acute and chronic CRPS with acute CRPS patients having significantly lower levels of SP than chronic CRPS patients (48). By contrast, the study by Blair et al. showed no difference in blood SP levels between CRPS patients and healthy controls (46). To our knowledge, these studies did not look at the correlation between SP and clinical symptoms (32, 46, 48). Based on the findings from these studies, we cannot determine the place of SP in the diagnosis and/or management of CRPS, however, we can conclude that there may be a role for SP in the pathophysiology of CRPS.

POTENTIAL BIOMARKERS OF IMMUNE DYSREGULATION IN CRPS

Local biomarkers: One of the first studies that proved inflammation plays a significant role in CRPS also gave us some of the first soluble local inflammatory biomarkers for this disease (33). In 2002, Huygen et al. conducted a study in which they assessed whether changes in levels of inflammatory mediators could be found in limbs of CRPS patients. The authors induced artificial skin blisters in the CRPS-affected and contralateral limb and subsequently extracted the fluid from these blisters. They found significantly elevated levels of the pro-inflammatory cytokines TNF- α and IL-6 in the affected limbs (33). Based on these findings, their group decided to treat two patients with the TNF- α inhibitor infliximab. This treatment resulted in considerable clinical improvement in both patients and was associated with a substantial decline of TNF- α and IL-6 levels in blister fluid (49). These results illustrate that blister-fluid TNF- α and IL-6 levels can be considered as local biomarkers with two potential applications: firstly, for determining the inflammatory component in CRPS and thus determining the eligibility of a patient for treatment with a TNF- α inhibitor such as infliximab, and secondly, for monitoring treatment response to this TNF- α inhibitor. However, it should be noted that randomized controlled trials are still required to assess the therapeutic effects of TNF- α inhibitors in CRPS (50).

Another potential local biomarker of inflammation in CRPS is the mast cell and its specific mediators. Activated mast cells release various mast cell-specific products, including

tryptase (51, 52). Significantly higher levels of tryptase have been found in blister fluid of the affected limb of CRPS patients than in the contralateral limb (53). In addition, blister fluid tryptase levels were found to have a significant positive correlation with patient pain scores (53). In further support of a role for mast cells in CRPS are the findings by Birklein et al. describing higher mast cell numbers in skin biopsies of the affected limb than in skin biopsies of the contralateral limb (54). Interestingly, mast cell numbers seem to be increased only in the early stages of CRPS and not in long-standing disease (54, 55). Consequently, determining local mast cells could represent a diagnostic biomarker of inflammation associated with early-stage CRPS. If elevated mast cell numbers or specific mast cell products are found, mast cell-directed therapy could be considered (56, 57) and determining local mast cell accumulation or their products (e.g. tryptase) could be used to monitor treatment effect.

Systemic biomarkers: Circulating monocytes can be subdivided into three phenotypically distinct subpopulations based on the expression of CD14 and CD16, i.e., CD14⁺CD16⁻ monocytes, CD14⁺CD16⁺ monocytes and CD14^{+/−}CD16⁺⁺ monocytes (58, 59). The monocyte subset composition is altered in peripheral blood in the case of inflammatory diseases, with mostly an increased fraction of the pro-inflammatory CD14⁺CD16⁺ monocytes (also designated as intermediate monocytes) (59, 60). Moreover, in sarcoidosis, for instance, the relative abundance of circulating CD14⁺CD16⁺ monocytes has been found to correlate positively with serum angiotensin-converting enzyme (ACE) levels of patients, suggesting that it represents a marker for disease activity (61). In CRPS patients, an elevated fraction of pro-inflammatory CD14⁺CD16⁺ monocytes in venous blood has also been observed in comparison with healthy controls (62). CD14⁺CD16⁺ monocytes are poor producers of the anti-inflammatory cytokine IL-10 as compared to the classical CD14⁺CD16⁻ monocytes, whereas both monocyte subpopulations produce similar amounts of pro-inflammatory cytokines (62, 63). These findings suggest that the increase in CD14⁺CD16⁺ monocytes in CRPS may contribute to and reflect a pro-inflammatory status (62). Moreover, the study by Ritz et al. observed a positive correlation between the relative abundance of the CD14⁺CD16⁺ monocyte subset and the clinical sign of cold allodynia which can indicate a role of these monocytes in the development of central sensitization in CRPS patients (62). When interpreted with caution, there might be a diagnostic role for monocyte subset determination in the assessment of the inflammatory status and central sensitization in CRPS patients. However, further research into this topic is clearly needed as it is not clear whether these pro-inflammatory monocytes have a pathogenic quality or are merely a result of an already ongoing inflammatory process in CRPS. Additionally, monocytes that infiltrate tissues differentiate further into macrophages of which different subtypes exist; future research on the relative tissue distribution of these pro-inflammatory M1 and anti-inflammatory M2 macrophages in CRPS could thus also be of interest (64, 65).

Circulating T lymphocyte subsets represent another example of potential cellular biomarkers in CRPS. To our knowledge, three studies have determined T lymphocyte subsets

in venous blood of CRPS patients (31, 62, 66). These studies, however, show conflicting results when it comes to alterations in circulating T lymphocyte subsets. For example, while Kaufmann et al. found significantly lower absolute numbers of cytotoxic CD8⁺ T lymphocytes in CRPS patients than in healthy controls, Ribbers et al. observed no difference in the absolute numbers of this subset, and Ritz et al. found no difference in the percentage of this subset between CRPS patients and healthy controls (31, 62, 66). Interestingly, Kaufmann et al. did not find any correlation between CD8⁺ T lymphocytes and pain, as measured on a Visual Analog Scale ranging from 0-10 in CRPS patients(66). Based on these conflicting results, we cannot draw a conclusion on whether T lymphocyte subset measurement in peripheral blood can currently be used as a biomarker for diagnosis and/or management of CRPS. Yet the existence of many different T lymphocyte subsets, including different types of T helper subsets, clearly warrants further study into the relation between T lymphocytes and CRPS(67, 68).

Not only can T lymphocyte involvement be assessed at a cellular level, but it can also be assessed using a soluble marker for T lymphocyte activity(69). The soluble interleukin-2 receptor (sIL-2R), also termed CD25, is a truncated protein that is released from activated T cells: hence, it is a surrogate marker for T cell activation (69, 70). Peripheral blood levels of the sIL-2R thus reflect the level of T cell activation in an individual and elevated blood levels of sIL-2R correlate with disease activity in for instance rheumatoid arthritis, systemic lupus erythematosus, and sarcoidosis, diseases in which enhanced T cell activity is centrally involved (71-75). Our group measured sIL-2R serum levels in CRPS patients and compared this to the serum sIL-2R levels of healthy controls (38). We found significantly higher levels of the sIL-2R in CRPS patients than in healthy controls (38). Serum sIL-2R further seems to be a good discriminator between patients with CRPS and healthy controls, showing a high sensitivity (90%) and specificity (89.5%) (38). These results seem promising: firstly, they may be indicative of a role for pathogenic T lymphocyte activation in CRPS, and secondly, they could lead to the use of the sIL-2R as a biomarker in the diagnosis and/or management of CRPS. However, the place of this marker in the diagnosis and/or management of this disease is yet to be determined and we are currently validating these findings in further studies.

Autoantibodies represent another example of soluble biomarkers. Autoantibodies are commonly used as biomarkers for diagnosis and monitoring of inflammatory diseases and autoimmune diseases (76). Multiple studies have described various autoantibodies in CRPS (34-37). One study identified autoantibodies against autonomic nervous system structures in a small subset of CRPS patients: five out of twelve CRPS patients were positive for these autoantibodies (34). Another study found that ~30-40% of their CRPS patient group had autoantibodies against an autonomic nervous system autoantigen(35). In a follow-up study, this group aimed to identify the antigens targeted by these autoantibodies and found agonistic autoantibodies against the β_2 -adrenergic receptor and/or the muscarinic-2 receptor in a

subset of their CRPS patients (36). A third study found a higher prevalence of anti-nuclear antibody (ANA) positivity in CRPS patients than in healthy controls (33% vs 4%, $P<0.001$) (37). Although these data support involvement of an autoimmune component in CRPS, it is still unclear whether these autoantibodies are pathogenic or whether they are a result of this disease. Consequently, their role as biomarkers in the diagnosis and/or management of CRPS needs further evaluation, especially in comparison with other diseases that are included in the initial differential diagnosis.

Finally, we would like to highlight the potential role of systemic micro-ribonucleic acids (miRNAs) in the diagnosis and management of CRPS (77). miRNAs are small non-coding RNA molecules that suppress protein synthesis through messenger RNA (mRNA) silencing (78, 79). Orlova et al. have suggested the potential application of blood miRNA profiling in the selection of treatments for CRPS patients and in the stratification of CRPS patients in, for example, clinical trials (80). They compared blood miRNA profiles of CRPS patients with those of healthy controls and found a significant differential expression of 18 miRNAs in the CRPS group (80). They were further able to stratify the study population into three clusters, with one cluster containing 60% of the CRPS patients and no healthy controls. Further analysis of this CRPS-only cluster revealed significant alterations in additional miRNAs and inflammatory markers compared to the rest of the CRPS patients and healthy controls (80). Though the clinical relevance is still not clear, these findings suggest that differentially expressed miRNAs could help to identify different CRPS subtypes and could further lead to the identification of additional inflammatory markers that are specific to these subtypes (77, 80). In addition, miRNA profiling could also be used as a prognostic biomarker to identify responders to specific therapies (81). Thus, the application of miRNA profiling could be useful in the diagnosis as well as the management of CRPS; however, research on this subject is still in its initial phase and more research is needed to validate current results.

BIOMARKERS IN CRPS: LIMITATIONS, CONSIDERATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Although the findings mentioned above are promising, there are still no biomarkers that have been implemented in the routine clinical practice surrounding CRPS patients. It is clear that we still have a long path ahead when it comes to the identification, validation and application of biomarkers in the diagnosis and management of CRPS. As this disease is currently diagnosed using a set of relatively subjective clinical criteria, an objective biomarker would be welcomed with open arms by pain physicians. There are, however, matters to be considered when choosing a biomarker (Table 3).

Table 3 An overview of the discussed biochemical biomarkers of inflammation in complex regional pain syndrome together with an overview of considerations for routine practice.

Biomarker	Acquisition	Simple to obtain in routine practice	Easy to measure	Minimal risk to patient
CGRP	Venous blood	+	+	+
SP	Venous blood	+	+	+
TNF-α	Blister fluid	-	+	-
IL-6	Blister fluid	-	+	-
Tryptase	Blister fluid	-	+	-
Mast cell numbers	Skin biopsies	-	-	-
CD14⁺CD16⁺ monocytes	Venous blood	+	+	+
CD8⁺ T lymphocytes	Venous blood	+	+	+
sIL-2R	Venous blood	+	+	+
Autoantibodies	Venous blood	+	+/-	+
miRNA	Venous blood	+	-	+

CGRP calcitonin gene-related peptide, *IL-6* interleukin-6, *miRNA* microRNA, *sIL-2R* soluble interleukin-2 receptor, *SP* substance P, *TNF- α* tumor necrosis factor- α , + indicates 'yes', - indicates 'no'.

First, acquisition of the marker is of great importance: is it acquired locally or systemically? Some of the markers mentioned above are acquired in blister fluid and/or skin biopsies while others are measured in venous blood. The techniques for skin blisters and skin biopsies are considered to be of a higher risk to the patient and more invasive than a venipuncture and require a close follow-up to assess healing of the damaged skin. The skin blister technique is also time consuming and requires pain physicians to have access to materials and devices not usually available in routine practice (33). Second, is it simple to obtain in routine practice? In general, a marker that can be measured in venous blood is easier to obtain in routine practice than a marker that needs to be measured in skin biopsies or blister fluid. Furthermore, certain techniques require a certain amount of training. Pain physicians would thus need to be trained in obtaining skin biopsies and inducing artificial skin blisters. Third, is the biomarker easy to measure? This question is largely dependent on whether an affiliated laboratory has the facilities to determine the aforementioned markers; for example, an enzyme-linked immunosorbent assay (ELISA) is a relatively simple technique to quantify substances such as autoantibodies, while a cell-based assay is a more complex technique which is often not routinely available. Lastly, and most importantly, is it at a minimal risk to the patient? Patients with CRPS have continuing pain of the affected limb that can be worsened by touch or any form of contact (allodynia and/or hyperalgesia). Performing a skin biopsy or inducing a skin blister in the affected limb can temporarily increase the pain a patient is experiencing. In contrast, venipuncture is usually conducted in the contralateral

limb, or in the case of the lower limb, it is conducted in an arm, thereby avoiding the painful limb.

We are aware that biomarkers of inflammation can also be detected in other tissues and fluids, for instance in cerebrospinal fluid (CSF) (82-84). However, we chose not to include findings in CSF as the technique to acquire this fluid, a lumbar puncture, is quite invasive and is currently only used in a research setting in CRPS. Furthermore, we chose to cover (mostly) biochemical and cellular markers of inflammation in CRPS, however, biomarkers are not only limited to biochemical or cellular findings but also can be clinical or radiographic in nature. In the future, it would be interesting to review all these different forms of biomarkers in CRPS.

In addition to the biomarkers discussed in this article, future research on the identification of other potential biomarkers of inflammation in CRPS is indicated. A molecule of interest is, for instance, the high mobility group box 1 (HMGB1) protein, a ligand for multiple immune receptors, that has been implicated in neuropathic pain and in various inflammatory diseases (85, 86). To our knowledge, HMGB1 has not yet been measured in CRPS patients. Furthermore, genetic and epigenetic analysis may represent an interesting future field of research, not only for identification of biomarkers but also to enhance our pathogenetic understanding of CRPS.

Future research should also focus on other non-invasive options for detection of biomarkers in CRPS, for instance salivary analysis (87). To our knowledge, only one study has used salivary analysis to analyze whether free radicals and oxidative stress are involved in the pathophysiology of CRPS (88). Although further optimization and validation of this approach for detection of most inflammatory markers is required (87), it may represent a promising method for non-invasive monitoring of inflammatory markers that warrants further exploration in CRPS.

While writing this article, we noticed that most biomarkers of inflammation that we have described have not yet been correlated to clinical symptoms and signs and thus a specific subtype or phenotype of CRPS. Future research should thus focus on identifying correlations between potential biomarkers of inflammation and clinical symptoms and signs of CRPS. Furthermore, validation studies are needed before any of the described markers can be implemented as routine biomarkers in the diagnosis and management of CRPS. In addition, considering the multi-mechanism pathophysiology of CRPS, it would be interesting for future studies to investigate biomarkers that that could identify the other pathophysiological mechanisms of disease in CRPS.

CONCLUSIONS

The importance of identification and validation of biomarkers in CRPS lies in the objective quality they bring to both the diagnosis and management of this disease. In the case of biomarkers of inflammation in CRPS, they can potentially be used to: 1) diagnose patients with CRPS; 2) aid phenotypical characterization in identifying underlying inflammatory mechanisms; 3) stratify patients according to who would or would not benefit from anti-inflammatory therapies; and 4) monitor the effects of these therapies.

Although there are a number of promising biomarkers of inflammation described in CRPS, it is still difficult to determine the place of these markers in the diagnosis and management of CRPS based on the current literature. Future studies should focus on finding correlations between clinical symptoms and signs and these biomarkers.

As CRPS is a multi-mechanism disease, we currently do not believe that there will be one biomarker specific to this disease. We believe that in the future, multiple biomarkers will be used together with phenotypical characterization to identify which mechanisms are prominent in each CRPS case. The results will then be used to guide physicians in the diagnosis and management of CRPS.

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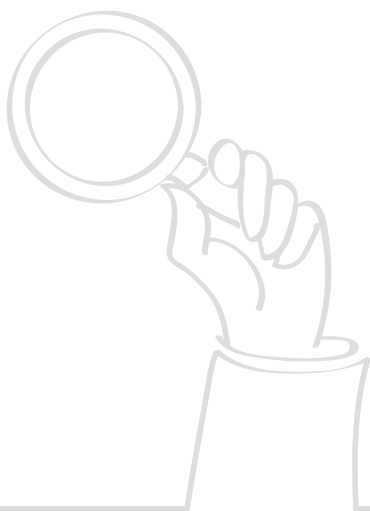
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Part 2



Chapter 4

Elevated plasma levels of sIL-2R in Complex Regional Pain Syndrome: a pathogenic role for T-lymphocytes?

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ABSTRACT

The immune system has long been thought to be involved in the pathophysiology of Complex Regional Pain Syndrome (CRPS). However, not much is known about the role of the immune system and specifically T-cells in the onset and maintenance of this disease.

In this study we aimed to evaluate T-cell activity in CRPS by comparing blood soluble interleukin-2 receptor (sIL-2R) levels between CRPS patients and healthy controls.

CRPS patients had statistically significant elevated levels of sIL-2R as compared to healthy controls (median sIL-2R levels 4151 pg/ml (Q3-Q1 = 5731pg/ml – 3546 pg/ml) versus 1907 pg/ml (Q3-Q1: 2206 pg/ml – 1374 pg/ml), $p < 0.001$, respectively). Furthermore, sIL-2R level seems to be a good discriminator between CRPS patients and healthy controls with a high sensitivity (90%) and specificity (89.5%).

Our finding indicates increased T-cell activity in patients with CRPS. This finding is of considerable relevance as it could point towards a T-cell mediated inflammatory process in this disease. This could pave the way for new anti-inflammatory therapies in the treatment of CRPS. Furthermore, sIL-2R could be a promising new marker for determining inflammatory disease activity in CRPS.

INTRODUCTION

Complex regional pain syndrome (CRPS) is a clinical disorder characterized by severe pain in an affected extremity that is accompanied by sensory, motor, vasomotor and sudomotor disturbances (1). CRPS is often preceded by an injury to an extremity such as a fracture or surgery (2).

The exact pathophysiology of CRPS is still unknown. CRPS is considered to be a multi-mechanism disease (3). The following mechanisms have been proposed to play a role in CRPS: inflammation; central and peripheral sensitization; altered sympathetic nervous system function; endothelial dysfunction; brain plasticity and psychological factors (3-5).

Inflammation as an underlying pathogenic mechanism for CRPS has long been a topic of debate, as systemic markers of inflammation such as erythrocyte sedimentation rate (ESR), c-reactive protein (CRP) and white blood cell count (WBC) are usually not elevated in CRPS patients (6-8). However, classic signs of inflammation such as pain, swelling and redness are often present during physical examination, especially in the initial stages of the disease, suggesting that inflammation does contribute to CRPS (9, 10). The latter is supported by research findings that demonstrated increased levels of the pro-inflammatory cytokines tumor necrosis factor alpha (TNF- α) and interleukin (IL)-6 in skin blister fluid of the affected limbs versus the unaffected limbs of CRPS patients (11, 12). Further, recent studies have confirmed evidence of systemic inflammation in venous blood of CRPS patients (7, 13).

An interesting theory on inflammation in CRPS was put forward by Goebel et al. suggesting that CRPS is a novel kind of antibody mediated autoimmune disease (14).

To expand on this theory, our research group previously analyzed the presence of anti-nuclear antibodies (ANA) and anti-neuronal antibodies in CRPS patients and demonstrated increased ANA positivity in CRPS patients as compared to healthy controls (33% vs 4% respectively, $p < 0.001$) (15). The frequency of positivity for anti-neuronal antibodies did not differ between the groups (15). Considering our findings and those of Goebel et al. we could not define CRPS as an antibody mediated autoimmune disease in accordance with Witebsky's criteria for autoimmune diseases (14-19). Therefore, we shifted our focus to exploring the role of T-cells in the inflammation seen in CRPS.

Hitherto, very few studies have been conducted on the role of T-cells in CRPS (6, 20, 21). These studies were conducted using different methodologies and had different outcome parameters, making comparison between the studies and thus establishment of a firm conclusion difficult. Furthermore, some data presented in these studies are contradictory, e.g. while one study showed no difference in blood lymphocyte populations (i.e. cytotoxic) CD8+ T-cells, CD4+ T-cells, B-cells and natural killer (NK)-cells between CRPS patients and healthy controls, another study found that CRPS was associated with a significant reduction in the number of CD8+ T-cells (6, 20).

Another approach to study T-cell involvement in CRPS is to analyze whether there are indications of increased T-cell activity in CRPS, for instance by measurement of soluble interleukin-2 receptor levels in peripheral blood.

Interleukin-2 (IL-2) is a cytokine crucially important in regulating activation, proliferation and survival of different T-cell subsets (22, 23). This effect of IL-2 is mediated through the IL-2 receptor which consists of the common γ -chain (CD132), a β -chain (CD122) and an α -chain (CD25) (22, 24). CD25 is strongly expressed on activated T-cells which also secrete this molecule as a soluble variant (referred to as soluble IL-2 receptor; sIL-2R) from the cell membrane into the circulation (22, 25, 26).

Peripheral blood levels of sIL-2R have been found to reflect the level of T cell activation and elevated sIL-2R levels correlate with disease activity in for instance rheumatoid arthritis and sarcoidosis, diseases in which T-cell activation is centrally involved (22, 24, 25, 27-30).

Finding increased levels of sIL-2R in CRPS could be indicative of a T-cell mediated inflammatory process in this disease. This finding could contribute to a better understanding of the underlying inflammatory pathophysiological mechanism in CRPS. This could further lead to the development and application of (new) therapies in the treatment of CRPS patients with (T-cell mediated) inflammation as an underlying mechanism of their disease.

Therefore, the aim of this study was to evaluate T-cell activity in CRPS by examining the levels of sIL-2R in a group of CRPS patients and comparing these to sIL-2R levels in a group of healthy controls.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Medical Ethics Committee of Erasmus MC University Medical Center Rotterdam (MEC-2016-172).

Patients and controls

Patients who visited the Center for Pain Medicine at Erasmus MC University Medical Center Rotterdam between 2001 and 2007 and fulfilled the Harden-Bruehl diagnostic criteria for CRPS were invited to participate in various ongoing studies (31).

In the context of these studies, venous blood samples were drawn from patients and plasma was stored in a refrigerator at -80 degrees Celsius for use in future research with permission of the patients.

For this study we examined the levels of sIL-2R in the plasma of 80 adult patients with CRPS type I and compared this to sIL-2R levels measured in 76 anonymous healthy blood bank donors who also had given permission to use their blood for future research purposes.

sIL-2R analysis

Venous blood samples were centrifuged at 3000 rpm immediately after collection for a duration of 10 minutes. Plasma was stored at -80 degrees Celsius and thawed to room temperature for sIL-2R analysis.

sIL-2R plasma levels were quantified using an enzyme linked immunosorbent assay (Human sCD25/sIL-2R ELISA KIT, Besancon, Cedex, France) in accordance with the manufacturer's instructions. sIL-2R levels are expressed in picograms per milliliter (pg/ml), and levels >2500 pg/ml are considered elevated.

Statistical analysis

Descriptive statistics were used to determine the frequencies of the demographic variables and plasma sIL-2R levels and to describe measures of central tendency and of variability. The Shapiro-Wilk test was used to test the distribution of these variables for normality. Results are reported in medians and interquartile ranges (Q3-Q1) if the distribution is skewed and otherwise in means and standard deviations (sd).

Differences in sIL-2R levels between the CRPS patients and the healthy blood bank donors were analyzed using the Independent-Samples Mann-Whitney *U* test (two-sided).

A possible association between sIL-2R levels of the CRPS patients, their age, gender and duration of the CRPS was also explored. An association with gender was evaluated using the Independent-Samples Mann-Whitney *U* test (two-sided). With regard to the age of patients and the duration of CRPS the Spearman's rank correlation was used (two-sided).

A binary logistic regression was used to evaluate the contribution of the level of sIL-2R to the prediction of the group (CRPS patients vs healthy controls). A Receiver Operating Characteristic (ROC) curve was computed. The sensitivity, specificity, positive (PPV) and negative predictive value (NPV) of sIL-2R were calculated.

The alpha level for statistical significance was set at 0.05. Analyses were performed using IBM SPSS Statistics 21.

RESULTS

Plasma was available from 80 CRPS patients. The characteristics of our CRPS patient group are depicted in Table 1.

The median sIL-2R level of the CRPS group was statistically significant higher compared to the median sIL-2R level of the control group. The median of the CRPS patients was 4151 pg/ml (Q3-Q1 = 5731 pg/ml – 3546 pg/ml) and that of the control subjects 1907 pg/ml (Q3-Q1: 2206 pg/ml – 1374 pg/ml), $p < 0.001$ (see Figure 1).

Plasma levels of sIL-2R between male and female CRPS patients were also compared. The sIL-2R plasma levels in men were statistically significant higher than in women (men

Table 1 Characteristics of the CRPS patients.

Characteristics	n=80
Women (n, %)	67 (83.8)
Age in years (mean, sd)	44.4 (12.25)
CRPS duration in months (median, Q3-Q1)	11 (36-5)
Upper limb (n, %)	46 (57.5)
<i>Warm/cold/unknown CRPS</i>	
Warm CRPS (n, %)	30 (37.5)
Cold CRPS (n, %)	44 (55.0)
Unknown (n, %)	6 (7.5)
<i>Precipitating injury</i>	
Trauma (n, %)	51 (63.8)
Surgery (n, %)	21 (26.3)
Spontaneous onset (n, %)	6 (7.5)
Missing (n, %)	2 (2.5)

5602 pg/ml, Q3-Q1= 5829 pg/ml – 3921 pg/ml vs women 4016 pg/ml, Q3-Q1 = 4951 pg/ml - 3286 pg/ml, $p=0.03$) (see Figure 2).

No association was found in the CRPS patient group between sIL-2R levels and the duration of disease ($r_s=-0.18$, $p=0.10$), nor between sIL-2R levels and the age of patients ($r_s=0.12$, $p=0.28$).

The level of sIL-2R showed a favorable discrimination between CRPS patients and healthy controls. The sensitivity was observed to be 90%, specificity 89.5%, PPV 90% and NPV 89.5%, using a cut value of 0.5, which corresponds with an sIL-2R level of 3730 pg/ml (see Table 2, Figure 3).

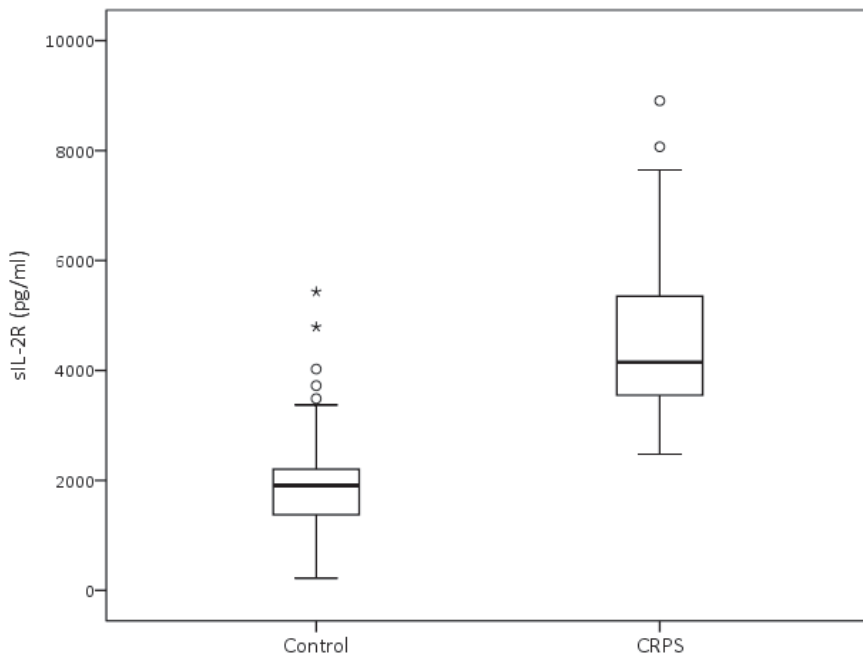


Figure 1 Boxplot of the sIL-2R level by experimental group.

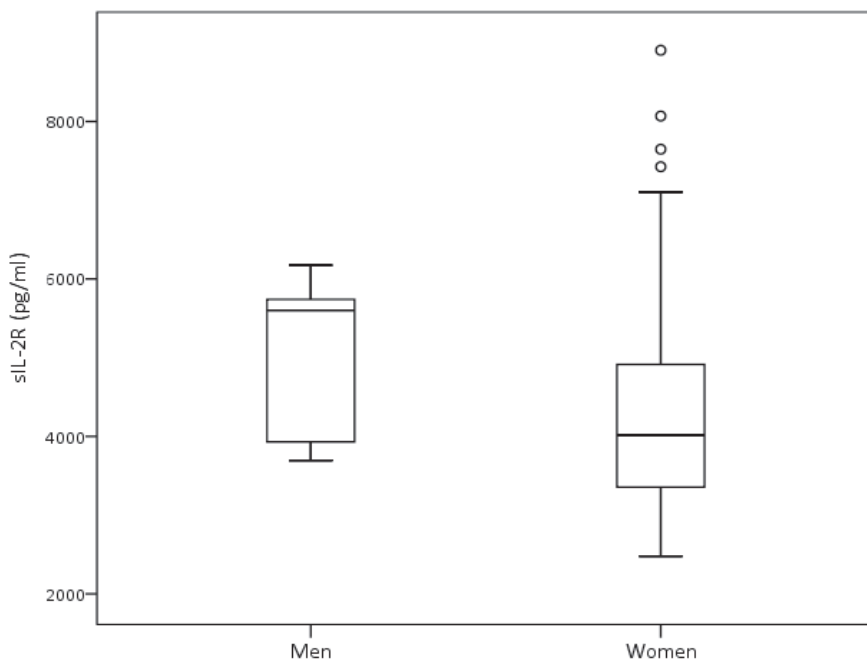


Figure 2 Boxplot of the sIL-2R level by gender in CRPS patients.

Table 2 Results of the binary logistic regression analysis.

	B (SE) [p-value]	95% CI for Odds Ratio		
		Lower	Odds Ratio	Upper
Included				
Constant	-6.96 (1.11) [<0.001]			
sIL-2R	0.002 (< 0.001) [<0.001]	1.002	1.002	1.003

$R^2 = .57$ (Cox & Schnell), $.76$ (Nagelkerke), Model $\chi^2_{(1)} = 130.33$, $p < 0.001$

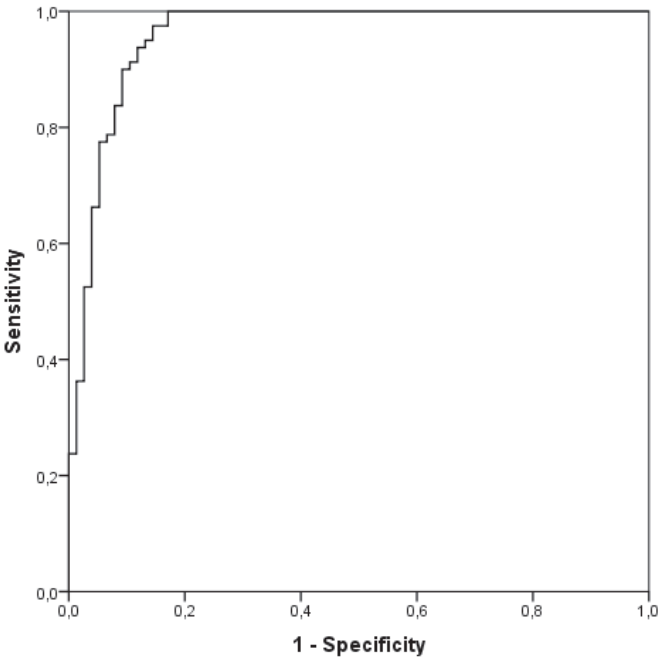


Figure 3 Receiver Operating Characteristic curve of sIL-2R as a predictor of group (CRPS patients vs healthy controls). Area under the curve 0.958 (se 0.016), $p < 0.001$.

DISCUSSION

This study was conducted to explore whether T-cell activation is involved in the pathophysiology of CRPS. To this end, venous blood levels of sIL-2R in CRPS patients were compared to those of healthy controls. Our data clearly demonstrate that plasma sIL-2R levels are significantly elevated in CRPS patients. This finding is of considerable relevance as it indicates increased T-cell activity in CRPS and therefore could point towards an underlying T-cell mediated inflammatory process in this disease.

There is one earlier study which has shown significantly elevated plasma levels of sIL-2R in CRPS patients (32). However, the aim of this study was to conduct an explorative analysis of various plasma analytes in CRPS patients and to subsequently derive different CRPS clusters based on their findings (32). The authors did not relate this finding of higher plasma levels of sIL-2R to T cell activity (32). This seems to be more an ancillary finding, but it strongly supports our results.

In our study we observed higher sIL-2R plasma levels in men with CRPS as compared to those in women with CRPS. We could not compare the gender distribution of sIL-2R levels in our CRPS group to the group of healthy controls as this was an anonymous group without any available demographic data. However other studies do not report elevated blood sIL-2R levels in healthy men compared to healthy women (22, 33-35).

Our finding of a significant difference in sIL-2R levels between CRPS men and women conflicts with the finding of the previously mentioned explorative study (32). This study found no differences in sIL-2R levels between men and women with CRPS (32). One explanation for the contradictory results could be the possibility of higher disease severity in our group of male CRPS patients, as sIL-2R levels have been shown to correlate with disease severity in other disease entities (28).

We found no association between sIL-2R levels and age in the CRPS group. Studies in healthy individuals have shown sIL-2R levels to vary with age (22). Children (age 1-14 years) and the elderly (age 67-99 years) have been shown to have higher sIL-2R levels as compared to (young) adults (age 22-67) (22, 33, 35). This could explain why no association was found between sIL-2R levels and age as our CRPS patient sample consisted mainly of (young) adults with a mean age of 44.4 years (sd 12.25).

No association was found between sIL-2R levels and duration of disease, suggesting immune system activation throughout the entire disease course in a (subgroup) of CRPS patients.

Finally, our findings show sIL-2R level to be a good discriminator between CRPS patients and healthy controls. This finding could not only lead to the use of sIL-2R as a marker of inflammatory disease activity in CRPS but also to the use of sIL-2R during the diagnostic work-up of this disease. As per our knowledge, no study has yet been performed on this subject. Consequently, further studies are required to validate sIL-2R as a marker of inflammatory disease activity in CRPS and to establish a validated diagnostic cut-off value to differentiate CRPS from other chronic pain diseases (e.g. fibromyalgia) and autoimmune and auto-inflammatory disorders (e.g. rheumatoid arthritis).

While our findings indicate increased T-cell activity in CRPS, it is still unclear what subset of T-cells is being activated as sIL-2R is T-cell subset nonspecific and is thus measured during activation of different T-cell subsets (22).

Previous studies have tried characterizing changes in lymphocyte subsets in CRPS (20, 21). In 2007, Kaufmann et al. investigated the influence of pain and stress on lymphocyte

numbers, lymphocyte subpopulations and T-helper 1/T-helper 2 (Th1/Th2) ratio in CRPS patients, fibromyalgia patients and healthy controls (20). They found a significant reduction of CD8+ T-lymphocytes in CRPS patients as compared to healthy controls (20).

In 2015, Osborne et al studied skin immune cell populations in long-standing CRPS and found no significant differences in overall immune cell infiltrates between CRPS affected and unaffected limbs (21).

As stated previously, while our findings indicate increased T-cell activity in CRPS, we cannot specify which subset(s) of T-cells are active, making it difficult to relate our findings to the findings from the studies mentioned above (20, 21). Moreover, both studies used different methodologies to study involvement of T-cells (i.e. skin punch biopsies in the study by Osborne et al. and venous blood samples in the study by Kaufmann et al.) (20, 21). Furthermore, Osborne et al. compared the unaffected and affected extremity in CRPS patients (side to side comparison) while Kaufmann et al. looked at differences between CRPS patients, fibromyalgia patients and healthy controls (20, 21). As a consequence, comparison of our results to those of Kaufmann et al. or to those of Osborne et al. is rather meaningless (20, 21).

Further, the internal and external validity of this current study should be evaluated taking into account the potential incomparability of the experimental groups in terms of age, gender, past history and medication as demographic data and medical history were unavailable for the control group.

Finally, our study consists of a cross-sectional measurement of sIL-2R levels in CRPS patients and healthy blood bank donors. It would be interesting to prospectively study sIL-2R levels during the course of this disease. This could increase our understanding on the role of T-cell activity in the onset and maintenance of CRPS.

Based on the findings of this study, we propose a role of T-cell-mediated inflammation as an underlying mechanism in the pathophysiology of CRPS. Our results are of considerable relevance as the involvement of T-cells in CRPS could lead to a better understanding of the rather complex pathophysiology of this disease. The findings of this study and the results of possible future research might lead to new therapeutic targets in the treatment of CRPS patients with (T-cell mediated) inflammation as an underlying mechanism of disease, thereby paving the way for new anti-inflammatory and/or immunomodulating therapies in the management of CRPS.

CONCLUSION

The median sIL-2R level of CRPS patients was found to be significantly increased as compared to that of a group of healthy blood bank donors. This result indicates increased T-cell activity in CRPS.

This finding could point towards a T-cell mediated inflammatory process in CRPS, which could pave the way for new anti-inflammatory therapies in the treatment of CRPS patients with (T-cell mediated) inflammation as their underlying mechanism of disease. However, the precise role of T-cells in the pathophysiology of CRPS has yet to be unraveled.

Furthermore, sIL-2R level seems to be a good discriminator between CRPS patients and healthy controls. CRPS is still a clinical diagnosis. Until now, no diagnostic test exists for monitoring inflammatory disease activity in CRPS. Based on our findings, sIL-2R could be a promising new marker to determine inflammatory disease activity in CRPS. However, this must be validated in future research.

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Chapter 5

**Serum soluble interleukin-2 receptor does not
differentiate Complex Regional Pain Syndrome from
other pain conditions in a tertiary referral setting**

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ABSTRACT

Previously, we showed that serum soluble interleukin-2 receptor (sIL-2R) levels, a marker for T-cell activation, were higher in Complex Regional Pain Syndrome (CRPS) patients than in healthy controls, suggesting pathogenic T-cell activation in CRPS. Additionally, sIL-2R levels discriminated well between CRPS and healthy controls with a high sensitivity (90%) and specificity (89.5%), suggesting a possible role for sIL-2R in the diagnosis of CRPS. In order to further validate this marker in the diagnostic workup of CRPS, we conducted this prospective cohort study in which we determined sIL-2R levels in patients that were referred to our tertiary referral center with a suspicion of CRPS in a limb, and subsequently compared sIL-2R levels between the patients that were diagnosed with CRPS (CRPS group) and those who were not (no-CRPS group). A group of anonymous blood bank donors were used as a healthy control group. Furthermore, we explored the relationship between sIL-2R and CRPS disease severity using the CRPS severity score. Median sIL-2R levels of both the CRPS group (2809.0 pg/ml; Q3-Q1: 3913.0-1589.0) and no-CRPS group (3654.0 pg/ml; Q3-Q1: 4429.0-2095.5) were significantly higher than that of the control group (1515.0 pg/ml; Q3-Q1: 1880.0-1150.0): CRPS vs controls, $p < .001$; no-CRPS vs controls, $p < 0.001$. Serum sIL-2R levels did not differ significantly between the CRPS and no-CRPS group. A statistically significant negative correlation was observed between sIL-2R levels and the CRPS severity score ($r_s = -0.468$, $p = 0.024$). Our results confirm our previous findings of higher sIL-2R levels in CRPS patients than in healthy controls. We further showed that serum sIL-2R cannot differentiate between CRPS and other pain conditions of a limb in a tertiary referral setting. Interestingly, a negative correlation was found between sIL-2R and CRPS disease severity, this finding warrants further research into the relationship between sIL-2R and CRPS disease severity.

INTRODUCTION

Complex regional pain syndrome (CRPS) is characterized by continuous pain which is accompanied by various sensory, motor, vasomotor, sudomotor and trophic disturbances (1). The onset of CRPS is preceded by damage to the tissues of a limb, for example, due to fracture or surgery (2). If CRPS is left untreated, it can have incapacitating consequences not only on the function of the affected limb, but also on the social life of patients (3). However, appropriate treatment is often initiated too late due to a delay in diagnosis (4).

This diagnostic delay is mostly due to two reasons. First, the diagnosis of CRPS is still based on a set of relatively subjective criteria: the New International Association for the Study of Pain clinical diagnostic criteria for CRPS (1). Thus, the (early) diagnosis of CRPS cannot yet be established by objective diagnostic testing. Second, the pathophysiology of CRPS is complex and still incompletely understood; this lack of understanding creates skepticism among physicians on whether this disease exists (5, 6) and further leads to a general lack of awareness on the symptoms and signs of this disease.

Although the pathophysiology of CRPS is still incompletely understood, it has been established that it comprises of multiple disease mechanisms (7). Inflammation is recognized as one of the pathophysiological mechanisms contributing to CRPS. This inflammation may, in part, be related to dysregulation of the immune system associated with altered T-cell activity (8-10). Our group previously assessed T-cell activity in CRPS patients by measuring serum levels of the soluble interleukin-2 receptor (sIL-2R): a marker for T-cell activation (8, 11, 12). We found significantly higher serum sIL-2R levels in the CRPS group than in healthy controls, supporting the notion of pathological T-cell activation in CRPS (8). Moreover, serum sIL-2R level discriminated well between CRPS patients and healthy controls, with a high sensitivity (90%) and specificity (89.5%) (8).

This last finding is especially noteworthy as it indicates that serum sIL-2R may represent a biomarker to facilitate the diagnosis of CRPS. Elevated serum sIL-2R levels are, however, not disease-specific as this is found in many different disease entities, including immune and rheumatic diseases, as well as malignancies (13). Yet, the potential diagnostic value of serum sIL-2R was recently demonstrated in a retrospective cohort study in patients suspected of sarcoidosis (14). On the basis of an established cut-off value, the sensitivity and specificity of serum sIL-2R for the detection of sarcoidosis was 88% and 85%, by far superior to angiotensin-converting enzyme (ACE; the classical biomarker for sarcoidosis with a sensitivity of 62% and specificity of 88%) (14). Therefore, we consider it of interest to further explore the potential application of serum sIL-2R measurement in establishing the diagnosis of CRPS. At this moment, biomarkers validated for use in the diagnosis of CRPS are not available. However, identification of potential diagnostic biomarkers could greatly aid in preventing a delayed diagnosis and starting appropriate and timely therapy in CRPS.

Previously, we determined serum sIL-2R levels only in CRPS patients and healthy controls and consequently, we could not draw conclusions on the role of serum sIL-2R in the diagnostic workup of CRPS (8). Therefore, in this current study, we examined whether serum sIL-2R can be used to differentiate CRPS from other pain conditions of a limb in patients referred to a tertiary referral center due to a suspicion of CRPS.

MATERIALS AND METHODS

Ethical approval

This study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO). The study was approved by the Medical Ethics Committee of Erasmus MC University Medical Center Rotterdam (MEC-2017-495). The trial was registered in the Netherlands Trial Registry (NTR7465).

Study design, recruitment and study population

This prospective cohort study was conducted at the Center for Pain Medicine (CPM) at Erasmus MC University Medical Center which is a teaching hospital located in Rotterdam, the Netherlands. The CPM is a tertiary referral center with CRPS being one of the fields of expertise. Patients are referred to our center by general physicians or other specialists such as orthopedic surgeons.

All patients referred to our center with a suspicion of CRPS in one limb were invited to participate in this study. Two weeks before their first outpatient clinic appointment, patients were approached by a study physician with both verbal and written information on the study. The patients could decide on the day of their appointment whether they wanted to participate in the study. Patients were informed that the results of this study would not influence the diagnosis or treatment of their disease. After obtaining informed consent, the inclusion and exclusion criteria described in table 1 were applied. Patients were included consecutively until the required sample size was reached. The inclusion period started in March 2018 and ended in August 2019.

Serum sIL-2R levels available from 101 anonymous healthy blood bank donors served as a reference for serum sIL-2R levels in the healthy population. Thus, the study population consisted of 3 groups: patients finally diagnosed with CRPS (CRPS group), patients finally diagnosed with a condition other than CRPS (no-CRPS group) and healthy controls.

Table 1 Inclusion and exclusion criteria applied in this study. Patients had to meet both the inclusion criteria and were excluded if they met any of the exclusion criteria.

Inclusion criteria	Exclusion criteria
Age \geq 18 years	History of an auto-inflammatory or autoimmune disease
Only one limb is affected	Current or past (within the last six months) treatment with immunomodulating medication such as steroids or TNF- α inhibitors
	Ill in the past two weeks or at the time of visit
	Potential pregnancy or confirmed pregnancy

Study measurements and data collection

The following data were collected during the outpatient clinic appointment: age; duration of disease (i.e. duration of symptoms and signs); precipitating injury (i.e. initiating factor of symptoms and signs); affected limb; medication; intensity of pain at the moment of the visit and in the past 24 hours using an 11-point Numeric Rating Scale (NRS); and symptoms and signs recorded using the CRPS severity score- Database Form developed by Harden et al. along with the resulting CRPS severity score (CSS) (15) (table 2). Permission was received from N. Harden for use of the CRPS severity score-Database Form (15). The study physicians followed the instructions of the CRPS severity score- Database Form to register symptoms and signs during physical examination. At the end of the appointment, one 5 milliliter tube of venous blood was drawn for sIL-2R analysis.

Table 2 Symptoms and signs assessed using the CRPS severity score- Database Form by Harden et al. (15)

Symptoms ^{*1}	Signs ^{*2}
Continuing, disproportionate pain	Hyperalgesia to single pinprick
Allodynia or Hyperalgesia	Allodynia
Temperature asymmetry	Temperature asymmetry by palpation
Color asymmetry	Color asymmetry
Sweating asymmetry	Sweating asymmetry
Edema	Asymmetric edema
Dystrophic changes	Dystrophic changes
Motor abnormalities ^{*3}	Motor abnormalities ^{*4}

^{*1} Symptoms as reported by the patient. All symptoms are categorical variables and are registered as absent or present.

^{*2} Signs as observed during physical examination by the physician. All signs are categorical variables and are registered as absent or present.

^{*3} Motor abnormalities as reported by the patient: weakness, tremor, dystonia, decreased range of motion, myoclonus.

^{*4} Motor abnormalities as observed by the examiner: tremor/myoclonus, dystonia, decreased active range of motion, weakness.

Diagnosis of CRPS group and no-CRPS group

CRPS was diagnosed using the widely accepted New International Association for the Study of Pain Clinical Diagnostic Criteria for CRPS (1). All other diagnoses were established using appropriate and up-to-date guidelines and when needed, patients were referred to the appropriate specialty. The diagnoses of patients in the no-CRPS group were divided into the following categories: neuropathic pain syndromes, myofascial pain syndromes, vascular diseases, inflammatory conditions and psychiatric problems/disorders. These categories were derived from the differential diagnosis of CRPS as described in the article by van Eijs et al. (16).

sIL-2R analysis

Venous blood samples were centrifuged at 3000 rpm after collection and serum was subsequently isolated. Soluble IL-2R levels were measured using an enzyme linked immunosorbent assay (Human sCD25/sIL-2R ELISA kit, Besancon, Cedex, France) according to the manufacturer's instructions at the diagnostic Laboratory Medical Immunology facility of Erasmus MC University Medical Center Rotterdam. The measurements were conducted under strict quality procedures (ISO15189).

Sample size calculation

Based on the results of our previous study (8), we chose a statistically detectable and clinically relevant effect size (d) of 1.0 on serum sIL-2R level using an independent t -test. The power of the study ($1-\beta$) was set at 0.8, the allocation ratio at 0.25 and the two-sided level of significance (α) at 0.05. The required sample size computed by this method was 52.

Statistical analysis

Descriptive statistics were used to calculate the frequencies of categorical variables and to calculate measures of central tendency and variability of continuous variables. The Shapiro-Wilk test was used to analyze whether continuous variables were normally distributed. Variables with a skewed distribution are reported in medians and interquartile ranges (Q_3-Q_1), otherwise means and standard deviations are used. The primary outcome parameter was the serum sIL-2R level in the CRPS group, no-CRPS group and healthy control group.

Depending on the shape of distribution, continuous variables were compared between two groups using either a two-sided independent t -test or a two-sided Mann-Whitney-U test. Comparison of continuous variables between more than two groups was conducted using either an ANOVA or a Kruskal-Wallis test, dependent on the shape of the distribution of the variable. Categorical variables were compared using the Fisher's exact test.

A possible association in the CRPS group between sIL-2R levels and age, sIL-2R levels and duration of disease and sIL-2R levels and the CRPS severity score was explored using either a Pearson's correlation or a Spearman's rank correlation, dependent on the shape of

the distribution of these variables. A possible association in the CRPS group between sIL-2R levels and gender was explored using a point-biserial correlation.

Where possible, data are presented in tables and graphs, such as box-and-whisker plots and scatterplots. For box-and-whisker plots that are created in SPSS, the box represents the interquartile range and the whiskers extend to the highest and lowest value in the data range which are no greater than 1.5 times the interquartile range. Circles in the box-and-whisker plots indicate outliers that are between 1.5 and 3 times the interquartile range. Analyses were performed using IBM SPSS Statistics 21. The alpha level for statistical significance was set at 0.05.

RESULTS

Figure 1 depicts the recruitment and inclusion of our study population. A total of 86 patients were approached to participate in this study. Twenty-nine patients did not participate in the study: one patient canceled the outpatient clinic appointment; one patient did not show up at the appointment; two patients had an incorrect referral; five patients declared, of their own accord, during the phone call that they have an autoimmune or auto-inflammatory disorder with or without use of immunomodulating medication; six patients were unwilling to participate in research; and fourteen patients were unreachable when called. Fifty-seven patients signed the informed consent form. Five patients were excluded after signing the form: two patients were excluded due to use of prednisolone; one patient did not have time to complete the outpatient visit; one patient backed out without further explanation and one patient was excluded because of a history of active psoriasis. This resulted in the required sample size of 52 patients for analysis.

Of the 52 patients, 23 patients (44%) were diagnosed with CRPS and 29 patients (56%) were diagnosed with other conditions (no-CRPS group). Of the no-CRPS group 7 patients (24.1%) were diagnosed with neuropathic pain syndromes, 17 (58.6%) with myofascial pain syndromes, 2 patients (6.9%) with inflammatory conditions and 3 patients (10.3%) had an unclear or unknown diagnosis. No diagnoses were made that could be categorized as vascular diseases or psychiatric problems/disorders. Full details of the no-CRPS group, including diagnoses that were made per category, can be found in table 3.

Patient characteristics such as age, gender, affected limb, precipitating injury and duration of disease were comparable among both the CRPS group and the no-CRPS group (table 4). Use of medication was also comparable among both groups (table 5). Median pain scores at the time of visit and 24 hours before the visit were also comparable among both groups (table 6).

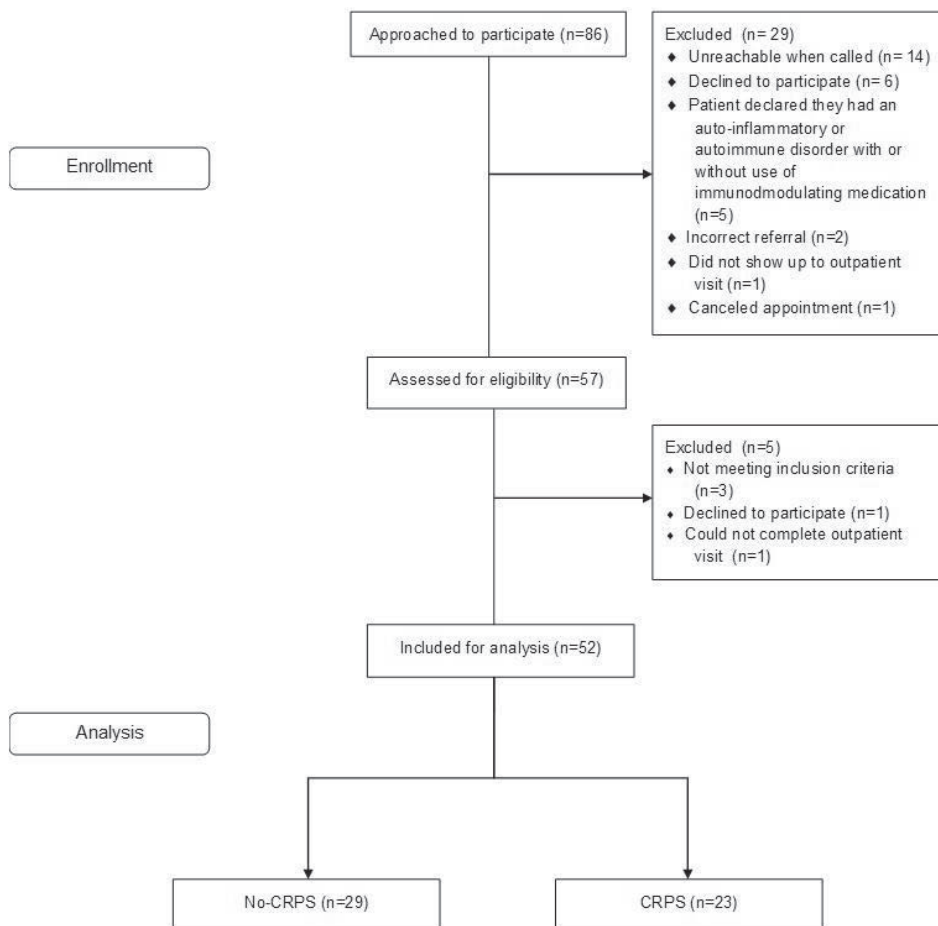


Figure 1 Flow diagram depicting the recruitment and inclusion of the study population.

Table 6 shows the proportion of symptoms and signs in each group recorded according to the CRPS severity score- Database Form (15). The prevalence of the following symptoms (i.e. subjective symptoms reported by patients) was significantly higher in the CRPS group than in the no-CRPS group: continuing pain, color asymmetry and decreased active range of motion of the affected limb. The prevalence of the following signs (i.e. objective signs observed by the physician) was significantly higher in the CRPS group than in the no-CRPS group: hyperalgesia to pinprick; allodynia and its corresponding subcategories; temperature asymmetry, with all affected CRPS patients having a cooler affected limb; color asymmetry and its corresponding subcategory 'red'; sweating asymmetry, with all affected CRPS patients experiencing increased sweating on the affected side; and asymmetric edema. The mean CRPS severity score was significantly higher in the CRPS group than in the no-CRPS group (CRPS 11.4 (sd=2.2) versus no-CRPS 8.1 (sd=1.9), $p<0.001$).

Table 3 Diagnosis and median sIL-2R level per group

Diagnosis	Total patients per group	Median sIL-2R pg/ml (Q3-Q1)
Healthy controls	101	1515.0 (1880.0-1150.0)
CRPS group	23	2809.0 (3913.0-1589.0)
No-CRPS group	29	3654.0 (4429.0-2095.5)
Neuropathic pain syndromes ^{*1} (n, % no-CRPS group)	7 (24.1)	4170.0 (5203.0-2050.0)
Myofascial pain syndromes ^{*2} (n, % no-CRPS group)	17 (58.6)	3529.0 (4253.5-2150.5)
Inflammation ^{*3} (n, % no-CRPS group)	2 (6.9)	N/A
Unknown ^{*4} (n, % no-CRPS group)	3 (10.3)	N/A

*1 Neuropathic pain syndromes: peripheral neuropathy (n=5); Cervical dermatomal pain (n=1); Radicular pain (n=1).

*2 Myofascial pain syndromes: post-fracture pain and osteoarthritis (n=1); osteoarthritis (n=1); disuse (n=1); myalgia (n=1); disability and impairment of hand related to fracture as diagnosed by plastic surgeon (n=2); shin splints (n=1); subacromial pain syndrome (n=1); unspecified pain of the shin (n=1); suspected patellofemoral pain syndrome (n=1); suspected clenched fist syndrome (n=1); pain related to healing process after trauma (n=4); post-surgical pain (n=2).

*3 Inflammation: osteomyelitis (n=1); arthritis of the wrist (n=1). Median sIL-2R levels were not calculated due to the size of the group.

*4 Median sIL-2R levels were not calculated due to the size of the group.

Table 4 Patient demographics and general characteristics of the no-CRPS and CRPS group.

Demographics and characteristics	No CRPS (n=29)	CRPS (n=23)	Significance
Age in years (median, (Q3-Q1))	43.0 (55.5-27.5)	37.0 (55.0-28.0)	NS
Duration of disease in months (median, (Q3-Q1))	20.0 (36.0-8.5)	26.0 (81.0-14.0)	NS
Gender			NS
Male (n,%)	10 (34.5)	4 (17.4)	
Female (n,%)	19 (65.5)	19 (82.6)	
Affected limb			NS
Right upper limb (n,%)	6 (20.7)	5 (21.7)	
Left upper limb (n,%)	5 (17.2)	4 (17.4)	
Right lower limb (n,%)	6 (20.7)	4 (17.4)	
Left lower limb (n,%)	12 (41.4)	10 (43.5)	
Precipitating injury			NS
Trauma	13 (44.8)	11 (47.8)	
Operation	11 (37.9)	9 (39.1)	
Spontaneous	5 (17.2)	0	
Other	0	2 (8.7)	
Unknown	0	1 (4.3)	

The median sIL-2R levels of both the CRPS group (2809.0 pg/ml; Q3-Q1: 3913.0-1589.0) and no-CRPS group (3654.0 pg/ml; Q3-Q1: 4429.0-2095.5) were significantly higher than the median sIL-2R level of the control group (1515.0 pg/ml; Q3-Q1: 1880.0-1150.0): CRPS group vs control group $p<.001$ and no-CRPS group vs control group $p<.001$. Serum sIL-2R levels did not differ significantly between the CRPS group and no-CRPS group (Figure 2 and Table 3).

Of the no-CRPS group, both the neuropathic-pain-syndromes group (4170.0 pg/ml; Q3-Q1: 5203.0-2050.0) and myofascial-pain-syndromes group (3529.0 pg/ml; Q3-Q1: 4253.5-2150.5) had median sIL-2R levels that were significantly higher than the median sIL-2R level of healthy controls (1515.0 pg/ml; Q3-Q1: 1880.0-1150.0): neuropathic-pain-syndromes group versus control group $p<0.001$ and myofascial-pain-syndromes group versus control group, $p<0.001$. There was no significant difference in the distribution of sIL-2R levels between the neuropathic-pain-syndromes group, myofascial-pain-syndromes group and the CRPS group (Figure 3 and Table 3).

Within the CRPS group, a statistically significant negative correlation existed between serum sIL-2R levels and the CRPS severity score ($r_s = -0.468$, $p=0.024$, Figure 4). No association was found between serum sIL-2R level and age, gender and disease duration in the CRPS group.

Table 5. Medications being used at the time of visit at the outpatient clinic center

Medication	No-CRPS (n=29)	CRPS (n=23)	Significance
Paracetamol (n,%)	10 (34.5)	9 (39.1)	NS
NSAIDs ¹ (n,%)	10 (34.5)	5 (21.7)	NS
Opioids (n,%)	5 (17.2)	8 (34.8)	NS
Antidepressants (n,%)	3 (10.3)	6 (26.1)	NS
Anti-epileptics (n,%)	3 (10.3)	6 (26.1)	NS
Calcium Channel Blockers (n,%)	1 (3.4)	2 (8.7)	NS
Phosphodiesterase-5 inhibitor (n,%)	0	0	N/A
Vitamin C (n,%)	6 (20.7)	3 (13.0)	NS
Fluimucil or N-acetyl cysteine (n,%)	0	1 (4.3)	N/A
DMSO ² (n,%)	2 (6.9)	0	N/A

¹NSAIDs: nonsteroidal anti-inflammatory drugs

²DMSO: dimethylsulfoxide cream

Table 6 CRPS severity score-Database Form: presence of symptoms and signs of CRPS in each group

Symptoms	No CRPS (n=29)	CRPS (n=23)	Significance
NRS at time of visit (median, Q3-Q1)	7.0 (8.0-3.0)	7.0 (8.0-6.0)	NS
NRS 24 hours before visit (median, Q3-Q1)	7.5 (8.0-6.3)	8.0 (8.0-7.0)	NS
Continuing pain (n, %)	18 (62.1)	23 (100)	P=0.001
Allodynia and/or Hyperalgesia	27 (93.1)	23 (100)	NS
Allodynia	14 (48.3)	17 (73.9)	NS
Hyperalgesia	24 (82.8)	23 (100)	NS
Temperature asymmetry	27 (93.1)	20 (87.0)	NS
Affected side warmer	11 (37.9)	7 (30.4)	NS
Affected side colder	9 (31.0)	5 (21.7)	NS
Affected side warm/cold	7 (24.1)	8 (34.8)	NS
Color asymmetry	23 (79.3)	23 (100)	P=0.028
Red	14 (48.3)	13 (56.5)	NS
Blue	5 (17.2)	8 (34.8)	NS
Other color	12 (41.4)	14 (60.9)	NS
Sweating asymmetry	12 (41.4)	14 (60.9)	NS
Edema	24 (82.8)	21 (91.3)	NS
Dystrophic changes	15 (51.7)	17 (73.9)	NS
Nails	10 (34.5)	12 (52.2)	NS
Hair	8 (27.6)	11 (47.8)	NS
Skin	6 (20.7)	10 (43.5)	NS
Motor abnormalities	29 (100)	23 (100)	N/A
Weakness	25 (86.2)	22 (95.7)	NS
Tremor	15 (51.7)	13 (56.5)	NS
Dystonia	13 (44.8)	10 (43.5)	NS
Decreased AROM	20 (69.0)	22 (95.7)	P=0.030
Myoclonus	4 (13.8)	9 (39.1)	NS
Signs	No CRPS (n=29)	CRPS (n=23)	Significance
Hyperalgesia to pinprick	11 (37.9)	17 (73.9)	P=0.013
Allodynia	18 (62.1)	22 (95.7)	P=0.007
Light touch	6 (20.7)	19 (82.6)	P<0.001
Deep joint pressure	9 (31.0)	18 (78.3)	P=0.002
Vibration	8 (27.6)	14 (60.9)	P=0.021
Cold	2 (6.9)	11 (47.8)	P=0.002
Heat	3 (10.3)	11 (47.8)	P=0.004
Temp asymmetry on palpation	2 (6.9)	8 (34.8)	P=0.015
Affected side cooler	1 (3.4)	8 (34.8)	P=0.007
Affected side warmer	1 (3.4)	0	NS

Table 6 CRPS severity score-Database Form: presence of symptoms and signs of CRPS in each group
(continued)

Signs	No CRPS (n=29)	CRPS (n=23)	Significance
Color asymmetry	4 (13.8)	12 (52.2)	P=0.006
Red	3 (10.3)	9 (39.1)	P=0.021
Blue or Pale	3 (10.3)	4 (17.4)	NS
Mottled	0	4 (17.4)	N/A
Scar	0	0	N/A
Sweating asymmetry	1 (3.4)	6 (26.1)	P=0.035
Increased on affected side	1 (3.4)	6 (26.1)	P=0.035
Decreased on affected side	0	0	N/A
Asymmetric edema	1 (3.4)	6 (26.1)	P=0.035
Dystrophic changes	4 (13.8)	7 (30.4)	NS
Nails	1 (3.4)	5 (21.7)	NS
Hair	3 (10.3)	2 (8.7)	NS
Skin	1 (3.4)	4 (17.4)	NS
Motor abnormalities affected side	20 (69.0)	21 (91.3)	NS
Tremor or Myoclonus	2 (6.9)	6 (26.1)	NS
Dystonia	2 (6.9)	7 (30.4)	NS
Decreased AROM	16 (55.2)	17 (73.9)	NS
Weakness 1/5^{*1}	0	3 (13.0)	N/A
Weakness 2/5^{*2}	0	5 (21.7)	N/A
Weakness 3/5^{*3}	4 (13.8)	5 (21.7)	NS
Weakness 4/5^{*4}	12 (41.4)	7 (30.4)	NS
CRPS severity score (mean, sd)	8.1 (1.9)	11.4 (2.2)	P<0.001

*1Weakness 1/5: flicker of movement

*2Weakness 2/5: movement with gravity

*3Weakness 3/5: movement against gravity

*4Weakness 4/5: weak

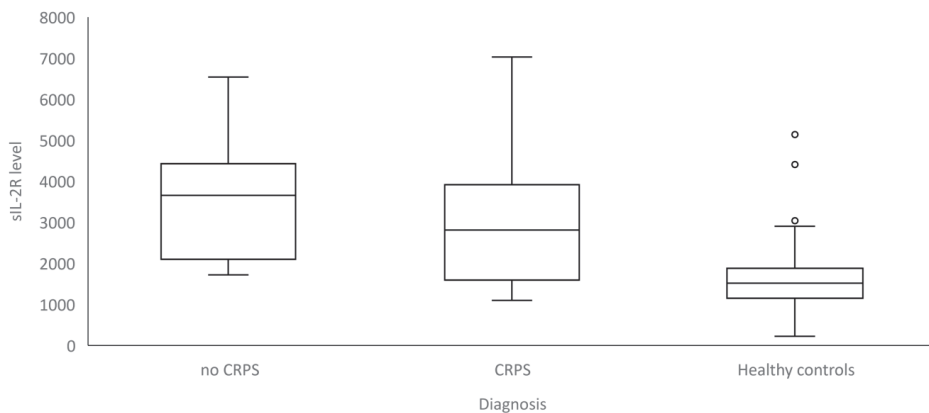


Figure 2 Boxplot of the median sIL-2R levels in the no-CRPS group (3654.0 pg/ml; Q3-Q1: 4429.0-2095.5), the CRPS group (2809.0 pg/ml; Q3-Q1: 3913.0-1589.0) and the control group (1515.0 pg/ml; Q3-Q1: 1880.0-1150.0): CRPS vs controls $p < .001$ and no-CRPS vs controls $p < 0.001$

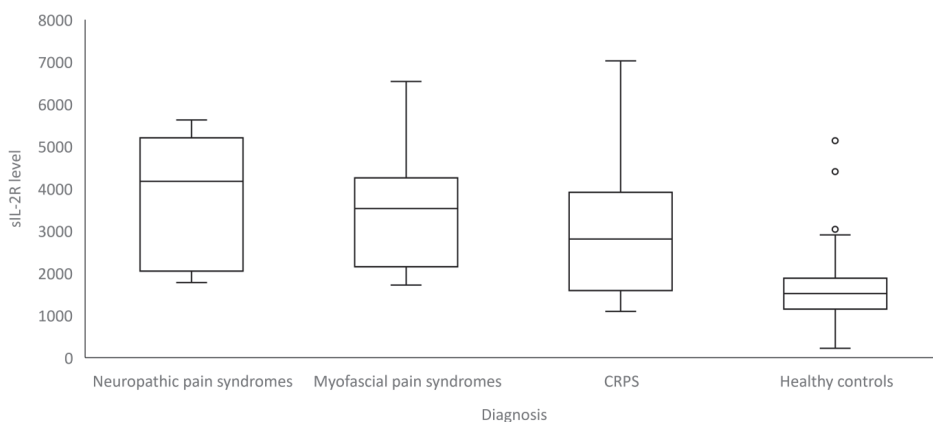


Figure 3 Boxplot of median sIL-2R levels in the neuropathic-pain-syndromes group (4170.0 pg/ml; Q3-Q1: 5203.0-2050.0), the myofascial-pain-syndromes group (3529.0 pg/ml; Q3-Q1: 4253.5-2150.5), the CRPS group (2809.0 pg/ml; Q3-Q1: 3913.0-1589.0) and the group of healthy controls (1515.0 pg/ml; Q3-Q1: 1880.0-1150.0): neuropathic pain syndromes versus controls $p < 0.001$ and myofascial pain syndromes versus controls, $p < 0.001$.

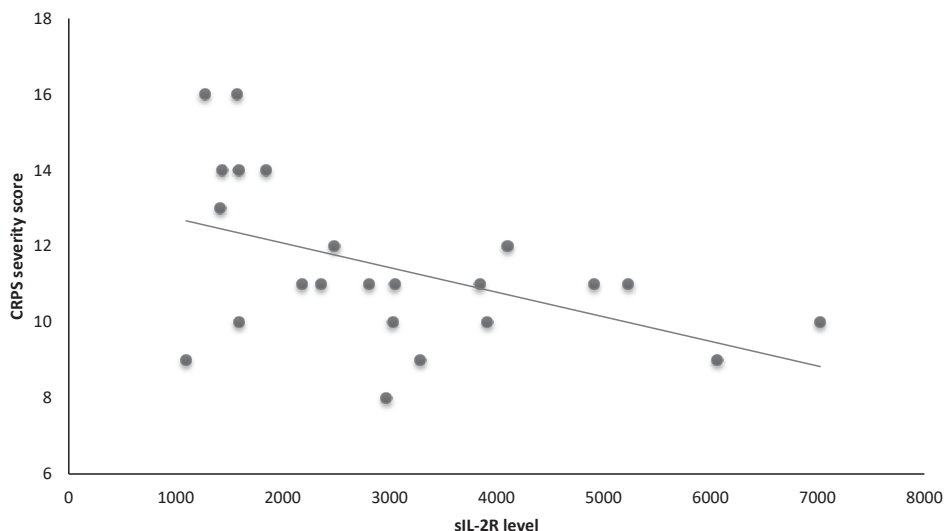


Figure 4 Scatter plot showing the correlation between serum sIL-2R level and CRPS severity score in CRPS patients: $r_s = -0.468$, $p = 0.024$

DISCUSSION

So far, objective diagnostic tests to diagnose CRPS are not available. This lack of objective tests hampers early diagnosis and timely initiation of appropriate therapies (17). Based on findings from our previous study in which sIL-2R levels were found to be significantly higher in CRPS patients than in healthy controls (8), we conducted this current study in which we investigated whether serum sIL-2R could be used to help establish the diagnosis CRPS in patients who were referred to a tertiary referral center with pain in a limb that was suspected to be caused by CRPS. To our knowledge, this is the first study assessing the differentiating capacity of serum sIL-2R in CRPS. Our results indicate that serum sIL-2R is not useful for differentiating CRPS from other pain conditions of a limb in patients referred with a suspicion of CRPS to a tertiary referral center.

One of the main explanations why serum sIL-2R may not be useful in differentiating CRPS from other pain conditions of a limb may be that altered T-cell activity occurs in various diseases that are part of the initial differential diagnosis of CRPS. For example, there are diseases in the differential diagnosis of CRPS that have been proven to involve T-cell activation and have been shown to have elevated sIL-2R levels, such as rheumatoid arthritis (13, 18). Recently, carpal tunnel syndrome (CTS) —which also needs to be considered in the differential diagnosis of CRPS of the upper limb — was shown to be associated with elevated percentages of central and effector memory CD4+ T-cells which is suggestive of changes in memory T-cell homeostasis in CTS (19). Therefore, we consider it likely that

serum sIL-2R levels may be elevated in CTS patients as well, although data on this is lacking so far. There is also evidence that altered T-cell activity may play a role in (the development of) neuropathic pain (20, 21). It is thus plausible, that there is altered T-cell activity in the various diseases that make up the differential diagnosis of CRPS, thereby diminishing any differentiating power serum sIL-2R may have in the diagnosis of CRPS. Moreover, as stated in the introduction, elevated serum sIL-2R levels are not disease-specific as elevated levels of sIL-2R can be found in many different diseases (12, 13).

In this study, we have confirmed our previous finding of elevated serum sIL-2R levels in CRPS, indicating that T-cell activation is involved in the pathogenesis of CRPS (8-10). It was further observed that the group of neuropathic pain syndromes was also associated with elevated serum sIL-2R levels, indicating that T-cell activation is likely to be involved in these pain syndromes. In line with this, recent observations in animal models support an important role for T-cells in (the development of) neuropathic pain (20, 21). We also found significantly higher sIL-2R levels in the myofascial-pain-syndromes group than in the group of healthy controls. This may be related to the various diagnoses we categorized into this group. For reasons of simplicity, we categorized diseases as myofascial pain syndromes if they were not considered neuropathic or 'classically inflammatory' by nature. However, it is not unthinkable that certain diseases we classified in this group, such as osteoarthritis, could reveal increased sIL-2R levels (table 3) (22). Nevertheless, studies in larger cohorts should separately explore the contribution of T-cells to the various diseases categorized into the group of myofascial pain syndromes.

Interestingly, we further found a statistically significant negative correlation between sIL-2R levels and the CRPS severity score in our CRPS patients. We propose three explanations for this negative correlation in our cohort of CRPS patients. First, it is possible that serum sIL-2R level reflects T-cell driven inflammatory disease activity (the intensity of the inflammatory process) rather than disease severity (the impact of the disease activity on the limb) in CRPS. Such would indicate that serum sIL-2R level measured in CRPS may be strongly related to the phase of disease. Patients in the acute phase of CRPS, often present with the warm subtype of CRPS (2, 23). As the disease progresses and becomes chronic, most patients undergo a change from a warm (acute) subtype to a cold (chronic) subtype (24). It is thought that this subtype transition is caused by a change in active underlying pathophysiological mechanisms during the course of this syndrome. For example, inflammatory mechanisms seem to be most prominent in the warm (acute) CRPS subtype and seem to diminish as the disease progresses (24). However, (tissue) damage inflicted by the early inflammatory phase may persist and even worsen because of other pathophysiological mechanisms that gain the upper hand. Considering that all CRPS patients in this study had chronic CRPS, it is possible that in this group of chronic CRPS patients, T-cell mediated inflammatory disease activity, and thus sIL-2R level, has diminished over time while the damage caused by this activity — the disease severity — remains extensive. It would be

interesting to test this hypothesis with serial measurements of sIL-2R in a prospective cohort of acute CRPS patients.

Second, this negative correlation may be explained by an immunosuppressive biological function of sIL-2R. The sIL-2R is the circulating form of the α -chain of the membrane-bound high-affinity trimeric interleukin-2 (IL-2) receptor. IL-2 is an important regulatory cytokine for the activation, proliferation, differentiation and survival of different T-cell subsets (12, 25, 26). It has been suggested that circulating sIL-2R competes for available IL-2 and may limit activation and proliferation of T-lymphocytes by sequestration of available IL-2 (12, 25, 27-31). It has further been proposed that sIL-2R presents IL-2 to CD4⁺T-helper cells, thereby inducing T-cell differentiation towards anti-inflammatory T-regulatory cells (Tregs) instead of pro-inflammatory Th1 or Th17 cells (25, 32). Considering that the discovered negative correlation suggests a higher sIL-2R is associated with less disease severity, it can be hypothesized that sIL-2R may have an immunosuppressive, and thus protective, biological function in CRPS. This idea is partially supported by the findings in the study by Heyn et al. in which the authors found a significantly lower percentage of pro-inflammatory Th17 cells, a lower Th17/Tregs ratio and a significantly higher proportion of anti-inflammatory CD39⁺Tregs in a group of CRPS patients, suggesting an anti-inflammatory T-cell shift in CRPS (9).

Third, the negative correlation may reveal an inability of our clinical observations to objectify a possible T-cell mediated inflammatory pathology and the related disease activity and severity in CRPS. This inability of our clinical observations to reflect an underlying pathology could explain the discrepancy between biochemical changes and clinical findings that is often found in CRPS.

Thus, although in our current study serum sIL-2R seems to lack diagnostic value when it comes to differentiating CRPS from other pain conditions of a limb with a similar presentation, it seems that this marker may have a potential role in the monitoring of disease activity and/or severity of CRPS. This warrants future research in which the relationship between serum sIL-2R levels and disease activity and severity of CRPS are explored.

We made two interesting observations in this study: first, our current study population had a relatively long disease duration; and second, at the time of measurement, patients who suffered from temperature changes all had a cool limb. Our Center for Pain Medicine is a tertiary referral center and it seems that the cases that are referred to us are usually the cases that are refractory to therapy and can be considered to have chronic (cold type) CRPS based on the disease duration. Thus, a limitation of our study is that there may be a referral bias in the study population resulting in a patient sample that may not be completely representative of the general CRPS patient population. Therefore, it is not unlikely that if this study were to be replicated in another setting such as a secondary hospital where patients are seen at an earlier stage and/or with a warm limb, it might return different results. We therefore suggest that future research replicate this study in a primary or secondary care setting. Furthermore,

future research should also focus on measuring other inflammatory markers in CRPS, for example, cytokines or other soluble surface molecules secreted from activated immune cells.

Another limitation of our study is that the current sample size was calculated based on the effect-size which was derived from our first study in which we investigated whether there was a difference in serum sIL-2R levels between CRPS patients and healthy controls (8). The observed effect-size from this previous study was rather large and may have led to an underestimation of the required sample size for the current study. Therefore, this study may have been underpowered for the primary outcome: the difference between sIL-2R levels in the CRPS group and no-CRPS group. Furthermore, we chose not to conduct corrections for multiple testing with regard to the secondary outcomes as it may have barred the discovery of potential associations that could be of interest to explore in future research.

Despite the limitations mentioned above, we believe that the greatest strength of our study is the selection of the study population. All patients included in this study were suspected of having CRPS. Therefore, our no-CRPS group consisted of various diseases that can display the same symptoms and signs as CRPS in a limb. Thus, our study design closely reflects clinical practice, especially in a tertiary care setting, and could be used as a model for replication studies.

CONCLUSION

In summary, we conclude that serum sIL-2R cannot be used in a tertiary referral setting to differentiate CRPS from other pain conditions of a limb in patients referred with a suspicion of CRPS. Our current findings confirm the findings from our previous study in which serum sIL-2R levels are shown to be higher in CRPS patients than in healthy controls, suggesting a role for pathogenic T-cell activation in CRPS (8).

Although serum sIL-2R may not be useful in establishing the diagnosis CRPS, future studies should focus on replicating this study in a primary and/or secondary care setting and should further focus on exploring the relationship between sIL-2R and (T-cell mediated) disease activity and disease severity in CRPS. These explorations could reveal a possible role for sIL-2R as a biomarker for disease activity and/or severity in CRPS and could further reveal a possible role for sIL-2R as a biomarker for selection of (anti-inflammatory) therapies in CRPS.

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Chapter 6

Elevated serum soluble CD163 indicates macrophage activation in Complex Regional Pain Syndrome

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ABSTRACT

Not much is known about the role of tissue-resident macrophages, and the monocyte-macrophage system, in the pathophysiology of Complex Regional Pain Syndrome (CRPS). However, studies into the role of these immune cells are warranted as various findings, such as increased local levels of the cytokines tumor necrosis factor(TNF)- α and interleukin(IL)-6 which are primarily released from local pro-inflammatory M1 macrophages, point towards a contribution of these cells to CRPS (immuno)pathophysiology. Examination of tissue-resident macrophages in CRPS is, however, seriously hampered by the fact that the process of obtaining tissue specimens from CRPS patients is perceived by the patients as extremely painful. An easily measurable, systemic marker for pro-inflammatory tissue-resident macrophage activation is soluble(s)CD163. In this retrospective cohort study, we determined sCD163 levels as a marker for tissue-resident macrophage activation in venous blood of CRPS patients and healthy controls. Twenty-two CRPS patients and 27 healthy controls were included for analysis. The median sCD163 level of the CRPS group was significantly higher than that of the healthy control group: CRPS 960.5 pg/ml (Q3-Q1: 1211.5-623.9) versus healthy controls 677.0 pg/ml (Q3-Q1: 828.0-469.5), $p=0.008$. Our findings show that sCD163 is increased in CRPS patients indicating activation of tissue-resident macrophages and thus the monocyte-macrophage system in CRPS. This system represents an interesting new target for future research on diagnosis and therapy in CRPS.

INTRODUCTION

Complex Regional Pain Syndrome (CRPS) is primarily a limb-confined syndrome that is mostly preceded by trauma to the affected limb. It is characterized by continuous pain in the affected limb which is accompanied by sensory, motor, vasomotor, sudomotor and trophic disturbances (1). CRPS is diagnosed using the new IASP clinical diagnostic criteria (1). There are still no objective biomarkers or tests to diagnose this disease. The treatment of CRPS is conducted in a mechanism-based manner: it targets the active pathophysiological mechanisms in each CRPS case (2).

The pathophysiology of CRPS is still incompletely understood. It is generally accepted that multiple mechanisms play a role in the onset and maintenance of CRPS (3). An important, and well-established, pathophysiological mechanism in CRPS is dysregulation of the immune system. Dysregulation of both the innate and adaptive branch of the immune system has been described in CRPS (4). Involvement of the adaptive immune system in CRPS is reflected by a higher prevalence of various autoantibodies, such as autoantibodies against autonomic nervous system autoantigens and anti-nuclear antibodies (5-8), and an altered regulation of T-cells, which is reflected by findings such as significantly lower absolute numbers of cytotoxic CD8⁺ T cells in CRPS patients than in healthy controls, significantly higher levels of the T-cell-specific soluble interleukin-2 receptor (sIL-2R) in serum of CRPS patients than in healthy controls, and a decreased number of pro-inflammatory Th17 cells and an increased proportion of CD39(+)Tregs in CRPS patients than in healthy controls (9-11). Involvement of the innate immune system in CRPS is supported by the increase in circulating CD14⁺CD16⁺ pro-inflammatory monocytes and elevated levels of the innate cytokines tumor necrosis factor(TNF)- α and interleukin(IL)-6 in blister fluid of CRPS affected limbs (12-14). These data point towards monocytes, and their descendent tissue-resident macrophages, as important cellular components of the (immuno)pathophysiology of CRPS. This is further supported by studies that indicate that the monocyte-macrophage compartment may be a valuable target for therapy in CRPS: studies have shown that Thalidomide, a potent immunomodulatory and anti-inflammatory drug with inhibitory effects on TNF- α production, especially from monocytes, seems to be effective in a subgroup of CRPS patients (15-18). Therefore, measuring the activity of the monocyte-macrophage system could be of value in the diagnostic work-up and the monitoring of disease activity and therapy efficacy in CRPS.

Although alterations in peripheral blood monocyte subset distribution can be indicative of inflammatory activation, it does not necessarily reflect pathological activation of macrophages within the tissues (19). Tissue-resident macrophages are important immune cells in maintaining tissue homeostasis and can react rapidly to disturbances in homeostasis, as seen under physiological or infectious stress. At sites of tissue injury, macrophages orchestrate the local inflammatory response and are responsible for scavenging cellular debris and mediating

tissue repair (19, 20). Not much is known from human studies about the role of tissue-resident macrophages in CRPS. In a recently published study using a mouse model— the chronic post ischemia pain model (CPIP) — of CRPS, it was shown that macrophages may play a role in sustaining chronic neuroinflammation and allodynia in CRPS (21). Although necessary caution must be exercised when extrapolating findings from animal models to humans, these findings, together with previously mentioned findings, warrant further investigation into the role of macrophages in the pathophysiology of CRPS. Assessing the activation of tissue-resident macrophages could lead to a better understanding on the role of the monocyte-macrophage compartment in the (local) inflammatory processes that take place in CRPS.

Examination of tissue-resident macrophages in CRPS is, however, seriously hampered by the fact that the process of obtaining tissue specimens from CRPS patients is perceived by the patients as extremely painful. In line with this, is the limited availability of histopathological studies on CRPS tissue so far, of which none, to our knowledge, explored macrophages (22-24). Yet, the supposed contribution of macrophages to CRPS pathophysiology warrants further studies on this topic, preferably using minimally invasive measures. Circulating soluble (s)CD163 is a systemic marker of pro-inflammatory activation of tissue-resident macrophages, which is easily measurable in serum using ELISA. Soluble CD163 is the circulating, extracellular portion of the membrane receptor CD163 (25), which is the high affinity scavenger receptor for haptoglobin-hemoglobin complexes. This receptor is solely expressed on monocytes and macrophages. Upon activation by pro-inflammatory stimuli, CD163 is enzymatically cleaved from the macrophage surface by ADAM17, the enzyme that also releases TNF- α into the circulation (25-27). In healthy individuals, circulating levels of sCD163 are considerably low, but during inflammation and macrophage activation, levels of sCD163 acutely increase (25). Soluble CD163 is now considered a useful biomarker of macrophage activation in various inflammatory and infectious conditions and may correlate with disease activity in diseases such as rheumatoid arthritis and sarcoidosis (25, 28-31).

In this study, we investigated whether there are indications of tissue-resident macrophage activation in CRPS by measuring serum sCD163 levels in venous blood of CRPS patients and healthy controls. Furthermore, we explored the relation between sCD163 and sIL-2R in CRPS.

MATERIALS AND METHODS

Ethical approval

This retrospective cohort study was approved by the Medical Ethics Committee of Erasmus MC University Medical Center Rotterdam (MEC-2020-0716).

Study population and data collection

The study population consists of patients with CRPS of one limb who were diagnosed at the Center for Pain Medicine using the new IASP clinical diagnostic criteria for CRPS (1), and a group of healthy controls.

Venous blood samples of CRPS patients (n=22) and healthy controls (n=27) were collected during previous studies that were conducted at the Center for Pain Medicine (MEC-2017-495(32)) and the Medical Immunology Laboratory (MEC-2016-202 (28)), respectively, at Erasmus MC University Medical Center Rotterdam. All participants included for analysis in this current study signed informed consent forms declaring their data and blood samples could be stored and used for future research.

For the CRPS group, data on demographics, pain scores (11-point Numeric Rating Scale: NRS), sIL-2R levels, signs and symptoms and CRPS disease severity as measured using the CRPS severity score-Database Form (33) were available from our previous study (32). Soluble CD163 levels and sIL-2R levels of healthy controls were available from a previous study and used for the analysis in this study ((28) MEC-2016-202). Demographic data of the healthy controls were unavailable for analysis in this study.

sCD163 analysis

Serum sCD163 was measured in CRPS patients using ELISA (Trillium Diagnostics/IQ Products BV, Groningen, the Netherlands) according to the manufacturer's instructions, without modification. Results are expressed in picograms/milliliter (pg/ml). Serum sCD163 levels of healthy controls were previously measured using the same technique and the data were available for analysis from a previous study (28).

sIL-2R analysis

Serum sIL-2R levels of the CRPS patients and healthy controls were available from previous studies (MEC-2017-495 (32); MEC-2016-202 (28)) and were used for analysis in this study. Serum sIL-2R levels were previously determined using ELISA (Diaclone, Besancon, France), according to the manufacturer's instructions, within the diagnostic facility of the Laboratory Medical Immunology, Erasmus MC Rotterdam and under strict quality regulations (ISO15189).

Statistical analysis

Descriptive statistics were used to determine the frequencies of categorical variables and to describe measures of central tendency and variability of continuous variables. A Shapiro-Wilk test was used to analyze whether continuous variables were normally distributed.

The primary outcome parameter of this study was the sCD163 level in the CRPS and the healthy control group. The secondary outcomes were the 1) association between sCD163

levels and sIL-2R levels between and within both groups and 2) the association between sCD163 level and CRPS severity score in the CRPS group.

Dependent on the shape of their distribution, continuous variables were compared across the CRPS group and healthy control group using either an independent samples T-test or an independent samples Mann-Whitney-U test. Categorical variables were compared using a Fisher's exact test.

The association between sCD163 level and sIL-2R level and sCD163 level and CRPS severity score was explored using either a Pearson correlation or Spearman's rank correlation depending on the shape of the distribution of these variables. In addition, in CRPS patients, the association between sCD163 and age, duration of disease and pain scores at the time of visit was also explored using either a Pearson correlation or Spearman's rank correlation depending on the shape of the distribution of these variables. The association between sCD163 and gender was explored using a point-biserial correlation.

Variables with a normal distribution are expressed in means and standard deviations (sd). Variables with a skewed distribution are expressed in medians and interquartile ranges (Q3-Q1). Categorical variables are expressed in frequencies. Analyses were conducted using IBM SPSS Statistics 25. The alpha level for statistical significance was set at 0.05.

RESULTS

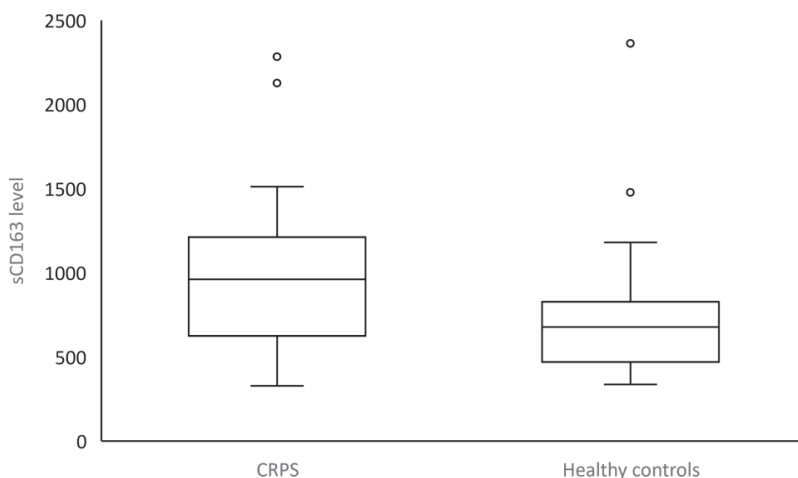
The characteristics of the CRPS group are presented in Table 1. The median sCD163 level of the CRPS group was significantly higher than that of the healthy control group: CRPS 960.5 pg/ml (Q3-Q1: 1211.5-623.9) versus healthy controls 677.0 pg/ml (Q3-Q1: 828.0-469.5), $p=0.008$ (Figure 1). The median sIL-2R level in the CRPS group was 2889.5 pg/ml (Q3-Q1: 3960.8-1592.8). The median sIL-2R level of the healthy controls was 2115.0 pg/ml (Q3-Q1: 2842.0-1757.0).

There was a statistically significant positive correlation between sCD163 and sIL-2R in the whole study population, i.e. the CRPS group and healthy controls: $r_s = 0.512$, $p < 0.001$. Further analysis of this association between sCD163 and sIL-2R revealed a statistically significant positive correlation in only the CRPS group ($r_s = 0.577$; $p=0.005$, Figure 2), but not in the healthy control group ($r_s = 0.359$; $p=0.066$).

In the CRPS group, no association was found between sCD163 and the CRPS severity score: $r_s = -0.324$, $p=0.14$. No association was found in the CRPS group between sCD163 and age ($r_s = 0.040$, $p=0.86$), gender ($r_s = -0.037$, $p=0.87$), durations of signs and symptoms ($r_s = -0.390$, $p=0.07$) and pain score at the time of visit ($r_s = 0.060$, $p=0.79$).

Table 1 Characteristics of CRPS group

	CRPS group (n=22)
Age in years (median, Q3-Q1)	42.5 (55.5-29.5)
Duration of disease in months (median, Q3-Q1)	26.0 (81.8-14.8)
Gender (n, %)	
Male	4 (18.2)
Female	18 (81.8)
NRS at time of visit (mean, sd)	7.1 (\pm 1.25)
Precipitating injury (n, (%))	
Trauma	10 (45.5)
Operation	9 (40.9)
Other	2 (9.1)
Unknown	1 (4.5)
Affected limb (n, (%))	
Right upper limb	4 (18.2)
Right lower limb	4 (18.2)
Left upper limb	4 (18.2)
Left lower lib	10 (45.5)
Temperature of affected limb during physical examination (n, (%))	
Warm	0
Cold	7 (31.8%)
No difference	15 (68.2%)
CRPS severity score (mean, sd)	11.2 (sd \pm 1.97)

**Figure 1** Boxplot of sCD163 level by group: CRPS 960.5 pg/ml (1211.5-623.9) vs healthy controls 677.0 (828.0-469.5), $p=0.008$

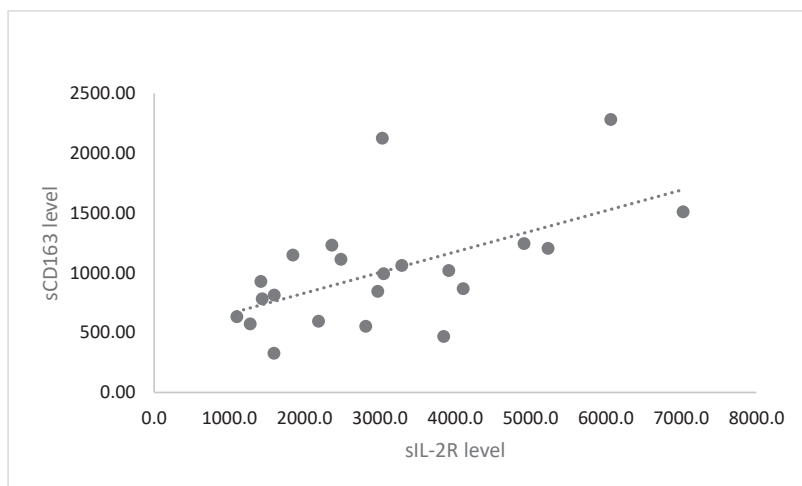


Figure 2 Scatterplot of the sIL-2R level and sCD163 level in the CRPS group ($r_s = 0.577$; $p = 0.005$)

DISCUSSION

In this study, we explored whether there are indications of activation of tissue-resident macrophages in CRPS by means of measuring serum sCD163 levels in CRPS patients and comparing these to those of healthy controls. The results from this study showed that serum sCD163 levels were significantly higher in CRPS patients than in healthy controls. This indicates enhanced activity of tissue-resident macrophages and further supports involvement of the monocyte-macrophage system in the pathophysiology of CRPS. In addition, a significant positive correlation was found between sCD163 and sIL-2R in the CRPS group. Taken together, our findings support activation of both the innate and adaptive immune system in CRPS.

To our knowledge, this is the first study to measure serum sCD163 levels in CRPS patients. Therefore, a direct comparison of the measured sCD163 levels in our study with other studies is not possible. However, there are multiple findings that support our finding of activation of the monocyte-macrophage system in CRPS. First, increased levels of TNF- α and IL-6, which are cytokines primarily released from local pro-inflammatory M1 macrophages (34, 35), have been found in blister fluid of the affected limb in CRPS patients (12). Notably, sCD163 is cleaved from the macrophage membrane upon activation by pro-inflammatory stimuli by the enzyme ADAM17/TACE (tumor necrosis factor α -converting enzyme), the same enzyme that releases TNF- α into the circulation (25, 26). Second, an increase in circulating levels of the pro-inflammatory CD14⁺CD16⁺ monocytes in CRPS patients has been found (14). These CD14⁺CD16⁺ monocytes have been shown to produce high levels of pro-inflammatory cytokines such as TNF- α , but low levels of anti-inflammatory

IL-10 (36). Third, beneficial clinical effects of Thalidomide, an anti-inflammatory and immunomodulatory drug that exerts its effects in part by inhibition of TNF- α production by monocytes, have been reported in CRPS patients (15, 16, 18). Thus, our results strengthen the notion that macrophages and monocytes may be important cellular components in the pathophysiology of CRPS.

Although we did not find a statistically significant correlation between sCD163 and CRPS disease severity in our CRPS group, we observed that the correlation that was found was a weak downhill (i.e. negative) correlation. Despite not being statistically significant, this finding is in line with findings in our previous article in which sIL-2R was shown to have a significant negative correlation with CRPS disease severity (32). Although not part of our primary results, we performed a post-hoc analysis of the correlation of sIL-2R and CRPS disease severity and also found a moderate negative correlation, although statistical significance was not achieved possibly due to this study being underpowered for this result: $r_s = -0.422$, $p = 0.051$ (a posteriori power $(1 - \beta) = 0.47$). Previously, we proposed, based on this negative correlation between sIL-2R and CRPS disease severity, that sIL-2R may be a marker for inflammatory disease activity (the intensity of the inflammatory process) and not disease severity (the damage caused by this inflammatory process) and thus may be related to the phase of disease (i.e. acute vs chronic) it is measured in (32). This hypothesis may also be applicable for sCD163: sCD163 may reflect inflammatory disease activity and not disease severity in CRPS. This hypothesis should be tested using serial measurements of this marker in early stage CRPS with a follow-up into chronic CRPS.

We further found a statistically significant moderate positive correlation between sCD163 and sIL-2R in our CRPS group. This finding indicates activation of both the monocyte-macrophage system and T-cell system in CRPS. As far as we are aware, this is the first study to measure both markers in a cohort of CRPS patients and the first study to indicate activation of both systems, at the same time, in CRPS patients. These findings indicate that in CRPS, there may be a dysregulation of both the innate and adaptive immune response. A recently published article has brought into perspective both the innate and adaptive immune components that may contribute to the pathophysiology of CRPS (4). Our findings further support this notion that both the innate and adaptive immune system play a role in CRPS.

Taken together, the current study shows that CRPS is associated with tissue-resident macrophage activation, as indicated by the elevated serum level of sCD163. These activated macrophages are considered to contribute to inflammatory disease pathology in CRPS and represent a new target for research on the diagnosis and management of CRPS. In addition, the findings from this study warrant further exploration of sCD163 as a potential new biomarker for diagnosis and therapy in CRPS. As this marker can be measured relatively easily in blood, it is less invasive than gathering tissue specimens to analyze macrophage activation. Further, sCD163 correlated positively with sIL-2R in CRPS patients, suggesting simultaneous stimulation and activation of both the monocyte-macrophage system (i.e. in-

nate immune system) and T-cell system (i.e. adaptive immune system) in CRPS. Based on this finding, it is possible that clinical therapies targeted against inflammation in CRPS may need to target one or both of these systems, however, future research still needs to explore whether sCD163 and sIL-2R can be used for therapeutic stratification in CRPS. It is already known that sIL-2R cannot be used to diagnose CRPS from other pain conditions, at least in a tertiary care setting (32). Such a study would also need to be conducted for sCD163 to explore its diagnostic value in CRPS.

Our study is not without limitations. First, this study is a retrospective cohort study with a single measurement of sCD163 level in CRPS patients with a relatively long disease duration (i.e. chronic (cold) type CRPS). Thus, conclusions cannot be drawn on changes in the level of this marker during the course of disease. Furthermore, patients in the acute phase of CRPS, i.e. with a short disease duration, often have an inflammatory phenotype (3), and inflammation is deemed to be most active in this phase of disease. It is thus not unthinkable that, should this marker be measured in a group of patients in the acute phase of CRPS, the levels may differ from the levels in our group of chronic CRPS patients. Second, in this study, only sCD163 for tissue-resident macrophage activation and sIL-2R for T-cell activation were measured. Therefore, this study does not reflect the full spectrum of activation of the different macrophage and T-cell subsets. Third, because demographic data of healthy controls was unavailable, patients and controls could not be matched on age and gender. However, we consider it unlikely that matching would have significantly influenced or changed the results of this study.

CONCLUSION

Soluble CD163 is increased in CRPS patients indicating activation of tissue-resident macrophages and thus the monocyte-macrophage system in CRPS. This system represents an interesting new target for diagnosis and therapy in CRPS and should be explored in future research. In addition, the role for sCD163 as a biomarker for diagnostic and therapeutic management in CRPS should be explored. Further, there is a significant positive correlation between sCD163 levels and sIL-2R levels in CRPS patients, indicating activation of both the innate (i.e. monocyte-macrophage) and adaptive (i.e. T-cell) immune system in CRPS. Future research should focus on exploring the contributions of these two systems to the pathophysiology of CRPS as this could have important clinical implications with regards to the initiation and selection of anti-inflammatory therapies in this syndrome.

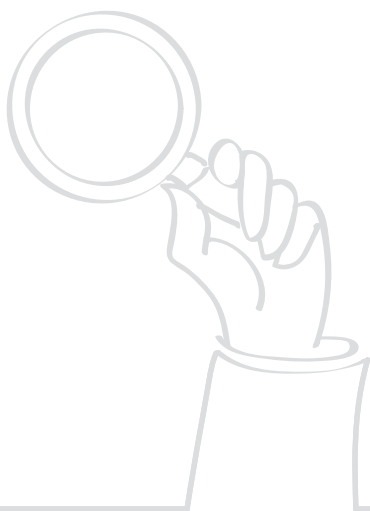
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Part 3



Chapter 7

Denying the truth does not change the facts: a systematic analysis of pseudoscientific denial of Complex Regional Pain Syndrome

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ABSTRACT

Background and objective: Several articles have claimed that complex regional pain syndrome (CRPS) does not exist. Although this is a minority view, it is important to understand the arguments presented in these articles. We conducted a systematic literature search to evaluate the methodological quality of articles that claim CRPS does not exist. We then examined and refuted the arguments supporting this claim using up-to-date scientific literature on CRPS.

Databases and Data treatment: A systematic search was conducted in MEDLINE, EMBASE and Cochrane CENTRAL databases. Inclusion criteria for articles were (a) a claim made that CRPS does not exist or that CRPS is not a distinct diagnostic entity and (b) support of these claims with subsequent argument(s). The methodological quality of articles was assessed if possible.

Results: Nine articles were included for analysis: 4 narrative reviews, 2 personal views, 1 letter, 1 editorial and 1 case report. Seven points of controversy were used in these articles to argue that CRPS does not exist: 1) the label 'CRPS'; 2) the 'unclear' pathophysiology; 3) the validity of the diagnostic criteria; 4) CRPS as a normal consequence of immobilization; 5) the role of psychological factors; 6) other identifiable causes for CRPS symptoms; and 7) the methodological quality of CRPS research.

Conclusions: The level of evidence for the claim that CRPS does not exist is very weak. Published accounts concluding that CRPS does not exist, in the absence of primary evidence to underpin them, can harm patients by encouraging dismissal of patients' signs and symptoms.

INTRODUCTION

Complex regional pain syndrome (CRPS) is defined by the International Association for the Study of Pain as “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” (1). Reported incidence rates vary between 5.5 and 26.2 per 100.000 person-years and women are more often affected than men (2, 3). A definitive pathophysiology of CRPS is still not completely clear but knowledge regarding its pathophysiological mechanisms has progressed significantly in recent years. Despite these advances, there are still voices questioning the legitimacy of this condition.

In the past few years, several articles have been published that claim that CRPS does not exist as a distinct clinical and diagnostic entity. While the large majority of the scientific literature provides evidence supporting the existence of CRPS, it is nonetheless important to understand the arguments and concerns that are presented in these critical articles.

There is a risk that published accounts concluding that CRPS does not exist, despite the absence of primary evidence to underpin these claims, may harm patients by encouraging dismissal of patients’ CRPS signs and symptoms. Consequently, vulnerable patients might feel invalidated and misunderstood, appropriate treatment withheld and recovery jeopardized. Therefore, we propose that to the extent that these critical claims are inaccurate or misleading, such claims should be challenged to mitigate their potential adverse effects. In addition, understanding the basis of these critical arguments and concerns could potentially help guide future research and support improvements in diagnosis and therapy protocols.

To our knowledge, there has been no prior integration of the critical literature that denies CRPS as a diagnostic entity. Therefore, we conducted a systematic search of the literature to evaluate the methodological quality of articles that claim CRPS is not a legitimate condition. Based on the results of this literature search, we then employed an empirical approach to analyze and refute the arguments used to support the claim that CRPS does not exist.

METHODS

With the help of an experienced biomedical information specialist, we searched the MEDLINE, EMBASE and Cochrane CENTRAL databases from inception until the 4th of July 2019 for English-language articles debating the existence of CRPS. The search strategies can be found in Appendix 1. All article types were included in this review.

Two investigators (AK, KB) independently selected articles based on title and abstract. The full-text of the identified articles was then screened, and articles were selected for inclu-

sion in this review if a) a claim was being made that CRPS does not exist or that CRPS is not a distinct diagnostic entity, and if b) the claim was subsequently supported by arguments or if controversies were described. When there was disagreement between the two investigators regarding the inclusion of an article, a third investigator was consulted (FH). The reference lists of included articles were also screened for relevant studies.

The two investigators further assessed the quality of the included articles by examining the study designs. The methodological strength of included narrative reviews was scored using the Scale for the Assessment of Narrative Review Articles (SANRA). The following elements are included in the SANRA scale: the importance of the article, the aim, the literature search, referencing, scientific reasoning and the presentation of data. Scores were compared and consensus was reached on the final scores.

Finally, the two investigators summarized the arguments used to support the claim that CRPS does not exist and identified seven main points of controversy raised across the identified articles.

RESULTS

Search results

Our search yielded 1048 articles which were screened on title and abstract. This resulted in 13 articles that were eligible for full-text screening. The reference lists of the 13 articles were screened, and 3 additional articles were found which were also screened on full-text. Of the 16 articles assessed on full-text, 9 articles were included in this review and seven articles were excluded: one article because it was written in Dutch (4) and six articles because they did not primarily focus on the claim that CRPS does not exist (5-10) (Fig. 1). The 9 included articles consisted of 4 narrative reviews, 2 personal views, 1 letter, 1 editorial and 1 case report (see Table 1).

Quality assessment of narrative reviews

The outcome of the quality assessment of the four identified narrative reviews using the SANRA scale is provided in Table 2. It is notable that in only one review did the authors express their aim, albeit vague, for writing the article. Also, in all four reviews, it is not clear whether a literature search was conducted. Referencing was inconsistent or minimal in three of the four articles. Overall, the review of Chang et al. scored the highest, with eight out of twelve points. The second highest scoring article was the review by Borchers et al., with six out of twelve points. After that, Pawl et al. and Pearce et al. followed with four out of twelve points.

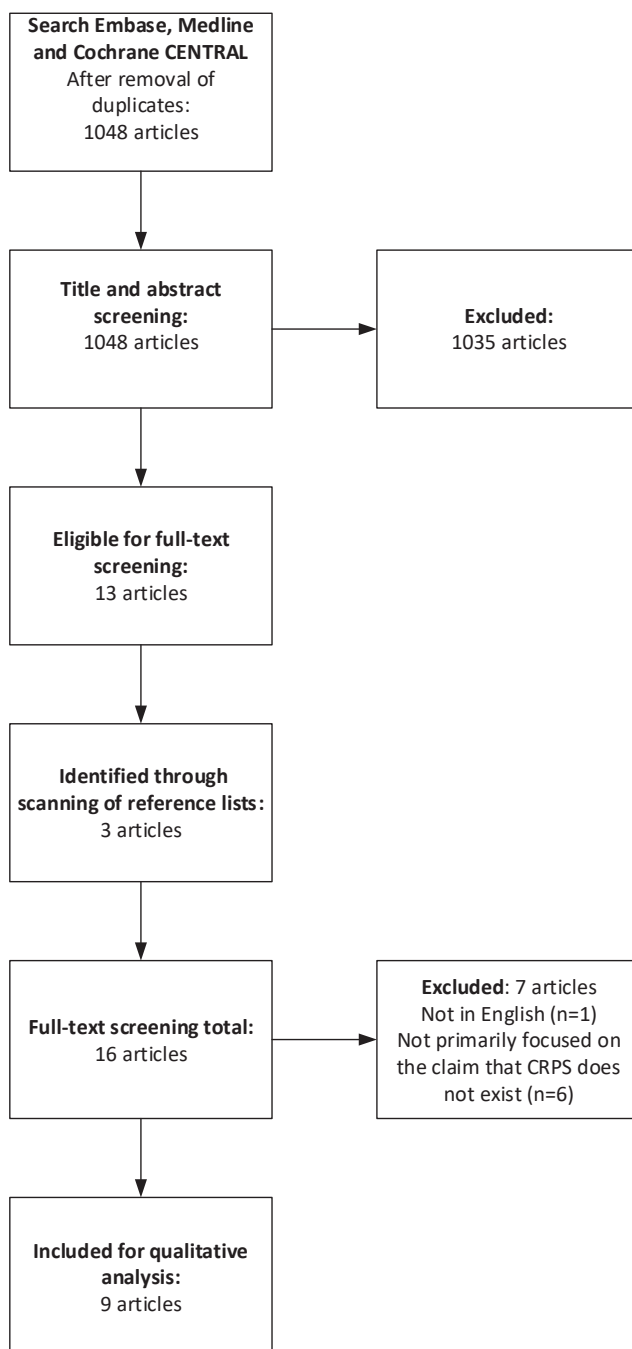


Figure 1 Flow diagram of article selection

Table 1 Studies included in systematic review

First Author	Article type	Title	Year	Journal
Basler	Letter	Diagnosis of complex regional pain syndrome needs to be tightened	2014	BMJ (Online)
Bass	Personal view	Complex regional pain syndrome medicalises limb pain	2014	BMJ
Borchers	Review	The clinical relevance of complex regional pain syndrome type I: The Emperor's New Clothes	2017	Autoimmun Rev
Chang	Review	Complex regional pain syndrome – False hopes and miscommunications	2019	Autoimmun Rev
Ek	Personal view (response)	Complex Regional Pain Syndrome type 1 does not exist. (can it be a normal reaction after immobilization?)	2014	BMJ
Pawl	Review	Controversies Surrounding Reflex Sympathetic Dystrophy: A Review Article	2000	Curr Rev Pain
Pearce	Review	Chronic regional pain and chronic pain syndromes	2005	Spinal Cord
del Piñal	Editorial	I have a dream... reflex sympathetic dystrophy (RSD or Complex Regional Pain Syndrome – CRPS I) does not exist	2013	J Hand Surg Eur Vol
Ring	Case report	Evidence-Based Medicine: Disproportionate Pain and Disability	2010	J Hand Surg Am

Table 2 Methodological strength of published narrative reviews assessed by the SANRA

	Borchers (2017)	Chang (2019)	Pawl (2000)	Pearce (2005)
Justification of the article's importance for the readership ^a	2	2	1	1
Statement of concrete aims or formulation of questions ^b	0	0	0	1
Description of the literature search ^c	0	0	0	0
Referencing ^d	1	2	1	0
Scientific reasoning ^e	1	2	1	1
Appropriate presentation of data ^f	2	2	1	1
Total score	6	8	4	4

^a 0=not at all; 1=superficially; 2=thoroughly. ^b 0=not at all; 1=vaguely; 2=clearly. ^c 0=not present; 1=briefly referred to; 2=present. ^d 0=sporadically; 1=inconsistently; 2=present for most/all key statements. ^e 0=hardly at all; 1=superficially; 2=thoroughly. ^f 0=hardly at all; 1=partially; 2=thoroughgoingly.

Points of controversy

Analysis of the nine included articles led to identification of seven different points of controversy that were used by the various authors to argue that CRPS is not a diagnostic entity (see Table 3). The arguments regarding the definition of CRPS and the diagnostic criteria were mentioned most often (by eight different authors). The argument that CRPS represents symptoms normally seen after immobilization was mentioned in five articles. Arguments that other causes explain the signs and symptoms of CRPS, that psychological factors cause CRPS, or that the pathophysiology is unclear are mentioned in four articles. One author mentioned weaknesses in the methodology of CRPS research.

Table 3 Critical arguments mentioned in each article.

	Basler (2014)	Bass (2014)	Borchers (2017)	Chang (2019)	Ek (2014)	Pawl (2000)	Pearce (2005)	Del Pinal (2013)	Ring (2010)
1) Definition	+	+	+	+	+	+	+		+
2) Pathophysiology			+	+			+		+
3) Diagnostic criteria	+	+	+	+		+	+	+	+
4) Psychological factors			+	+		+			+
5) Immobilization		+	+	+	+		+		
6) Other causes than CRPS			+	+				+	+
7) Methodology				+					

DISCUSSION

Concerning the methodological quality of the articles summarized above, it is remarkable that the identified articles consisted exclusively of narrative reviews, personal views, a letter, an editorial and a case report. The level of evidence presented by these articles against the existence of CRPS as a diagnostic entity is therefore weak. When assessing the quality of the narrative reviews more in depth, only two out of the four narrative reviews scored more than half of the possible points on the SANRA. This illustrates that the methodological quality of the narrative reviews was low.

The arguments used to support the claim that “CRPS does not exist” are summarized below, followed by counterarguments based on available scientific literature on CRPS that refute each argument.

1. CRPS should not be called CRPS

Seven articles criticize the label ‘CRPS’ for various reasons (11-18). It is stated that the “label” does not say anything about the underlying pathophysiology and that it medicalizes non-specific symptoms (11, 13, 17, 18). In the view of Borchers and Gershwin, CRPS is merely a description of signs and symptoms that can be observed in many other diseases (12), implying that CRPS is not a distinct diagnostic entity. From this critical perspective, once such a diagnosis that medicalizes non-specific symptoms is communicated to the patient, the physician’s diagnosis causes a nocebo effect within the patient, in which hearing about potential symptoms or negative treatment outcomes can induce or increase symptoms. Thus, it is argued that patients develop symptoms consistent with CRPS simply by learning about the diagnosis and the expected pattern of symptoms. This argument implies that patients who believe that they are ill with ‘CRPS’ then adopt the sick-role assigned to them (11). Subsequently, this nocebo effect can also encourage immobilization and pain avoidance behavior (16). Concerns are also raised that other psychosocial conditions which may be primary drivers of dysfunction are masked by applying the diagnostic label ‘CRPS’ to patients (17). It is also argued that the CRPS diagnostic label legitimizes the overuse of pain medication (13) and may contribute to secondary gain (e.g. sickness benefits) that then maintains the symptoms (15). Finally, these critics note that beliefs about CRPS-recovery influence the chronicity of the condition more than the severity of the injury itself (11), again implying that the condition is maintained by patients adopting the ‘role’ of the CRPS patient.

Refutation

The term CRPS was indeed coined specifically as a means to describe clinical signs and symptoms without implying any specific pathophysiology (19). CRPS is explicitly described as a syndrome, which is “a group of signs and symptoms that occur together and characterize a particular abnormality or condition” (Merriam-Webster Dictionary) (20). Although the criticism that CRPS is a descriptive label is true, this has no bearing on the existence of CRPS as a syndrome. In 1993, at the Orlando conference at which diagnostic terminology issues regarding the condition were first addressed in an international consensus format, the term CRPS was proposed because prior diagnostic labels, such as reflex sympathetic dystrophy (RSD), implied a specific pathophysiology that had been proven incorrect or incomplete (19). Therefore, there was a need for a new name independent of the presumed pathophysiology. The task force that created the diagnostic label CRPS was concerned that new scientific discoveries regarding the pathophysiology of this condition would constantly require a new classification or name.

Other objections concerning the label CRPS, such as the nocebo effect accounting for patients’ CRPS signs and symptoms, are based on the a priori assumption that CRPS does not, in fact, exist. Nocebo effects, in which the patient’s knowledge of expected symptoms

may induce or increase symptoms, are not unique to CRPS. The same criticism could potentially apply to any medical diagnosis, particularly to pain disorders due to the inherently subjective nature of pain. The extent of these nocebo effects depends on how physicians communicate with patients about symptoms, diagnoses and treatment expectations (21-24). There is no research to support the argument that providing CRPS as a diagnostic label for patients who meet Budapest criteria induces the nocebo effect any more than providing, for example, a migraine headache label for patients who meet criteria for that diagnosis.

Of course, secondary gain factors could contribute to chronic disease and influence symptoms in most chronic pain condition (e.g., chronic back pain; (25)). It is a logical fallacy to argue that documented individual cases, in which secondary gain appears to account for self-induced CRPS-like features (26), necessarily mean that CRPS features in all patients are accounted for by secondary gain.

2. There is no clear pathophysiology

In four papers, critical remarks are made regarding the lack of a definitive pathophysiology of CRPS (12, 13, 17, 18). Some of these arguments focus on the heterogeneity in signs and symptoms of CRPS. For example, patients may display either a cold or a warm extremity, with skin that may appear either blue or red. These very different signs and symptoms are interpreted as pointing towards very different pathophysiological mechanisms, which in turn lead the critics to believe that there can be no common underlying pathophysiological mechanism of CRPS (12, 15). Likewise, the variable clinical course of the disease leads to uncertainty about a common underlying pathophysiology (17). Even though different possible pathophysiological mechanisms are hypothesized in the literature, Chang et al. assert that a direct relationship between trauma and prolonged chronic CRPS has not been proven and that there is no biological explanation for the development of CRPS following minor traumas, such as cuts, bumps, contusions and bruises that occur in everyday life (13). Chang et al. further write that studies on CRPS often do not elaborate on details such as the inciting event or the duration of immobility, so the association between these factors and the development of CRPS is unknown (13). Interestingly, the second author in the manuscript by Chang et al. is a corporate defense attorney routinely defending large corporations against claims of injury-related CRPS. Thus, the arguments presented by Chang et al. must be considered in light of the potentially biased opinions of this author (13). Nevertheless, the confusion surrounding the causes and pathophysiology of CRPS must be addressed.

Refutation

Historically, the medical field has assumed psychological or psychosomatic origins of symptoms when there is a lack of knowledge or understanding of pathophysiological mechanisms. A typical example is dystonia like spasmodic torticollis. This movement disorder was long considered to be a psychogenic disorder (turning the head away) due to a lack of structural

changes. However, as research progressed, findings from electrophysiological and imaging studies revealed torticollis as a disorder of sensory-motor integration circuits in the brain, which can be successfully treated with botulinum toxin injections instead of ineffective psychotherapy which has been used for decades. Further, neurogenetic studies showed genetic involvement in certain subtypes of dystonia and at present, the classification of dystonia is even informed by neurogenetics (27).

Similar arguments apply to widely-accepted pain syndromes, such as fibromyalgia and chronic nonspecific low back pain, which have a pathophysiology that is multifactorial and not entirely clear (28-36). No credible sources argue that these conditions are not legitimate and/or are not distinct chronic pain conditions simply because their pathophysiology is not completely understood. While the pathophysiology of CRPS is also not completely understood, several mechanisms have been uncovered in recent studies, which may have been overlooked by the authors of these reviews (37, 38). It has become clear that patients meeting CRPS diagnostic criteria regularly display a pattern of findings indicating several likely pathophysiological mechanisms.

Although Chang et al.'s argument that there is no conclusive biological explanation yet for the development of CRPS following minor trauma, may in a strict sense be correct, there is evidence from both human and animal studies consistent with the idea that minimal distal nerve injury (such as might occur after minor trauma) may be involved in CRPS (39-42), even in the absence of obvious nerve dysfunction on clinical examination or electromyography (EMG)/ nerve conduction velocity (NCV) testing. Work in a post-fracture CRPS-Type I animal model further suggests that biochemical changes in the local milieu after injury such as B-cell activation, increased interleukin(IL)-1 β and enhanced substance P (SP) signaling, contribute to the development of CRPS after injury (43-45). Findings such as these certainly weaken arguments raised by critics that the occurrence of CRPS after minor injury is unexplainable and therefore existence of the condition itself is implausible. To further address claims that CRPS pathophysiology is unknown, we now briefly review the extensive research literature on CRPS mechanisms.

Autonomic Nervous System Mechanisms

A variety of studies indicate that autonomic nervous system alterations contribute to CRPS. CRPS is associated with both regional disturbances in sympathetic nervous system (SNS) activity in the affected limb (46) and a range of systemic autonomic disturbances and central changes in autonomic drive (47-49). Consistent with the latter, early bilateral reductions in SNS-mediated vasoconstrictor responses post-fracture prospectively identify patients who subsequently go on to develop CRPS, suggesting a potential contributory role (50, 51). Regarding specific autonomic changes, early studies suggested a regional reduction in turnover of the SNS neurotransmitter norepinephrine (52, 53), which together with upregulated adrenoceptor responses (54), might contribute to altered vasoregulation in the

CRPS affected limb. Evidence from more recent experimental studies evaluating the effects of the α 1-adrenoceptor agonist phenylephrine (46) and the α 1-adrenoceptor antagonist prazosin (55) in CRPS-affected limbs specifically support activation of α 1-adrenoceptors on primary afferent fibers by norepinephrine as a contributor to the allodynia and hyperalgesia associated with CRPS.

Central Nervous System Mechanisms

Evidence for central nervous system involvement in CRPS predominantly emerges from neuroimaging studies. Cortical reorganization, as evidenced by a reduction in size of the representation of the CRPS-affected limb in the somatosensory cortex compared with the contralateral limb, has been observed in CRPS and is thought to explain some sensory features of CRPS such as its glove- or stocking-like distribution (56-61). Furthermore, the degree of cortical reorganization has been shown to positively correlate with the pain intensity and the amount of hyperalgesia, as well as tactile acuity, in CRPS patients (56, 57, 59). However, it must be stated that these very consistent findings were recently challenged (62).

Patients with CRPS also exhibit impairments in endogenous pain inhibitory pathways in the brain (63, 64), increased glucose metabolism in pain-related brain regions (65), and alterations within the default mode network during resting-state fMRI (66, 67). Longitudinal studies suggest that successful treatment of CRPS may normalize changes in brain structure and function, such as a reversal of primary somatosensory cortex reorganization (58); an increase in gray matter in the dorsolateral prefrontal cortex, thalamus, basal ganglia, amygdala, and hippocampus and enhanced functional connectivity between the dorsolateral prefrontal cortex and the periaqueductal gray, a primary source of endogenous pain inhibition (68).

Inflammatory Mechanisms

Growing evidence indicates a role for inflammatory changes, including autoimmunity, neurogenic inflammation and neuroinflammation as a contributor to CRPS. For example, recent brain imaging studies suggest increased microglial activation in CRPS patients, and thus a potential role for neuroinflammation in CRPS (69, 70). In the periphery, multiple studies indicate that patients with CRPS exhibit a pro-inflammatory cytokine pattern (71-81). These pro-inflammatory changes have been observed at multiple biological levels, ranging from mRNA (76) to suction blister fluid and skin biopsies obtained from the CRPS-affected extremity (71, 81-83).

Neurogenic inflammation occurs when peptidergic primary afferents are activated and release SP or calcitonin gene-related peptide (CGRP). These substances mainly bind to the neurokinin-1 (NK-1) receptor and the calcitonin receptor-like receptor (CRLR), respectively, and they induce protein extravasation, vasodilatation and warming of the skin, all of which are characteristic features of CRPS. In early posttraumatic CRPS, patients exhibit enhanced electrically evoked plasma extravasation and vasodilatation responses (84). In chronic CRPS,

the total number of epidermal nerve fibers including the peptidergic nerve fibers is reduced in most cases (39) or unchanged in some (85). Furthermore, the anti-inflammatory protease network in patients with CRPS may be less effective than in both healthy controls and other chronic pain conditions. Evidence for this comes from CRPS-linked differences in degradation pathways for the inflammatory mediator bradykinin (86) and increased sensitivity of SP receptors (87) in CRPS patients' affected limbs compared to their contralateral limbs.

Immune Mechanisms

Autoimmune processes appear to represent another mechanism that may contribute to CRPS, based on the presence of antinuclear antibodies and surface binding immunoglobulin G (IgG) serum-autoantibodies in a substantial subset of patients with CRPS (88-90) and abnormalities in both circulating and tissue-resident T-cell sub-populations (91, 92). Autoimmune mechanisms are further supported by studies using a passive transfer model demonstrating that administration of IgG from CRPS patients can induce CRPS-like features (e.g., prolonged swelling, hyperalgesia) in animals following injury (93), a finding that depends on spinal IL-1 β mediated mechanisms (37). Nociceptors (A-delta and C fibers), but not touch-related fibers, are abnormally activated in mice following transfer of IgG from donor CRPS patients, with IgG from CRPS patients with severe pain eliciting the strongest mechanical hyperalgesia in mice (94). Passive transfer of serum immunoglobulin M from patients in the early stages of CRPS also consistently elicits hyperalgesia in a passive-transfer tibia fracture-casting model (95). Importantly, the changes induced in these passive transfer models are strictly unilateral — affecting only the injured rodent limb — and are 'invisible' to normal assessment methods: there is no systemic inflammatory response and no regional tissue destruction; the main antibody effect is limb-confined pain-sensitization (93-95).

Genetic Factors and Epigenetic Changes

Familial aggregation of CRPS cases suggests a potentially heritable component of CRPS risk (96-98). The most consistent genetic findings regarding CRPS suggest genetic differences in the human leukocyte antigen (HLA) system: the system underlying the adaptive immune response (99-105). Epigenetic modifications provide an additional path for transmitting CRPS risk, and the most widely studied of these is DNA methylation (106, 107). A recent study revealed 48 differentially methylated cytosine-phosphate-guanine dinucleotide (CpG) sites between CRPS and non-CRPS neuropathic pain patients, despite comparable pain intensity, with the top two sites being in the *COL11A1* and *HLA-DRB6* genes (108). The latter finding mirrors the genetic evidence for HLA system involvement in CRPS, as well as results of a small study suggesting differential gene expression in the *HLA-DRB6* gene between CRPS patients and healthy controls (109). This work also revealed significantly enriched methylation in immune-related genetic pathways, consistent with a role for immune mechanisms in CRPS (108).

Putting the Pieces Together

In summary, extensive literature using a variety of research methods indicates that a set of mechanisms reflecting autonomic, brain plasticity, inflammatory, immune, and genetic/epigenetic factors, is consistently associated with a diagnosis of CRPS (110, 111). If CRPS pain and symptoms were purely the result of being given a label, or represented broad nonspecific complaints or underlying psychosocial issues, there would be no reason for the studies summarized above to show consistent patterns of association with a CRPS diagnosis compared to a non-CRPS pain diagnosis, to show unilateral clinical and biochemical changes, or for these changes to reverse in some cases with CRPS-focused treatment. Critics argue that seemingly very divergent clinical presentations, such as a warm/red limb versus a cold/blue limb, imply opposite mechanisms and therefore different pain conditions. However, as substantiated above, enhanced inflammation and altered autonomic function provide mechanisms that could explain both abnormally warm and cold skin. Results of a large prospective clinical study of CRPS, including both acute CRPS and chronic CRPS patients, demonstrated that the characteristic warm CRPS presentation evident in acute CRPS often changes over time within individuals to the cold CRPS presentation more common in chronic CRPS (112). Furthermore, it has been shown that signs and symptoms of CRPS are dynamic, i.e., can change at different time points dependent for example on surrounding temperature (113) and that different clinical phenotypes of CRPS exist (114).

Other prospective work suggests that elevations in inflammatory mediators (cytokines and peptides), which can contribute to edema as well as skin redness, are present in acute CRPS patients but generally normalize within the first 18 months post-onset (81). Evidence that the presentation and mechanisms of CRPS can change over time within individuals argues for a single syndrome that evolves in character over time rather than these differing CRPS presentations reflecting two different, unrelated syndromes. Findings such as these are more consistent with clinical patient subtypes within the CRPS diagnostic label rather than distinct conditions.

3. The diagnostic criteria lack validity

A criticism often mentioned is the lack of validity of the diagnostic criteria for CRPS (11-15, 17, 18, 115) (See Table 4). Several problems are noted, including the specificity, validity, interobserver reliability, and objectivity of the criteria; the presence of different sets of criteria; and lastly the existence of a condition named CRPS-Not Otherwise Specified (NOS). The individual criteria are considered non-specific and subjective, and the symptoms described in the criteria (at least when considered individually) are very common in other diseases (11-13, 115). These critics argue that CRPS is often overdiagnosed, implying that this overdiagnosis relates to poor diagnostic criteria. These critics support this claim with a study in which CRPS was found to be diagnosed incorrectly in 72% of the cases by family physicians or community specialists (116), and another study with a similar design

which found that CRPS was diagnosed incorrectly in 77% of the cases (117). These critical authors argue that CRPS symptoms are grouped under the assumption that they share the same pathophysiology, but that this has not been objectified (13), and furthermore, that the symptoms can often be either decreased or increased, which indicates opposite activity of the underlying mechanism (15). Related to this is the argument that the somewhat heterogeneous clinical presentations and multiple mechanisms associated with CRPS imply that CRPS is a “garbage basket” diagnosis, and that its diagnostic criteria inappropriately group together multiple unrelated conditions (12). It is claimed that CRPS is a diagnosis of exclusion, but in scientific research it is unclear in what way and how thoroughly other diagnoses have been ruled out (12). One author even claims that only hand surgeons should be allowed to diagnose CRPS of the hand, because they are the only ones who are able to properly rule out other causes for the pain in the hand (115). While the point regarding overdiagnosis of CRPS has some merit, particularly with use of the older 1994 IASP criteria, the 2012 Budapest criteria have shown evidence of validity when properly applied (118).

However, there are also some points of criticism regarding the methods used to validate the Budapest criteria (118). The criteria were validated in a group of 113 CRPS-I patients and 47 non-CRPS neuropathic pain patients, and the relative ability to distinguish between CRPS patients and non-CRPS patients using the former IASP versus the new Budapest diagnostic criteria was tested (118). The first objection is that there is no gold standard against which the criteria can be validated (12). The second point of criticism is about the control group in this validation study, of which it is said that 85% had easily recognizable neuropathies in specific dermatomes without autonomic symptoms, making it uncomplicated to distinguish these conditions from CRPS (13). The third point is about the specificity, on which Chang et al. conclude that in 30% of cases a physician would incorrectly identify a patient with an uncomplicated neuropathy as having CRPS and confirm a diagnosis of CRPS in 99% of the cases with criteria that only have a specificity of 40% (13). Lastly, it is noted that the existence of the CRPS-NOS category enables everyone with chronic pain to be diagnosed with CRPS (12, 15).

Refutation

The studies cited above regarding dramatic rates of CRPS misdiagnosis do not indicate problems with the criteria themselves. Borchers and Gershwin (12) use the results of Mailis-Gagnon et al. (55) to argue broadly against CRPS as a diagnosis. In fact, results of both this study and the study by Frölke et al. (56) highlight problems in application of the diagnostic criteria rather than the criteria themselves, with misdiagnosis in both studies due in most cases to failure to properly rule out other conditions that might account for the signs and symptoms (criterion 4 of the Budapest criteria). It needs to be pointed out that a diagnosis of CRPS can only be made when all four criteria are fulfilled (See Table 4). Improper ap-

plication of the CRPS criteria, particularly by non-specialist clinicians, does not invalidate the condition as a diagnosis.

Other criticisms of the CRPS diagnostic criteria are in part justified, because there are indeed limitations to the criteria. Regarding comments that the criteria are too subjective, this is accurate with regards to the self-reported symptoms, and to some degree, certain signs that require patient input for evaluation (allodynia, hyperalgesia, weakness, limited active range of motion). However, by their nature, existence of pain and descriptions of pain characteristics are always subjective. There is no objective tool yet that can be used to validate the presence of specific pain characteristics. Other diagnostic signs including changes in skin color, temperature, sweating and edema are truly objective (observable by clinicians without patient input). Partial reliance on self-report of subjective symptoms for diagnosis is, however, common in all psychiatric conditions and many medical conditions, particularly those involving an inherently subjective phenomenon such as pain (119-121). In contrast to the previous (1994) IASP criteria in which the diagnosis could be made based on self-reported symptoms only, the 2012 IASP (Budapest) criteria explicitly require that clinical signs be present, with this change implemented specifically to address this subjectivity issue.

Regarding methodology, validation of the Budapest criteria as done by Harden et al. (118), in which the new criteria are compared to old criteria in order to validate the new criteria, can indeed be considered limited because these older criteria cannot truly serve as a gold standard. These efforts to validate the CRPS criteria therefore addressed incremental validity rather than absolute validity. Research is being carried out into a possible role for biomarkers in CRPS (122-124), but because no definitive biomarker has yet been found, a clear gold standard for diagnosis remains elusive. At present, CRPS is a clinical diagnosis that can in some cases be supported by objective tests, such as three-phase-bone scintigraphy (125). Arguments highlighted above would imply that there should be no diagnostic criteria for CRPS until a definitive biomarker has been identified, or further, that the condition should not be considered valid until a gold standard biomarker is identified. This argument runs contrary to accepted practice in other medical (e.g., primary headache disorders, seronegative rheumatic arthritis) and psychiatric diagnostic systems, in which similar methods to those used to validate the CRPS criteria are considered standard and acceptable even in the absence of a clear gold standard (126).

We do not view the criticism of the control groups used in validation of the CRPS criteria as justified, because it is inevitable that control groups in scientific research do not consist of the 'borderline' cases, as such cases minimize the ability to identify real group differences. The results of the Harden et al. validation study showed a sensitivity of 0.99 and a specificity of 0.68 for the Budapest criteria, which is a substantial improvement compared to the 1994 IASP criteria (118). From one perspective, this means that in 32% of the cases the diagnosis of CRPS is still made in patients that do not actually have CRPS, a situation that is less than ideal. Nonetheless, the Harden et al. validation work indicated that patients meeting the

Budapest criteria for CRPS can be distinguished from other neuropathic pain conditions with a reasonable degree of specificity (0.68) (118). This highlights that despite the absence of a clear gold standard, the signs and symptoms in the criteria, taken as a set, are reasonably distinct from non-CRPS neuropathic pain conditions (i.e., CRPS looks different than non-CRPS pain). This issue of treating criteria as a set of features is crucial – while individual criteria may be common to other disorders, a point highlighted by critics, their occurrence in combination with each other in a single patient is what distinguishes CRPS from other pain conditions. The current diagnostic criteria (i.e. the Budapest criteria), despite the flaws mentioned above, remain the only validated tool to diagnose CRPS.

From a broad perspective, criticism of the methods used to validate CRPS as a means of dismissing the condition are unwarranted. Until recently, efforts to create validated criteria for diagnosing chronic pain disorders in a systematic way were limited to very few specific conditions (e.g., headache disorders, fibromyalgia). In this sense, the validated criteria for CRPS, even if limited, are a step beyond criteria available for diagnosing most other chronic pain conditions, yet no one seems to be arguing that those other pain conditions do not exist simply because they do not have validated criteria. Validation of the CRPS criteria has in fact been used as an exemplar in ongoing efforts to create a comprehensive, validated chronic pain taxonomy (126, 127).

Criticisms that focus on the existence of multiple competing diagnostic criteria are easily dismissed. The international standard for CRPS diagnosis is the criteria contained in the official updated (2012) IASP taxonomy. These are the only criteria that have been subjected to systematic validation research, even if such research has limitations. All other CRPS diagnostic criteria were published by individual authors and are not endorsed by any large organization of pain professionals. Clinicians and researchers who rely on these other diagnostic criteria are not using state-of-the-art diagnostic criteria, and CRPS diagnoses using these other criteria are questionable.

Criticisms of CRPS-NOS as a “diagnosis” are valid, and this term is subject to potential misinterpretation. CRPS-NOS was added to the taxonomy to parallel standard practice in the much more well-developed psychiatric diagnostic taxonomy at the time (i.e., DSM-IV). As with psychiatric diagnosis, it was intended to be a descriptive label for patients not fully meeting CRPS criteria who appeared to show features of CRPS and in whom no other condition could be identified to explain the symptoms. It is not a formal diagnosis and does not have any diagnostic criteria, so it cannot be validated. Valid criticisms of the descriptive term CRPS-NOS, however, imply nothing about the validity of the actual CRPS criteria.

Table 4. Budapest clinical diagnostic criteria for CRPS (118)

1. Continuing pain, which is disproportionate to any inciting event
2. Must report at least one symptom in <i>three of the four</i> following categories: <ul style="list-style-type: none"> • <i>Sensory</i>: reports of hyperesthesia and/or allodynia • <i>Vasomotor</i>: reports of temperature asymmetry and/or skin color changes and/or skin color asymmetry • <i>Sudomotor/edema</i>: reports of edema and/or sweating changes and/or sweating asymmetry • <i>Motor/trophic</i>: 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 <i>two or more</i> of the following categories: <ul style="list-style-type: none"> • <i>Sensory</i>: evidence of hyperalgesia (to pinprick) and/or allodynia (to light touch and/or deep somatic pressure and/or joint movement) • <i>Vasomotor</i>: evidence of temperature asymmetry and/or skin color changes and/or asymmetry • <i>Sudomotor/edema</i>: evidence of edema and/or sweating changes and/or sweating asymmetry • <i>Motor/trophic</i>: 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

4. Psychological factors play a role in the development of CRPS

Categorizing CRPS as primarily a psychological disorder is done in two different ways, namely by stating that CRPS is caused by psychological factors or stating that CRPS is a psychiatric disease. Pawl et al. (15) consider psychosocial factors to play a significant role in the generation of CRPS, because psychiatric comorbidity and personality abnormalities are claimed to be more often seen in CRPS patients than in healthy people (128). Borchers et al. suggest that CRPS is a psychiatric disorder that requires psychological therapy as the primary form of treatment (12). The authors point out that many of the signs and symptoms of CRPS can be easily artificially induced, that it is impossible to distinguish CRPS from somatoform disorders or malingering, and that there is a great deal of secondary gain for patients (12). The secondary gain specifically reveals itself in the medico-legal setting (13).

Refutation

The conclusion drawn by Pawl et al. in the year 2000, namely that psychosocial factors play a role in the generation of CRPS, can be refuted by more recent studies. A systematic review from 2009 shows that there is no association between a variety of psychological factors and the development of CRPS and that only the occurrence of stressful life events is associated (not necessarily causally) with CRPS (129). Additionally, a prospective study was carried out in 596 patients presenting with a single fracture in the emergency room which found no relation between standard psychological factors and subsequent development of CRPS (130). Moreover, in a prospective study of patients following knee arthroplasty surgery, there

was no association between preoperative psychosocial status and CRPS status at 6 month follow-up (131). Furthermore, a retrospective case-control study assessing the medical history of CRPS patients prior to the onset of disease found that psychological factors were not associated with CRPS (132). Finally, we note that there is no doubt that the intensity of pain symptoms in CRPS may be associated with psychosocial factors (e.g., enhancing or enhanced by negative affect). However, links between psychosocial factors and chronic pain status are well-known to occur across chronic pain syndromes as a class (133-136).

We disagree with the idea that CRPS cannot be distinguished from somatoform disorders for two reasons. The first reason is the difference in clinical presentation, as somatoform disorders are characterized by excessive thoughts, feelings or behaviors related to the somatic symptoms in the absence of significant physical findings, whereas in CRPS this is not the case. That is, a somatoform disorder would not be preferentially diagnosed rather than CRPS for a patient showing sufficient objective signs of CRPS (edema, warm and red skin, allodynia), even if they showed a high level of distress and intense focus on those symptoms. Second, in CRPS, objective biochemical changes are found (detailed above). These point to an underlying pathophysiology that would not be seen in psychiatric (somatoform) disorders. Furthermore, the fact that there may be malingerers does not undermine the existence of CRPS, because malingerers can be seen in any field of medicine.

5. Symptoms of CRPS are a normal reaction to immobilization

In five different articles, the authors draw the conclusion that CRPS is just a normal reaction to immobilization based on studies that find temperature and color differences after short-term immobilization (11-13, 16, 17). Borchers and Gershwin also point out that normal healing is associated with pain and inflammation and that recovery can take a long time for some patients (12).

Refutation

The three articles cited by critics to prove that CRPS symptoms are a normal reaction to immobilization, when examined in detail, were not intended to refute the existence of CRPS (45, 137, 138), but to explain underlying pathophysiology. All immobilization models used for the investigation of CRPS pathophysiology evoke some but not all symptoms of CRPS. In the study by Terkelsen et al., the authors draw the conclusion that forearm immobilization (via casting) does not represent a complete human model of acute CRPS (137) and in the study by Guo et al., the authors draw the conclusion that in their animal model, immobilization via casting only causes a CRPS-like syndrome of brief duration (45). Singh et al. found that immobility and dependency cause temperature and color differences, but they did not try to model CRPS (138). The only conclusion that can possibly be drawn from these studies is that immobilization may play a role in generating signs and symptoms of CRPS. However, this is no controversy as activation and mobilization to counteract immobilization is a

generally accepted treatment for CRPS (139, 140). Furthermore, in the study by Terkelsen et al., forearm immobilization in humans induced cold and mechanical hyperalgesia, but spontaneous pain was absent on cast removal and during the follow up period of 28 days (137). The other two studies did not study the effect of immobilization on pain (45, 138). In the absence of studies demonstrating that immobilization alone can induce not only skin color and temperature changes, but also intense chronic pain for months or years, arguments attributing CRPS solely to immobilization are pure speculation. In conclusion, these articles cannot be used as an argument to support claims that CRPS does not exist.

6. There are always other causes that explain the CRPS symptoms

In order to diagnose someone with CRPS, other causes for the signs and symptoms should be excluded. However, two authors firmly state that there is always another cause for the signs and symptoms of patients, leading to the conclusion that the diagnosis of CRPS can almost never be made (12, 115). The list of causes they consider for CRPS-like symptoms consists of unstable fractures, subclinical nerve entrapments, dysvascular states, pain-triggering pathology such as glomus tumors, varicella zoster reactivation, Lyme disease, mononeuropathies, and psychiatric conditions. Further, one author states that complications of surgery are masked when diagnosing the pain with CRPS, because the doctors can easily blame CRPS as the cause of the symptoms while the actual cause lies in mistakes or complications created by the doctors themselves (13).

Refutation

Critics' statements that there is always another cause other than CRPS for signs and symptoms that look like CRPS are made entirely as a matter of belief, with no evidence to support this, and make the unwarranted a priori assumption that CRPS does not exist. The list of differential diagnoses for CRPS is long and consists of the following categories of disease: neuropathic pain-like syndromes, myofascial pain syndromes, inflammation, vascular diseases and psychological disorders (140). These other causes for the signs and symptoms must be considered thoroughly and diagnostic tests can be used to exclude other disorders (141). However, after doing so, there still remain patients who meet the criteria for CRPS and who do not have any of the other disorders as mentioned by Borchers and Gershwin (12) and Del Piñal (115). These are the patients that fulfil the 2012 IASP (Budapest) criteria for CRPS. Eventually, even Del Piñal admitted that there may be a number of patients with CRPS (115) and we share this view.

7. Methodology in CRPS studies is flawed

Although only one article pays attention to the methodology in CRPS studies (13), we still chose to include this argument in this review because we consider this an important point. Chang et al. claim that good quality scientific studies on CRPS with a proper sample size do

not exist, because CRPS is so rare that it is impossible to include sufficient participants in a study (13). He supports this argument by calculations that researchers would need to follow 400,000 participants for 5 years to see 100 cases of CRPS develop of which only 26 patients would develop chronic CRPS.

Refutation

We agree that the rarity of this disease often makes it difficult to achieve optimal sample sizes in CRPS research studies. However, this problem is not new and is shared by many other rare diseases. In addition, Chang et al. base their claim on the argument that it is impossible to include sufficient participants (and thus reach sufficient statistical power) with a disease incidence of 5.5 per 100,000 person years as found by Sandroni et al. (3). Although this calculation by Chang et al. is not incorrect, it is important to consider that incidence rates in person years are most applicable in the same (or similar) setting that they were measured in and cannot easily be generalized to other populations. This is illustrated by the higher CRPS incidence rate that was found in a study by de Mos et al. in a Dutch population: 26.2 per 100,000 person years (2). In addition, a Dutch prospective cohort study in 596 patients with a single fracture of a limb (rather than in the general population) showed a CRPS incidence rate of 7% using the Budapest criteria (142). If this incidence rate were to be used in the calculation by Chang et al., it would return a more positive outcome. Additionally, Chang et al.'s comments are applicable only to prospective studies seeking to study factors influencing the onset of CRPS. Other study designs, such as prospective studies of patients with recent onset CRPS can be used instead of prospective studies enrolling individuals before they develop CRPS, and these can achieve the required sample sizes much more easily (although these designs also have limitations). We agree that in common with other rare disorders, it can be challenging to reach the 'generally accepted' power of 80% and level of significance of 0.05 when comparing with controls, but increased focus on registries (143, 144), long duration studies and multisite studies to expand the pool of CRPS patients are ways around these issues and have been and are currently ongoing.

CONCLUSION

Articles have appeared repeatedly in the peer-reviewed literature that argue against the existence of CRPS as a distinct chronic pain syndrome. When such articles are read by clinicians not intimately familiar with the CRPS literature, the critical arguments raised may appear compelling on the surface, and lead to potential harm to patients in terms of both hindering a positive provider-patient relationship and delaying appropriate treatment. This review indicates that the criticisms raised by these authors, on the whole, are not supported by the CRPS evidence base. Critical arguments presented either ignore (intentionally or otherwise)

or selectively interpret the CRPS research literature, which provides ample evidence to refute the criticisms raised. In the highest quality critiques, many of the criticisms raised seek to apply higher standards of validation to CRPS than are applied to most other chronic pain conditions, many of which are also incompletely understood, yet no one disputes that they are real conditions. Contrary to what many authors suggest, research into CRPS is not led by blind-faith in the condition. Rather, we believe research into CRPS represents the core of science: trying to uncover the unknown. There is a grain of truth in some of the criticisms raised, and there are indeed limitations to the CRPS diagnostic criteria due to limitations of the scientific literature. It is hoped that this review, by presenting both criticisms and relevant literature to refute each, may help guide future research and enhance scientific understanding and the clinical care of CRPS patients.

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APPENDIX 1

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('complex regional pain syndrome'/exp/mj OR ((complex NEAR/3 regional NEAR/3 pain) OR crps OR (reflex NEAR/3 sympathetic NEAR/3 dystroph*)):ti) AND ('reliability'/de OR validity/exp OR 'conflict'/de OR 'health personnel attitude'/de OR 'diagnostic accuracy'/de OR 'public opinion'/de OR uncertainty/de OR editorial/de OR 'evidence based practice'/exp OR (reliab* OR unreliab* OR reproducib* OR controvers* OR debate OR objectivit* OR ambigu* OR inappropriat* OR validit* OR view OR views OR confus* OR conflict* OR conflict* OR dispute* OR argument* OR ((personnel* OR doctor* OR physician*) NEAR/3 attitude*) OR (diagnos* NEAR/3 accur*) OR unclear* OR opinion* OR uncertain* OR editorial* OR myth* OR Factitious OR evidence-based OR (systematic* NEAR/3 review*) OR (exist* NEAR/3 (does OR 'not'))):ab,ti OR response:ti) AND [english]/lim

Medline Ovid

(* complex regional pain syndromes/ OR ((complex ADJ3 regional ADJ3 pain) OR crps OR (reflex ADJ3 sympathetic ADJ3 dystroph*)):ti.) AND (Reproducibility of Results/ or Validation Studies as Topic/ OR "Conflict (Psychology)"/ OR Attitude of Health Personnel/ OR Public Opinion/ OR Uncertainty/ OR editorial.pt. OR Evidence-Based Practice/ OR (reliab* OR unreliab* OR reproducib* OR controvers* OR debate OR objectivit* OR ambigu* OR inappropriat* OR validit* OR view OR views OR confus* OR conflict* OR conflict* OR dispute* OR argument* OR ((personnel* OR doctor* OR physician*) ADJ3 attitude*) OR (diagnos* ADJ3 accur*) OR unclear* OR opinion* OR uncertain* OR editorial* OR myth* OR Factitious OR evidence-based OR (systematic* ADJ3 review*) OR (exist* ADJ3 (does OR "not"))).ab,ti. OR response.ti.) AND english.la.

Cochrane CENTRAL

((complex NEAR/3 regional NEAR/3 pain) OR crps OR (reflex NEAR/3 sympathetic NEAR/3 dystroph*)):ti) AND ((reliab* OR unreliab* OR reproducib* OR controvers* OR debate OR objectivit* OR ambigu* OR inappropriat* OR validit* OR view OR views OR confus* OR conflict* OR conflict* OR dispute* OR argument* OR ((personnel* OR doctor* OR physician*) NEAR/3 attitude*) OR (diagnos* NEAR/3 accur*) OR unclear* OR opinion* OR uncertain* OR editorial* OR myth* OR Factitious OR evidence-based OR (systematic* NEAR/3 review*) OR (exist* NEAR/3 (does OR 'not'))):ab,ti OR response:ti)

Chapter 8

General Discussion

SUMMARY OF THIS THESIS

The aim of this thesis was threefold: 1) to explore the need for diagnostic and therapeutic biomarkers in Complex Regional Pain Syndrome (CRPS); 2) to study the role of the T-cell-specific sIL-2R and macrophage-specific sCD163 as potential biomarkers in CRPS; and 3) to address (recent) concerns that CRPS is not a distinct diagnostic entity. To this end, this thesis was divided into three parts with each part exploring one of the three sub-aims.

In Part 1 (chapters 1, 2 and 3), the need for diagnostic and therapeutic biomarkers in CRPS was explored. In Chapter 1, the introduction of this thesis, the importance of biomarker research in CRPS is explained and the aim of this thesis was outlined. In chapter 2, a concise and up-to-date overview on the diagnosis and management of CRPS is presented which also highlights the lack of objective tests to diagnose and manage this syndrome. In Chapter 3, an extensive review is given on the various potential biomarkers of inflammation that have been identified in CRPS and their current place in the diagnosis and management of CRPS (chapter 3).

In Part 2 (chapters 4, 5 and 6) we build on the findings from part 1 through hands-on investigation of two potential immunological biomarkers in CRPS: the T-cell-specific soluble interleukin-2 receptor (sIL-2R) and the macrophage-specific soluble CD163 (sCD163). In chapter 4, we showed that levels of sIL-2R were significantly increased in CRPS patients suggesting pathological activation of T-lymphocytes. In addition, this marker had a high differentiating capacity between CRPS patients and healthy controls. In chapter 5, we explored whether this marker could be used to differentiate patients with CRPS from patients with other pain conditions of a limb in a tertiary referral setting. We found that this marker could not differentiate between these two groups, however, that there still may be a role for this marker in the monitoring of inflammatory disease activity and/or severity in CRPS. In Chapter 6, we measured levels of the macrophage-specific sCD163 in CRPS patients and a group of healthy controls and found that this marker was significantly higher in the CRPS group, suggesting activation of local tissue-resident macrophages and thus the monocyte-macrophage system in CRPS.

Finally, in Part 3 (chapters 7 and 8), we addressed recently published critical articles that claimed that CRPS is not a distinct diagnostic entity (Chapter 7). The arguments presented in these critical articles are refuted in a review article using the extensive empirical literature available on CRPS. The need to address these accounts stems from concerns that these critical articles may be harmful to patients by encouraging dismissal of their CRPS signs and symptoms and further leading to patients feeling invalidated and misunderstood. Moreover, they may lead to appropriate treatment being withheld and may jeopardize recovery of patients. It becomes clear that the level of evidence for the arguments used to argue against CRPS being a distinct diagnostic entity is weak to very weak and should be taken with

a grain of salt. Part 3 ends with this current chapter, in which we summarize the major findings from this thesis and make recommendations for future research.

TYING IT ALL TOGETHER

Complex regional pain syndrome is a syndrome that is usually preceded by tissue injury to the affected limb, for example due to trauma, fracture, or surgery (1, 2). It is characterized by continuous pain, that is accompanied by sensory, vasomotor, sudomotor/edema, and motor/trophic disturbances (3). The diagnosis CRPS is made using the new International Association for the Study of Pain clinical diagnostic criteria for CRPS (3). There is currently no diagnostic test available to diagnose this syndrome and diagnostic tests are mainly used to exclude other diagnoses in the differential diagnosis of CRPS. It is advised to conduct treatment in a mechanism based manner, i.e., treatment should target the prominent underlying pathophysiological mechanisms in each CRPS case. If treatment is not initiated in a timely manner, CRPS can lead to a debilitating loss of function of the affected limb and can severely impact the quality of life of patients (4, 5).

Early diagnosis and therapy in CRPS are, however, hampered by the fact that there are no objective tests available to diagnose this syndrome, nor are their objective tests available to identify the underlying pathophysiological mechanisms to subsequently manage this syndrome. The demand for these objective tests can be met by researching potential clinical and biochemical biomarkers of the various identified pathophysiological mechanisms in CRPS. Mechanisms that have been identified in CRPS are inflammation (neurogenic, immune, neuroinflammation), central and peripheral sensitization, altered sympathetic nervous system function, changes in circulating catecholamines, brain plasticity changes, contribution of genetic factors and psychological factors (3, 6).

Various articles, both original and review articles, have been published indicating potential clinical and biochemical biomarkers of these mechanisms in CRPS (6, 7). The main setback is that most of these biomarkers have not yet been validated in CRPS, nor for the diagnosis nor for the management of this syndrome. It is, however, clear that because of the multi-mechanism pathophysiology of CRPS, not one biomarker will be available to diagnose or manage this syndrome. It is likely that in the future, a panel of biomarkers, be it clinical and/or biochemical, will be used to diagnose and manage this syndrome.

In this thesis, we focused on the role of biomarkers of inflammation, especially due to immune dysregulation, in CRPS. Inflammation is an important pathophysiological mechanism in CRPS, both for the onset and maintenance of this syndrome. Inflammation in CRPS seems to originate from three sources: neurogenic inflammation, neuroinflammation and inflammation due to dysregulation of the immune system (6). The latter has been demonstrated both locally — in the affected limb — and systemically, and both the

innate and adaptive immune system have been shown to be dysregulated in CRPS (8). An example of dysregulation of the innate immune system is the finding that local levels of the pro-inflammatory cytokines tumor necrosis factor (TNF)- α and interleukin(IL)-6 are increased in CRPS affected extremities (9). An example of dysregulation of the adaptive immune system is the higher prevalence of various autoantibodies that has been found in CRPS patients (10-13). Thus, both the innate and adaptive immune system represent interesting targets for biomarker research in CRPS.

In this dissertation, two new biomarkers of inflammation in CRPS are studied and presented: soluble IL-2R as a marker for T-cell activity and thus dysregulation of a component of the adaptive immune system; and soluble CD163 as a marker for tissue-resident macrophage activation and thus dysregulation of a component of the innate immune system.

The soluble interleukin-2 receptor is an easily measurable systemic marker for T-cell activation (14, 15). Increased levels of this marker have been found in diseases in which T-cell activity is centrally involved, such as rheumatoid arthritis and sarcoidosis, and have been shown to correlate with disease activity in these diseases (16-21). Furthermore, the diagnostic value of sIL-2R was recently demonstrated for sarcoidosis in a large retrospective study: the sIL-2R had a sensitivity of 88% and specificity of 85% for the detection of sarcoidosis, which is by far superior to the classical diagnostic biomarker angiotensin-converting enzyme (ACE; sensitivity 62%, specificity 88%) (22). In CRPS, significantly higher levels of sIL-2R were found than in healthy controls, indicating pathological activation of T-cells in CRPS patients (23). The sIL-2R, however, showed no discriminative capacity between patients with CRPS and patients who were initially suspected of having CRPS but were diagnosed with another (pain)condition (24). This was unsurprising as most of the syndromes and diseases that make up the differential diagnosis of CRPS (25) have been shown to have altered T-cell activity (24).

Interestingly, sIL-2R had a statistically significant negative correlation with CRPS disease severity in our CRPS group (24). This negative correlation led us to hypothesize that sIL-2R may be a suitable marker for inflammatory disease activity (the intensity of the inflammatory process) and not disease severity (the impact of disease activity on the affected limb) in CRPS. In addition, this would also indicate that serum sIL-2R level may be related to the phase of disease it is measured in. In the acute phase of CRPS, patients often present with a warm CRPS subtype which is characterized by the classic signs of inflammation, such as redness, swelling, warmth, edema and loss of function of the affected limb (1, 2, 26). As the syndrome progresses and transitions into the chronic phase, a majority of the patients experience a transition from a warm (acute) subtype to a cold (chronic) subtype which is characterized by a less edematous, cold, and blue/pale limb (26). This transition in subtypes is thought to be caused by a transition in active pathophysiological mechanisms during the course of this syndrome. For example, it appears that inflammatory mechanisms are most prominent in the acute phase of this syndrome in which a warm subtype presentation is

usually observed (26). During the course of this syndrome, these inflammatory mechanisms seem to diminish, however, the (tissue) damage caused by these inflammatory mechanisms persists and may even worsen due to other pathophysiological mechanisms gaining the upper hand.

Based on this transition in pathophysiological mechanisms and the negative correlation between sIL-2R and CRPS disease severity, we propose a disease-model for the relationship between T-cell mediated inflammatory disease activity and CRPS disease severity based on duration of CRPS. Figure 1 illustrates this disease-model in which as CRPS progresses, T-cell mediated inflammatory disease activity — and thus sIL-2R — diminishes over time, while (tissue) damage caused by this inflammatory activity — the CRPS disease severity — persists, and may even worsen due to other pathophysiological mechanisms gaining the upper hand. In our study in Chapter 5 (24), all patients had chronic CRPS with a median syndrome duration of 26 months. The median level of sIL-2R in this group was lower than that of the CRPS group described in Chapter 4 which had a median syndrome duration of 11 months (23) and can thus be considered to have a relatively short (acute) duration of CRPS. Therefore, based on this careful observation and based on the proposed disease-model hypothesis, it is conceivable that, T-cell mediated inflammatory disease activity, and thus sIL-2R level, diminishes over time. In line with this notion that sIL-2R may represent disease activity in CRPS, serum sIL-2R has previously been shown to be a marker of disease activity in several immune and rheumatic diseases, including sarcoidosis, rheumatoid arthritis and IgG4-related disease (16, 18, 20, 27). It would be interesting to test this disease-model hypothesis with serial measurements of sIL-2R in a prospective cohort of acute CRPS patients. It is clear, however, that T-cells, and thus the adaptive immune system, are involved in the pathophysiology of CRPS and future research should focus on determining in which phase T-cells are active in CRPS so that appropriate therapies such as steroids can be started in a timely manner.

The other biomarker assessed in this thesis was sCD163, which is a marker for tissue-resident macrophage activation, and by default activation of the monocyte-macrophage system and thus innate immune system in CRPS. Soluble CD163 is the circulating, extracellular portion of the CD163 membrane receptor for haptoglobin-hemoglobin complexes which is solely expressed on monocytes and macrophages. Soluble CD163 is enzymatically cleaved from the macrophage surface during activation by various pro-inflammatory stimuli (28-30) and is considered to be a useful biomarker of macrophage activation in various inflammatory diseases, such as macrophage activation syndrome, sepsis, liver disease and obesity (28, 31, 32). This marker is also easily measurable in serum using a validated ELISA system. In our group of CRPS patients, we found that serum levels of sCD163 were significantly higher than in healthy controls, suggesting activation of tissue-resident macrophages in CRPS.

Interestingly, sCD163 also correlated negatively with CRPS disease severity, although this correlation did not achieve statistical significance in our CRPS patient sample. In addi-

tion, there was a significant positive correlation between sIL-2R and sCD163 in the CRPS group. This latter finding suggests simultaneous activation of the monocyte-macrophage and T-cell system in CRPS. While the use of sCD163 in the diagnosis and management of CRPS still needs to be validated in further studies, we can possibly explain the place of this marker in the diagnosis and management of CRPS by placing it in our pathophysiological model (Figure 1, see red bar indicating other inflammatory mechanisms). It is possible that, as with sIL-2R, sCD163 represents inflammatory disease activity and not disease severity in CRPS and the level of this marker may differ during different phases of this disease. Further, it is also possible, seeing that a statistically insignificant, weak downhill correlation was found between sCD163 and CRPS disease severity, that the local macrophage system, and thus innate immune system, remains active for longer during the biological course of this syndrome, thereby, running somewhat parallel to the disease course and contributing, at least partially, to the disease severity in later phases of the syndrome. This is indirectly supported by previous findings by our group in which patients with the cold (chronic) CRPS subtype were shown to have local, i.e. skin blister fluid, TNF- α levels that did not differ from patients with the warm (acute) CRPS subtype (33). Serial measurements of sCD163 in a cohort of acute CRPS patients are needed to test this hypothesis.

Taken together, our findings show that there is pathological activation of both the monocyte-macrophage system and T-cell system in CRPS and thus a dysregulation of the innate and adaptive immune system in this syndrome. Serial measurements of sIL-2R and sCD163 in a prospective cohort of acute CRPS patients are needed to identify the place of these markers in the diagnosis and/or management of CRPS.

Ultimately, the pathophysiology of CRPS is a confluence of various mechanisms ranging from inflammation to endothelial dysfunction. Therefore, it is unlikely that there will ever be one biomarker that will be specific for CRPS, rather, in the future, multiple clinical and/or biochemical biomarkers will be used to assess which mechanisms are prominent in each CRPS case. However, the crux in forming such a biomarker panel still is the incomplete understanding of the pathophysiology of CRPS. Until the pathophysiology of CRPS becomes completely clear, numerous published and unpublished studies on CRPS are and will be focused on trying to understand the pathophysiological basis of this syndrome and subsequently on trying to objectify its diagnosis, and ultimately legitimizing its existence, possibly even as a disease.

LIMITATIONS OF THIS THESIS AND RECOMMENDATIONS FOR FUTURE RESEARCH

This thesis is not without its limitations. First, most of the conducted studies are cross-sectional studies. Therefore, conclusions cannot be drawn on changes in the levels of sIL-2R

and sCD163 during the biological course of this syndrome. Future studies conducting serial measurements of these markers in a cohort of acute CRPS patients, for example right after fracture, are warranted to assess not only the level of these markers in the acute phase, but also the role of T-cells and macrophages during the course of this syndrome. Second, there are various other inflammatory biomarkers that warrant testing in CRPS, this thesis focused on only two soluble markers of T-cell activation and macrophage activation. However, the choices for biomarker research are in some way limitless and both soluble and cellular markers for inflammation can be tested in future research. Third, the patient population in the studies in this thesis may not be representative of the whole CRPS population as the majority of patients had what can be considered chronic (cold) type CRPS. This was mainly related to the fact that our outpatient clinic center is a tertiary referral center which receives the most complex CRPS cases with a long duration of disease. Therefore, future research should focus on replicating this research in a primary and/or secondary care setting in which patients in the warm (acute) phase of CRPS are also included.

CONCLUSIONS FROM THIS THESIS

CRPS is a multi-mechanism syndrome and diagnosis and management should be focused on assessing and treating the mechanisms that are prominent in each CRPS case. Inflammation is a prominent mechanism especially in the early phase of CRPS, however, no inflammatory biomarkers have yet been validated to assess the level of inflammatory disease activity and severity in CRPS.

Serum levels of the soluble interleukin-2 receptor and soluble CD163 are increased in patients with CRPS indicating activation of both the T-cell system and macrophage system, respectively, in CRPS. These findings further support the notion that both the innate and adaptive immune system play a role in CRPS pathophysiology. Soluble IL-2R cannot be used in a tertiary referral setting to diagnose CRPS from other pain conditions of a limb, however, this marker may have a role in the assessment of inflammatory disease activity in CRPS. The latter is yet to be determined in future research. The value of sCD163 as a biomarker of diagnosis and management in CRPS is yet to be determined in future research.

Although there is skepticism surrounding the existence of CRPS, the amount of evidence supporting the existence of this syndrome and the level of this evidence significantly outweigh the arguments presented in recently published (pseudoscientific) articles suggesting this syndrome is fabricated. Therefore, this skepticism might even be considered to be misplaced. Patients with CRPS deserve to be heard and taken seriously regarding their symptoms and signs and future research on this syndrome should continue to unravel its pathophysiology and to find methods to objectify the diagnosis and management of this syndrome.

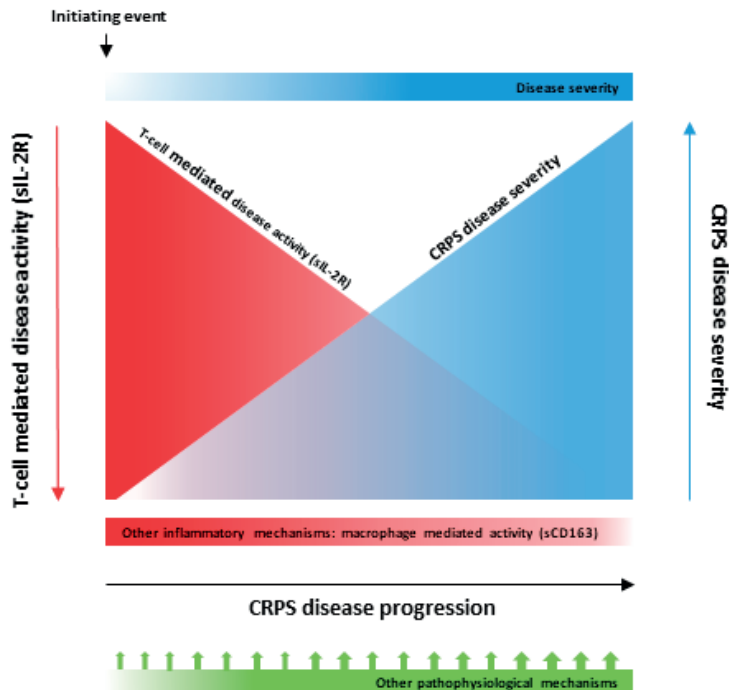


Figure 1 Hypothetical model of the relationship between T-cell mediated inflammatory disease activity and CRPS disease severity: as CRPS progresses, T-cell mediated inflammatory disease activity — and thus sIL-2R — diminishes over time, while (tissue) damage caused by this disease activity — the CRPS disease severity — persists, and may even worsen. Based on current understandings, inflammatory pathophysiological mechanisms seem to be most prominent in the warm (acute) CRPS subtype and seem to diminish as the syndrome progresses (26), however, (tissue) damage from these inflammatory pathophysiological mechanisms may persist and possibly worsen due to other pathophysiological mechanisms gaining the upper hand.

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Chapter 9

English Summary and Dutch summary

ENGLISH SUMMARY

Chapter 1 - General introduction

Chapter 1 introduces the rationale and aim of this thesis which is broadly to 1) highlight the need for biomarker research in Complex Regional Pain Syndrome (CRPS) and 2) address present skepticism on the existence of this syndrome. CRPS is still diagnosed using relatively subjective clinical criteria and there is no diagnostic test available yet to diagnose this disease. This subjectivity leads not only to a delay in diagnosis, but also to skepticism on the existence of this syndrome, both being issues which could be harmful to patients suffering from this debilitating condition. The identification of diagnostic (and therapeutic) biomarkers in CRPS would not only add an objective component to the diagnostic workup of CRPS, but also facilitate earlier identification and treatment of this syndrome. Although the exact pathophysiology of CRPS is yet to be established, it is now generally accepted that inflammation is an important mechanism not only in the onset, but also in the maintenance of CRPS. Therefore, inflammation is an interesting target for biomarker research in CRPS and the role of inflammatory biomarkers, especially for T cell and tissue-resident macrophage activity, in the diagnosis and management of CRPS is explored.

Chapter 2 - Complex Regional Pain Syndrome: diagnosis and treatment

In this chapter, a concise overview is given of the current understandings on the pathophysiology of CRPS and present recommendations for the diagnosis and treatment of CRPS are reviewed. Pathophysiological mechanisms that are discussed are inflammation, neurogenic inflammation, autoimmunity, ischaemia-reperfusion injury, genetic involvement, cortical reorganization, small fibre neuropathy and psychological factors. The cornerstones for diagnosis, which are history taking and physical examination, are discussed. Treatment is recommended to be conducted in a mechanism-based manner. Treatments that are discussed are anti-inflammatory drugs, analgetics/co-analgetics, vasodilators, muscle relaxants/spasmolytics, psychological intervention and invasive treatments.

Chapter 3 - Highlighting the role of biomarkers of inflammation in the diagnosis and management of Complex Regional Pain Syndrome

In this chapter, the role of inflammation in the multi-mechanism pathophysiology of CRPS is discussed and the application of potential biomarkers of inflammation in the diagnosis and management of this syndrome is highlighted. The reviewed biomarkers are divided into local biomarkers (TNF- α , IL-6, Tryptase, Mast cell numbers) and systemic biomarkers (CGRP, SP, CD14⁺CD16⁺ monocytes, CD8⁺ T lymphocytes, sIL-2R, autoantibodies and miRNA), with a further subdivision into cellular and soluble biomarkers. It becomes clear that, until date, no biomarker has yet been validated for use in the diagnosis and management of

CRPS, however, there are a number of promising biomarkers that warrant investigation in future research.

Chapter 4 - Elevated plasma levels of sIL-2R in Complex Regional Pain Syndrome: a pathogenic role for T-lymphocytes?

Not much is known about the role of T cells in the onset and maintenance of CRPS. In this retrospective cohort study, we evaluated T cell activity in CRPS by comparing blood soluble interleukin-2 receptor (sIL-2R) levels between CRPS patients and healthy controls. Soluble IL-2R is a marker for T cell activity and has been shown to be increased in diseases in which T cell activity is centrally involved, such as rheumatoid arthritis and sarcoidosis. CRPS patients had statistically significant higher levels of sIL-2R than those of healthy controls. Furthermore, sIL-2R level was found to be a good discriminator between CRPS patients and healthy controls with a high sensitivity and specificity. The findings from this chapter indicate increased T cell activity in CRPS patients suggesting a possible T cell mediated inflammatory disease process. Furthermore, this chapter introduces sIL-2R as a potential diagnostic biomarker in CRPS.

Chapter 5 - Serum soluble interleukin-2 receptor does not differentiate Complex Regional Pain Syndrome from other pain conditions in a tertiary referral setting

In this prospective cohort study, we explored whether sIL-2R could be used as a diagnostic marker in CRPS. To this end, sIL-2R levels were determined in patients who were referred to our pain center with a suspicion of CRPS. Subsequently, sIL-2R levels of the patients diagnosed with CRPS were compared with sIL-2R levels of the patients who were not diagnosed with CRPS. Soluble IL-2R levels of healthy volunteers were used as controls. The study confirmed the findings in Chapter 4: sIL-2R levels of CRPS patients are higher than those of healthy controls. Soluble IL-2R could not, however, be used to distinguish CRPS patients from patients with other pain conditions of a limb which were initially suspected to have CRPS. However, this does not exclude its use in monitoring inflammatory disease activity in CRPS; this should be explored in future research.

Chapter 6 – Elevated serum soluble CD163 indicates macrophage activation in Complex Regional Pain Syndrome

Various findings point towards activation of the monocyte-macrophage system in CRPS. For example, TNF- α , which is primarily released by local pro-inflammatory M1 macrophages, has been shown to be increased in blister fluid of affected CRPS extremities and increased levels of circulating pro-inflammatory CD14⁺CD16⁺ monocytes have been found in venous blood of CRPS patients. Furthermore, immunomodulating medication such as thalidomide, which exerts part of its effects on monocytes thereby reducing production of TNF- α by these

cells, seem to be effective in certain CRPS cases. Therefore, exploration of this system is warranted in CRPS as this system could be an interesting target for future therapies. To this end, in this retrospective cohort study, levels of the macrophage-specific soluble CD163 (sCD163) receptor were measured in CRPS patients and compared with sCD163 levels of healthy controls. The study revealed sCD163 was significantly higher in CRPS patients than in healthy controls, suggesting activation of local tissue-resident macrophages, and by extension the monocyte-macrophage system, in CRPS. Future studies should focus on exploring whether this marker can be used to diagnose CRPS, whether this marker can be used to monitor disease activity in CRPS, and whether this marker can be used to select therapies targeting monocytes/macrophages in CRPS.

Chapter 7 – Denying the truth does not change the facts: a systematic analysis of pseudoscientific denial of Complex Regional Pain Syndrome

Several articles have claimed that CRPS not exist. These articles not only undermine current empirical evidence that suggests otherwise, but also could potentially harm patients by encouraging dismissal of their signs and symptoms. In this chapter, we conducted a systematic literature search to evaluate the methodological quality of articles that claim CRPS does not exist. We then examined and refuted the arguments supporting this claim using up-to-date scientific literature on CRPS. Four narrative reviews, 2 personal views, 1 letter, 1 editorial and 1 case report were identified and included in this review. We identified seven points of controversy that were used in these articles to argue that CRPS does not exist: 1) the label 'CRPS'; 2) the 'unclear' pathophysiology; 3) the validity of the diagnostic criteria; 4) CRPS as a normal consequence of immobilization; 5) the role of psychological factors; 6) other identifiable causes for CRPS symptoms; and 7) the methodological quality of CRPS research. We concluded that the level of evidence for the claim that CRPS does not exist is very weak. Furthermore, most arguments could be refuted by the extensive empirical literature on CRPS.

Chapter 8 - General discussion

In the final chapter of this thesis, the general discussion, the findings from this thesis are summarized and discussed in a broader context, i.e., in terms of clinical and research application. Recommendations are made for future research on biomarkers in CRPS. It is important to note that although this thesis was focused on biomarkers of inflammation in CRPS, the pathophysiology of CRPS is complex and consists of multiple mechanisms. Therefore, it is likely that there will never be one biomarker or diagnostic test specific for CRPS, rather, it is imaginable that in the future a panel of diagnostic biomarkers or tests will be used to not only diagnose CRPS, but also to identify which pathophysiological mechanisms are involved in each CRPS case and to also identify and monitor effect of therapies.

SAMENVATTING PROEFSCHRIFT

Hoofdstuk 1 - General introduction

Hoofdstuk 1 bespreekt de achtergrond en het doel van dit proefschrift welke in grote lijnen zijn: 1) de noodzaak van biomarker-onderzoek in Complex Regionaal Pijn Syndroom (CRPS) benadrukken en 2) het aanpakken van de huidige scepticisme over het bestaan van dit syndroom. CRPS wordt nog steeds gediagnosticeerd aan de hand van relatief subjectieve klinische criteria en er is nog geen objectieve test beschikbaar om deze ziekte te diagnosticeren. Deze subjectiviteit leidt niet alleen tot een vertraging bij de diagnose, maar ook tot scepsis over het bestaan van dit syndroom; beide kunnen schadelijk zijn voor patiënten die aan deze aandoening lijden. De identificatie van diagnostische (en therapeutische) biomarkers bij CRPS zou niet alleen een objectieve component toevoegen aan het diagnostische proces van CRPS, maar ook eerdere identificatie en behandeling van dit syndroom vergemakkelijken. Hoewel de exacte pathofysiologie van CRPS nog moet worden onderzocht, wordt momenteel algemeen aangenomen dat inflammatie een belangrijk mechanisme is, niet alleen bij het ontstaan, maar ook bij het in stand houden van CRPS. Daarom is inflammatie een interessante 'target' voor biomarker-onderzoek bij CRPS en wordt de rol van inflammatoire biomarkers, vooral voor T-cel- en lokale macrofaagactiviteit, bij de diagnose en behandeling van CRPS onderzocht.

Hoofdstuk 2 - Complex Regional Pain Syndrome: diagnosis and treatment

In dit hoofdstuk wordt een beknopt overzicht gegeven van de huidige inzichten over de pathofysiologie van CRPS en worden de huidige aanbevelingen voor het stellen van de diagnose en behandeling van CRPS besproken. Pathofysiologische mechanismen die aan de orde komen zijn inflammatie, neurogene inflammatie, auto-immuniteit, ischemie-reperfusieletsel, genetische betrokkenheid, corticale reorganisatie, dunne vezel neuropathie en psychologische factoren. De hoekstenen voor diagnose, namelijk anamnese en lichamelijk onderzoek, worden besproken. De aanbeveling is om de behandeling van CRPS op een mechanism-based manier uit te voeren, m.a.w. op basis van de actieve onderliggende pathofysiologische mechanismen. Behandelingen die aan bod komen zijn ontstekingsremmers, analgetica/co-analgetica, vaatverwijders, spierverslappers/spasmolytica, psychologische interventie en invasieve behandelingen.

Hoofdstuk 3 - Highlighting the role of biomarkers of inflammation in the diagnosis and management of Complex Regional Pain Syndrome

In dit hoofdstuk wordt de rol van inflammatie in de (multi-mechanisme) pathofysiologie van CRPS besproken en wordt de toepassing van mogelijke biomarkers van inflammatie bij de diagnose en behandeling van dit syndroom belicht. De beoordeelde biomarkers zijn

onderverdeeld in lokale biomarkers (TNF- α , IL-6, Tryptase, Mestcel aantallen) en systemische biomarkers (CGRP, SP, CD14 + CD16 + monocyt, CD8 + T lymfocyten, sIL-2R, auto-antilichamen en miRNA), met een verdere onderverdeling in cellulaire en oplosbare (soluble) biomarkers. Het wordt duidelijk dat er tot op heden nog geen biomarker is gevalideerd voor gebruik bij de diagnose en behandeling van CRPS, maar er zijn wel een aantal veelbelovende biomarkers die in toekomstig onderzoek onderzocht zouden moeten worden.

Hoofdstuk 4 - Elevated plasma levels of sIL-2R in Complex Regional Pain Syndrome: a pathogenic role for T-lymphocytes?

Er is niet veel bekend over de rol van T-cellen bij het ontstaan en in stand houden van CRPS. In deze retrospectieve cohortstudie hebben we T-celactiviteit bij CRPS geëvalueerd door de waarde van de soluble interleukine-2-receptor (sIL-2R) in bloed te vergelijken tussen CRPS-patiënten en gezonde controles. Soluble IL-2R is een marker voor T-celactiviteit en het is aangetoond dat deze marker verhoogd is bij ziekten waarbij T-celactiviteit een centrale rol speelt, zoals bij reumatoïde artritis en sarcoidose. CRPS-patiënten hadden statistisch significante hogere waarden van sIL-2R vergeleken met die van gezonde controles. Daarnaast bleek het sIL-2R-gehalte een goede discriminator te zijn tussen CRPS-patiënten en gezonde controles met een hoge sensitiviteit en specificiteit. De bevindingen uit dit hoofdstuk duiden op een verhoogde T-celactiviteit bij CRPS-patiënten, wat een mogelijke T-cel gemedieerd ontstekingsproces zou betekenen. Verder bespreekt dit hoofdstuk sIL-2R als een potentiële diagnostische biomarker bij CRPS.

Hoofdstuk 5 - Serum soluble interleukin-2 receptor does not differentiate Complex Regional Pain Syndrome from other pain conditions in a tertiary referral setting

In deze prospectieve cohortstudie hebben we onderzocht of sIL-2R kan worden gebruikt als diagnostische marker bij CRPS. Hiervoor zijn de sIL-2R-waarden bepaald bij patiënten die met verdenking op CRPS naar ons pijncentrum waren verwezen. Vervolgens werden de sIL-2R-waarden van de patiënten met de diagnose CRPS vergeleken met de sIL-2R-waarden van de patiënten bij wie de diagnose van CRPS niet werd gesteld. Soluble IL-2R-waarden van gezonde vrijwilligers werden als controles gebruikt. De studie bevestigde de bevindingen uit hoofdstuk 4: de sIL-2R-waarden van CRPS-patiënten zijn hoger dan die van gezonde controles. Soluble IL-2R kon echter niet worden gebruikt om CRPS-patiënten te onderscheiden van patiënten met andere pijnandoeningen van een ledemaat waarvan aanvankelijk werd vermoed dat ze CRPS hadden. Deze bevinding sluit echter het gebruik van deze marker bij het monitoren van inflammatoire ziekteactiviteit bij CRPS niet uit. Dit zal in toekomstig onderzoek verder moeten worden onderzocht.

Hoofdstuk 6 – Elevated serum soluble CD163 indicates macrophage activation in Complex Regional Pain Syndrome

Er zijn diverse aanwijzingen voor activering van het monocyt- en macrofaagsysteem bij CRPS. Zo is TNF- α , dat voornamelijk wordt geproduceerd door lokale pro-inflammatoire M1-macrofagen, verhoogd in blaarvocht van aangedane CRPS-extremiteten en zijn verhoogde niveaus van circulerende pro-inflammatoire CD14⁺CD16⁺-monocyten in veneus bloed gevonden van CRPS-patiënten. Bovendien lijken immuunmodulerende medicijnen zoals thalidomide, die een deel van zijn effecten op monocyten uitoefent en daardoor de productie van TNF- α door deze cellen vermindert, effectief te zijn in sommige CRPS-patiënten. Onderzoek doen naar de activatie van dit systeem bij CRPS lijkt dan ook van belang, aangezien dit systeem een interessante target zou kunnen zijn voor toekomstige therapieën. In deze retrospectieve cohortstudie werden de waarden van de macrofaag-specifieke soluble CD163 (sCD163) receptor gemeten bij CRPS-patiënten en werden deze vergeleken met sCD163-waarden van gezonde controles. De studie liet zien dat sCD163 significant hoger was bij CRPS-patiënten dan bij gezonde controles, wat duidt op activering van lokale weefsel-macrofagen, en dus het monocyt- en macrofaagsysteem bij CRPS. Toekomstige studies zouden zich moeten richten op het onderzoeken of deze marker kan worden gebruikt om CRPS te diagnosticeren, of deze marker kan worden gebruikt om ziekteactiviteit bij CRPS te monitoren en of deze marker kan worden gebruikt om therapieën gericht op monocyten en macrofagen te selecteren.

Hoofdstuk 7 – Denying the truth does not change the facts: a systematic analysis of pseudoscientific denial of Complex Regional Pain Syndrome

In verschillende artikelen wordt beweerd dat CRPS niet bestaat. Deze artikelen ondermijnen niet alleen het huidige empirische bewijs dat anders suggereert, maar kunnen patiënten mogelijk ook schaden door (para)medische specialisten aan te moedigen de CRPS tekenen en symptomen van patiënten te negeren. In dit hoofdstuk hebben we een systematisch literatuuronderzoek uitgevoerd om de methodologische kwaliteit van artikelen die claimen dat CRPS niet bestaat te beoordelen. Vervolgens hebben we de argumenten die deze bewering ondersteunen onderzocht en weerlegd met behulp van actuele wetenschappelijke literatuur over CRPS. Vier narrative reviews, 2 personal views, 1 letter, 1 editorial artikel en 1 case report werden geïdentificeerd en opgenomen in deze review. We identificeerden zeven hoofdargumenten die in deze artikelen werden gebruikt om te beweren dat CRPS niet bestaat: 1) het label 'CRPS'; 2) de 'onduidelijke' pathofysiologie; 3) de geldigheid van de diagnostische criteria; 4) CRPS als normaal gevolg van immobilisatie; 5) de rol van psychologische factoren; 6) andere aanwijsbare oorzaken van CRPS-symptomen; en 7) de methodologische kwaliteit van CRPS-onderzoek. We concludeerden dat het bewijsniveau (level of evidence) voor de bewering dat CRPS niet bestaat, erg zwak is. Bovendien konden de meeste argumenten worden weerlegd met behulp van de uitgebreide empirische literatuur over CRPS.

Hoofdstuk 8 – General Discussion

In het laatste hoofdstuk van dit proefschrift, de general discussion, worden de bevindingen uit dit proefschrift samengevat en besproken. Er worden aanbevelingen gedaan voor toekomstig onderzoek naar biomarkers bij CRPS. Alhoewel dit proefschrift zich met name richt op inflammatoire biomarkers bij CRPS, is het belangrijk op te merken dat de pathofysiologie van CRPS complex is en bestaat uit meerdere mechanismen. Het is hierdoor aannemelijk dat er nooit één biomarker of diagnostische test specifiek voor CRPS zal zijn, maar dat er in de toekomst een panel van diagnostische biomarkers of tests zullen worden gebruikt om niet alleen CRPS te diagnosticeren, maar ook om te bepalen welke pathofysiologische mechanismen betrokken zijn bij iedere casus van CRPS.

Appendices

Words of appreciation

List of publications

About the author

PhD Portfolio



WORDS OF APPRECIATION

Here it is then, my thesis. A product of hard work, fun, laughter, tears and unconditional support from friends, family and co-workers. So many people deserve so much credit for helping me get here. Although I know these (short) words of appreciation will never be enough to thank all of you, I hope that each and every one of you understands how special and dear you are to me and that without your support, I would not be here.

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LIST OF PUBLICATIONS

Bharwani KD, Dirckx M, Stronks DL, Dik WA, Huygen FJPM. Serum Soluble Interleukin-2 Receptor Does Not Differentiate Complex Regional Pain Syndrome from Other Pain Conditions in a Tertiary Referral Setting. *Mediators Inflamm.* 2020 Sep 28;2020:6259064. doi: 10.1155/2020/6259064. PMID: 33061828; PMCID: PMC7539124.

Bharwani KD, Dik WA, Dirckx M, Huygen FJPM. Highlighting the Role of Biomarkers of Inflammation in the Diagnosis and Management of Complex Regional Pain Syndrome. *Mol Diagn Ther.* 2019 Oct;23(5):615-626. doi: 10.1007/s40291-019-00417-x. PMID: 31363934; PMCID: PMC6775035.

Bharwani KD, Dirckx M, Huygen FJPM. Complex regional pain syndrome: diagnosis and treatment, *BJA Education*, Volume 17, Issue 8, August 2017, Pages 262–268, <https://doi.org/10.1093/bjaed/mkx007>

Bharwani KD, Dirckx M, Stronks DL, Dik WA, Schreurs MWJ, Huygen FJPM. Elevated Plasma Levels of sIL-2R in Complex Regional Pain Syndrome: A Pathogenic Role for T-Lymphocytes? *Mediators Inflamm.* 2017;2017:2764261. doi: 10.1155/2017/2764261. Epub 2017 May 28. PMID: 28634419; PMCID: PMC5467333.

Bijl, EJ. **Bharwani, KD** (2013) 'The long-term outcome of the Kasai operation in patients with biliary atresia: a systematic review.', *The Netherlands Journal of Medicine*, 71(4), pp. 170-3.

ABOUT THE AUTHOR

Krishna Deepak Bharwani was born on the 13th of September 1990 in Mumbai, India. In 1991, her family migrated to the Caribbean island of Curaçao. After obtaining her high school diploma at Radulphus College, she moved to the Netherlands in 2009 to pursue her degree in Medicine at Erasmus MC University Medical Center Rotterdam. On the 16th of April 2016, she obtained her Master's in Medicine and went on to pursue a PhD at the Center of Pain Medicine at Erasmus MC (Supervisor/Promotor: Prof. dr. F.J.P.M. Huygen; co-supervisor/co-promotor: dr. M. Dirckx). During her PhD she also worked as a clinical resident at the Center for Pain Medicine and was involved in organizing the 10-week Pain Medicine Minor for 3rd year medical and clinical technology students. On the 1st of January 2021, she started her residency in Anesthesiology at the Department of Anesthesiology at Erasmus MC University Medical Center Rotterdam (Residency Program Director/Opleider: Prof. dr. R.J. Stolker).

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: Krishna Deepak Bharwani	PhD period: 01/06/2016 – 31/12/2020
Erasmus MC Department: Center for Pain Medicine, Department of Anesthesiology	Promotor/Supervisor: Prof. dr. F.J.P.M. Huygen
	Co-promotor/Co-supervisor: dr. M. Dirckx

1. PhD training

	Year	Workload (Hours/ECTS)
General courses		
- EndNote	2015	0.20
- Research Integrity	2017	0.30
- OpenClinica	2017	0.30
- Biomedical English Writing and Communication	2018	3.00
- Biostatistical Methods I: Basic Principles Part A Methodology	2018	2.00
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2018	1.50
- Basic Course on 'R'	2019	2.00
Seminars and workshops		
- Workshop 'Complex Regional Pain Syndrome' - Mainz, Germany	2017	1.00
Presentations		
- CRPS Patient Association: the role of T-cells in Complex Regional Pain Syndrome	2017	0.50
- CRPS Patient Association: an update on Complex Regional Pain Syndrome	2018	0.50
- NERASS Conference: Complex Regional Pain Syndrome: diagnosis and treatment	2018	1.00
- Refereeravond Pijn geneeskunde: Inflammation in Complex Regional Pain Syndrome	2019	1.00
(Inter)national conferences		
- EFIC Valencia: 'The soluble interleukin-2 receptor in Complex Regional Pain Syndrome: can it discriminate CRPS from other pain conditions in routine clinical practice?' (poster presentation and poster walk)	2019	1.00
- EFIC Copenhagen	2017	
Grants		
- St. Erasmus Pijnfonds: soluble interleukin-2 receptor levels in the onset and development of Complex Regional Pain Syndrome. An observational cohort study.	2017	
- St. Esperance – CRPS patient association: association between soluble interleukin-2 receptor and CRPS disease severity	2018	

2. Teaching activities

	Year	Workload (Hours/ECTS)
Lecturing		
- Praktijkopleiding Handtherapie: Diagnosis and Treatment of Complex Regional Pain Syndrome	2018	1.00
- Praktische Pijngeneeskunde: Complex Regional Pain Syndrome: diagnosis and management	2019	1.00
Supervising theses		
- Supervision of bachelor thesis/critical appraisal of a topic: 'TNF- α inhibitors in CRPS patients' by A.B. Kersten	2019	1.00
- Supervision master thesis: 'Effect of intravenous S-ketamine infusion on pain in patients with Complex Regional Pain Syndrome' by T.J.P. Mangnus	2019	7.50
- Supervision master thesis: 'Correlation between soluble interleukin-2 receptor levels and disease severity in Complex Regional pain Syndrome' by A.B. Kersten	2020	5.70
Other		
- Organization and coordination of Minor Pijngeneeskunde/Pain Medicine Minor	2018	10.00
- Organization and coordination of Minor Pijngeneeskunde/Pain Medicine Minor	2020	10.00

