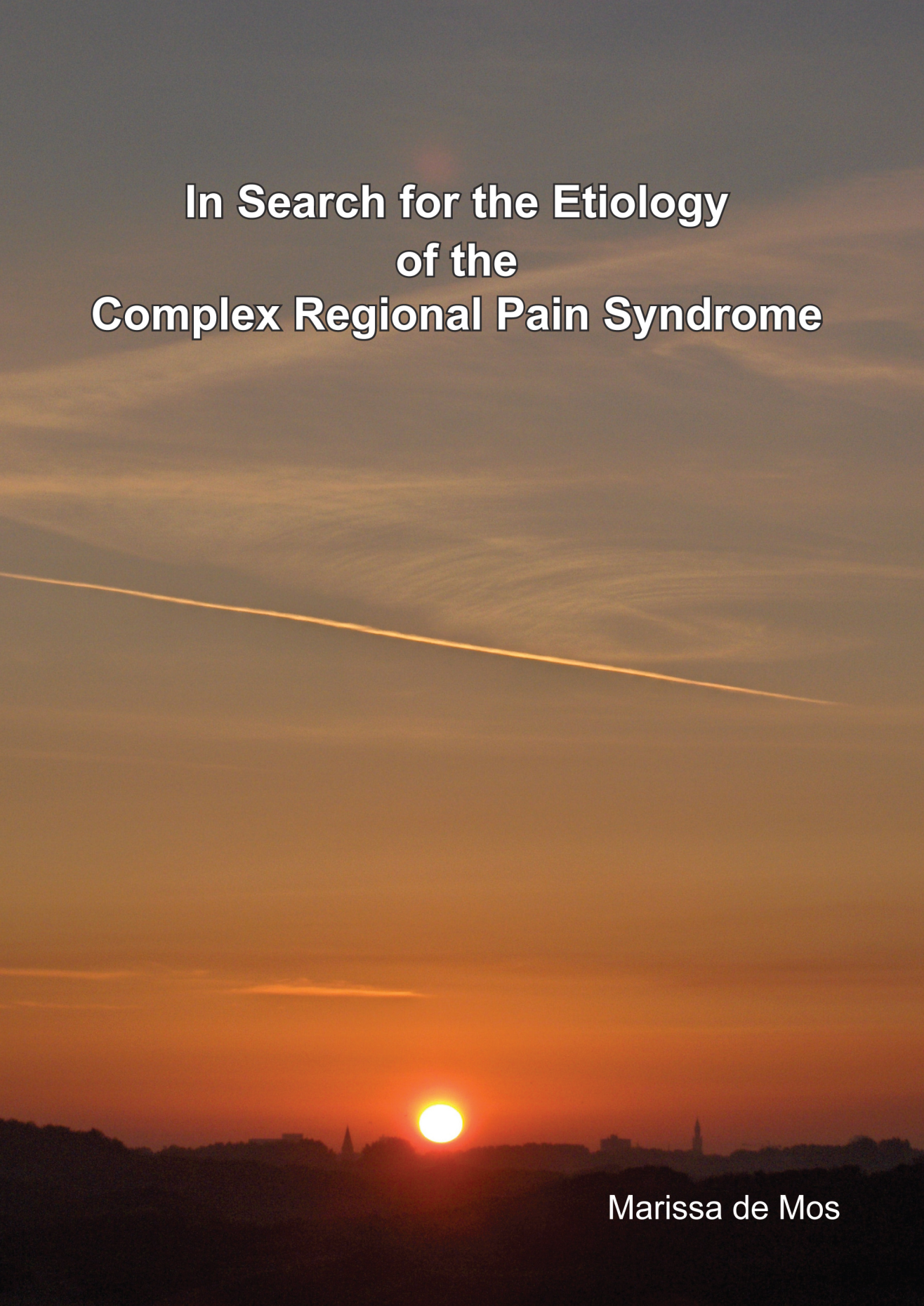
In Search for the Etiology
of the
Complex Regional Pain Syndrome
Marissa de Mos

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The work presented in this thesis was conducted at the Department of Medical Informatics in collaboration with the Pain Treatment Center, both of the Erasmus Medical Center, Rotterdam. Parts of the work were conducted at Astra Zeneca in Mölndal, Sweden, and at the Anesthesia research department of the McGill University, Montréal, Canada.

This PhD project was performed within TREND (Trauma RElated Neuronal Dysfunction, www.trendconsortium.nl), a scientific knowledge consortium that integrates research on Complex Regional Pain Syndrome type 1. The project was supported by a Dutch government grant (BSIK03016). One chapter was performed in collaboration with Infobiomed, a European funded network that aims to enforce biomedical informatics as an integrative discipline. One chapter was performed in collaboration with the Anesthesia research department of the McGill University.

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In Search for the Etiology
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Complex Regional Pain Syndrome

Zoektocht naar de etiologie van het complex regionaal pijn syndroom

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

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Marissa de Mos
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Overige leden:	Prof.dr. J.J. van Hilten Prof.dr. J. Klein Prof.dr. H.J. Stam
Copromotor:	Dr. F.J.P.M. Huygen

Quam saepe forte temere eveniunt, quae non audeas optare.

Terentius

Contents

Chapter 1

General Introduction

1.1 | Aim and Outline of the Thesis

The complex regional pain syndrome is poorly elucidated. In line with this its diagnosis and clinical management have remained suboptimal. The multifaceted nature makes it a fascinating study topic for scientists with varying interests, but unraveling the etiology has been proven a laborious mission.

The first notification of what could have been (what is currently named) complex regional pain syndrome (CRPS) stems from 1634, when the surgeon Ambroise Pare described that King Charles IX suffered from persistent pain and contractures of his arm following a bloodletting procedure.[1] The next remarks came from the military physician Scott Mitchell and date from the American Civil War: "…Long after the trace of the effect of a wound has gone neuralgic symptoms are apt to linger, and too many carry with them throughout long years this final reminder of the battle field...".[2] The first scientific publication on CRPS was issued in 1900 from a German surgeon named Paul Sudeck.[3] His name became tied to the syndrome for long (Sudecks' dystrophy).

Today we know CRPS as a complication of a physical trauma, for example a fracture. The affected extremity is marked by a broad variety of symptoms[4], including pain and sensory disturbances such as allodynia and hyperesthesia, swelling, changes in color, temperature and transpiration, trophic skin alterations, hair and nail growth changes, weakness, decreased motion and neuromotor disturbances. As a consequence CRPS patients may come to suffer from severe discomfort and invalidity. In rare cases a state is reached wherein amputation of the affected limb becomes inevitable. Although debatable, CRPS is classically divided in type I, without demonstrated nerve injury, and type II, with demonstrated nerve injury.

For decades CRPS has been considered mainly as a disease of the central nervous system, and still autonomic (sympathetic) and sensory disturbances are assumed to underlie many features of the disorder.[5-7] However, more recently an additional interest raised towards the peripheral disease mechanisms that are potentially involved in CRPS.[8-10] Most patients express signs of inflammation[4,11], while there are also indications that local oxygen deprivation may play a role.[12] Moreover, some authors have specifically emphasized the potential contribution of psychological factors, such as somatisation and anxiety.[13,14]

The cloudy and controversial insights in CRPS etiology are also reflected in the history of its nomenclature. Since its first description the syndrome has been addressed in the Angelsaksian literature alone by already more than 70 different names, that often referred to presumed underlying disease causes. In 1995 the International Association for the Study of Pain (IASP) strived to end the taxonomic diversity by introducing the term "complex regional pain syndrome (CRPS)", a name based on description rather than on pathophysiology.[15]

Aim and Outline of the thesis

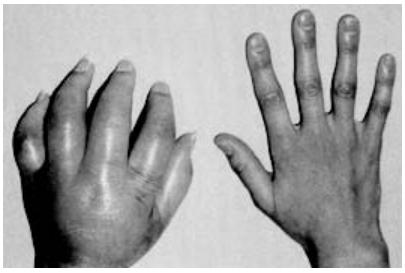
The aim of the research described in the present thesis was to gather more insight in CRPS as a clinical entity and into its pathogenesis and etiology. CRPS has been extensively studied before and we have started by reviewing the current understandings based on these valuable efforts (chapter 1.2).

In the second chapter we have attempted to achieve better insight in some general disease characteristics of CRPS. We studied the incidence (chapter 2.1), disease course (chapter 2.2), and treatment and referral patterns (chapter 2.3).

In the third chapter we sought for potential determinants of CRPS, with the purpose to identify risk factors and clues for the underlying disease mechanisms. In this view we studied the medical history of CRPS patients for specific pre-existing morbidities (chapter 3.1). Also, we investigated the association between CRPS and the use of specific types of medications, since some drugs might interfere with mediators in the pathogenesis of CRPS (chapter 3.2). Finally, as CRPS has a pronounced female predominance, we studied the relation between CRPS and both endogenous and exogenous exposure to estrogens (chapter 3.3).

In the fourth chapter we describe how we used an experimental approach to develop new ideas about potential underlying disease mechanisms of CRPS. In this, we combined information from abstracts in Medline by applying PathwayAssist, which is tool for automated information retrieval based on text mining (chapter 4.1). The evolving new hypothesis adressed the role of NFκB in CRPS and was explored further in an animal model for CRPS type I (chapter 4.2).

The thesis closes with a general discussion of the main findings and methodology, and with some suggestions for future research.



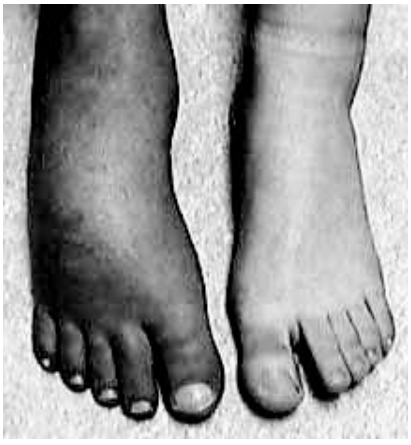


figure 1. Left hand and right foot are affected by CRPS. Pictures used with kind permission of dr. Roland Glinz (hand), www.schmerzzentrum.ch, and dr Schwartz (foot), www.robertgschwartz.homestead.com

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1.2 | Current Understandings on Complex Regional Pain Syndrome

Submitted

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Abstract

The mechanisms underlying complex regional pain syndrome (CRPS) have been increasingly studied over the past decade. Classically, this painful and disabling disorder was considered to emerge from pathology of the central nervous system. However, the involvement of additional peripheral disease mechanisms is likely and recently these mechanisms have also attracted scientific attention. The present article provides an overview of the current understandings regarding the pathology of the autonomic and somatic nervous system in CRPS, as well as the roles of neurogenic inflammation and hypoxia, and the contribution of psychological factors. Potential connections between the separate disease mechanisms are discussed. Additionally, currently known risk factors for CRPS will be addressed. Insight in risk factors is of relevance, since it facilitates early diagnosis and tailored treatment. Moreover, it may provide clues for further unraveling of the pathogenesis and etiology of CRPS.

Introduction

The complex regional pain syndrome (CRPS) is a painful and disabling disorder that can affect one or more extremities. Usually, its onset is precipitated by a physical injury, for example a fracture, sprain or surgery.[1,2] The spectrum of symptoms and signs is broad, including pain and sensory dysfunction, characteristics of inflammation, impaired motor function and trophic disturbances.[3] Differentiated by the presence of a demonstrated nerve lesion, CRPS can be classified into type I and type II, of which type I, without a nerve lesion, is the most common.[4] CRPS can impose great impact on the daily functioning and quality of life of the patient who suffers from it.[5]

Classically, CRPS has been regarded as a disorder of the central nervous system with sympathetic dysfunction as its major pathogenic mechanism. Recently, the interest shifted towards the contribution of peripheral disease mechanisms, including inflammation and hypoxia. Hereby we aim to provide an overview of the current understandings on the pathogenic mechanisms that underlie CRPS. Additionally, risk factors and determinants as far as presently known, will be summarized.

Methods

Five disease mechanisms were pre-specified as theoretic main contributors to CPRS. The choice of mechanisms was based on discussions with internal and external CPRS experts, including the investigators within TREND (Trauma RElated Neuronal Dysfunction), a Dutch government funded knowledge consortium that integrates research on CRPS-I (BSIK03016). The pre-specified mechanisms included: 1. autonomic (sympathetic) nervous system dysfunction; 2. somatic nervous system dysfunction; 3. inflammation; 4. hypoxia; and 5. psychological factors.

For each mechanism a separate search was conducted in Medline, using the query *"Complex Regional Pain Syndromes"[MAJR] OR sudeck* combined with one of the following queries: for autonomic nervous system dysfunction: *"sympathetically maintained pain" OR adrenerg* OR "sympathetic nervous system"*; for somatic nervous system dysfunction: *sensitization OR "neuropathic pain" OR motor*;* for inflammation: *inflammation OR cytokine OR neuropeptide*; for hypoxia: *hypoxia OR ischaemia OR vascular OR microcirculation OR "free radical";* and for psychological factors: *psychology OR psychiatry OR behavior*. Case reports and non research articles were excluded. All recovered abstracts (419 in total) were screened manually. Full text articles were studied if the abstract suggested an experimental or observational study design focused on the etiology or pathogenesis of CRPS, a review that discussed one or more of the pathogenic mechanisms, or a clinical trial regarding therapy.

Second, with the purpose to overview known determinants and risk factors, the query *"Complex Regional Pain Syndromes"[MAJR] OR sudeck* was combined with the following terms: *incidence, epidemiology, risk factor, genetics, infection, autoimmunity, and comorbidity*. Case reports as well as all other types of research articles were included.

Again, abstracts were screened manually and full text papers were considered if the abstract suggested an association between CRPS and any risk factor.

Only articles written in English or Dutch that were published in the past ten years (after 1998) were included. The retrieved literature was supplemented with referenced articles from the original yield or with manuscripts that were not indexed in Medline, but that were known otherwise by one of the authors.

Pathogenesis and Etiology

Autonomic nervous system

Increased sweating, trophic changes, and vasoconstriction related coldness of the affected limb have long been considered as a result of autonomic (sympathetic) hyperactivity. Additionally, in the past the phenomenon called sympathetically maintained pain (SMP) was considered almost synonymous for CRPS. In SMP, painful sensations are provoked by sympathetic outflow through sympathetic-afferent coupling, in which adrenergic receptors are expressed on primary afferent nerve endings.[6,7] The classic treatment of CRPS with sympatholytic blocks was aimed to attenuate the pain and vasoconstriction induced by sympathetic hyperactivity.

However, the role of sympathetic dysfunction in CRPS has currently become somewhat debatable. Sweating and trophic disturbances are not the most predominant features of CRPS[3] and can also be explained as neuropeptide effects[8] (see later). Vasoconstriction does not always reflect sympathetic activity[9] and alternative mechanisms than sympathetic overstimulation may account for the observed vasotonic impairments.[10,11] SMP may result from pathological failure of spinal inhibitory mechanisms to suppress nociceptive input by normal, instead of increased sympathetic stimulation.[12] Finally, from a clinical viewpoint, many CRPS patients do not benefit from sympatholytic blocks.[13]

Apart from the actual role of sympathetic dysfunction in CRPS, the mechanism behind it also has become subject to controversy. Increased sympathetic outflow (hyperactivity) is one possibility. Abnormal sensitivity of adrenergic receptors for normal sympathetic outflow is another.[16,20,21] The first mechanism is endorsed by the observation that central sympathetic arousal provokes pain and abnormal vasoconstriction patterns in CRPS.[14,15] Additionally, the sympathetic skin reflexes in CRPS patients are increased.[16] The underlying cause may be sprouting of new sympathetic nerves centrally in the dorsal horn[17] or peripherally in the upper dermis[18], which can be triggered by inflammatory mediators or nerve injury.[19] On the contrary, adrenergic receptor hypersensitivity is plausible based on the observed increases of pain after intradermal noradrenalin injections in CRPS patients, but not in controls.[24] Morever, the density of α-adrenoreceptors is enlarged in hyperalgesic skin from CRPS affected limbs[25], while the sympathetic innervation of sweat glands and vasculature is abnormal.[26] Catecholamine levels in serum derived from the affected side of CRPS patients are usually decreased instead of elevated[23], which argues in favor of a local hypersensitivity. Temporarily diminished sympathetic stimulation has been suggested as an underlying cause of the adrenergic receptor upregulation and sensitization in CRPS patients.[22,23]

A generally acknowledged view today is that SMP and sympathetic dysregulation can be, but are not an obligatory part of CRPS.[27] Sympathetic blocks remain still widely administered for the treatment of CRPS, with beneficial results in a subset of patients.

Somatic nervous system

Pain and sensory disturbances in CRPS have been attributed to pathology of the sensory somatic nervous system. Histopathological changes in skin innervation have been described, such as a substantial loss of normal C and Aδ fibers and the presence of fibers with aberrantly branched endings.[26] Compared to controls a 29% reduction of axonal density was reported in CRPS affected skin.[28] Because of similarities in pain characteristics between CRPS and other neuropathic pain disorders, analogies in the underlying mechanisms of neuroplasticity and sensitization are generally assumed, although they have never been well studied particularly in CRPS patients.

Spinal neuronal sensitization can follow upon continuous nociceptive input or nerve injury and comprises a state of hyperexcitability and disinhibition, causing a decreased stimulation threshold.[29] Eventually, this can cause a normally non-painful stimulus to become painful. Animal models have revealed several biochemical processes that underlie spinal sensitization with roles for post-synaptic receptors, including the N-methyl-D-aspartate (NMDA) receptor, the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, and the neurokinin-1 (NK-1) receptor.[30] The actual contribution of these mechanisms to human CRPS is difficult to study, since it would require investigation of spinal cord neuronal tissue. However, the NMDA-receptor antagonist memantine was effective in relieving pain and motor symptoms in CRPS patients.[31] Anti-epileptics however, that increase neuronal excitation thresholds and are applied generally for neuropathic pains, are only moderately effective in CRPS patients, as was demonstrated in one randomized crossover trial with gabapentin.[32]

In addition to spinal sensitization, supra-spinal alterations are also present in CRPS, as suggested by findings of impaired perceptual learning[33] and of a relation between pain severity and impaired tactile discrimination.[34] Brain imaging studies have indicated changes of cerebral blood flow, particularly in the hypothalamus.[35,36] Some CRPS patients experience referred sensations[37,38] or body perception disturbances[39-41], which is in line with studies that demonstrate altered brain activation patterns[42,43] and sensory mapping.[44,45]

CRPS affects not only afferent sensory systems; efferent motor pathways are also hampered, which leads to clinical signs of a decreased range of motion, involuntary movements and dystonia.[46,47] Post-synaptic motor reflex inhibition was found impaired in CRPS patients.[48] Moreover, measures of cortical reorganization of motor units correlated with the extent of motor dysfunction.[49,50] Experiments using transcranial stimulation revealed hyperexcitability of both the sensory and motor cortex.[51,52] Based on all these findings, graded motor imaginary and mirror therapy have been proposed as beneficial in CRPS treatment, since this is considered to reconcile motor output and sensory feedback.[53,54] Beneficial effects of mirror therapy have indeed been observed in relatively small open label studies.[54-56]

Inflammation

In the early phase, CRPS affected limbs often display the classic ‘dolor, rubor, calor, tumor’ aspects of an inflammatory disorder. Despite the fact that this clinical presentation was appreciated for a long period, it was not until recently that inflammatory mediators actually were demonstrated as involved in CRPS. Classic inflammation is marked by

typical immune cells like lymphocytes, phagocytes and mast cells, which excrete classic pro-inflammatory cytokines. In fluid derived from artificially produced blisters on CRPS affected extremities, compared to unaffected sides, levels of interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and tryptase were increased.[57,58] Analysis of blister fluid with a multiplex array, testing for 25 different cytokines, revealed an even stronger pro-inflammatory expression profile, with increased markers for activated monocytes and macrophages.[59] A pro-inflammatory cytokine expression profile was also demonstrated in liquor[60,61] (IL-1β and TNFα increased) and occasionally in venous blood[62,63] (mRNA levels of TNFα and IL-2 increased; levels of soluble TNFα receptor increased; mRNA levels of anti-inflammatory cytokoines IL-4, IL6 and tissue growth factor-β decreased). Additionally, there is an enhanced migration of injected radiolabelled autologous leucocytes or non-specific immunoglobulines towards the CRPS affected location.[64,65] However, systemic parameters of inflammation that are usually applied in clinical settings, including white blood cell count and C-reactive protein, are normal in CRPS patients.[66,67]

Neurogenic inflammation is mediated by neuropeptides, which are excreted by nociceptive C-fibers in response to various triggers and that possess vasoactive and immunologic properties.[8,68,69] The secretory nerve endings of these nociceptives are mainly located in the distal parts of extremities, the typical location for CRPS.[69] However, primary afferent depolarisation can also induce neuropeptide release within the dorsal horn, where they can mediate central sensitization. Cardinal mediators in neurogenic inflammation are Substance P (SP) and calcitonin gene-related protein (CGRP).[70] In rats SP application induced or increased CRPS-like symptoms[71,72], while in human CRPS patients intradermal SP administration provoked abnormal plasma extravasation.[73] Both SP and CGRP have been measured systemically elevated in CRPS patients.[66,70,74] Bradykinin, another peptide involved in inflammation and peripheral nociceptor sensitization[75,76], was four times higher in venous blood of CRPS patients compared to controls[74]. Neuropeptide Y (NPY) and perhaps angiotensin converting enzyme (ACE) have been suggested as potential modulators of the neuroinflammatory responses.[77] The involvement of vasoactive intestinal protein (VIP) has also been suggested, but could not be demonstrated.[78] Compared to controls CRPS patients displayed a facilitated neuro-inflammatory response upon electrical C-fibre stimulation, even in the unaffected extremity, as measured by plasma protein extravasation and axon reflex vasodilation.[79,80]

The important contribution of inflammation to CRPS is underlined by the successful reports from open label studies on treatment with immuno-modulating agents such as infliximab[81,82] and thalidomide.[83] Additionally, one clinical trial showed benefit from treatment with oral corticosteroids.[84]

Hypoxia

The presence of hypoxia in CRPS is endorsed by several observations. In skin, employing micro-lightguide spetrophotometry has revealed decreased capillary oxygenation[85], and dermal microdialysis has demonstrated increased lactate levels.[86] In muscle, nuclear magnetic resonance spectroscopy revealed signs of acidosis and impaired high energy phosphate metabolism.[87] CRPS affected limbs display histo-pathological characteristics consistent with oxidative stress.[88] Hypoxia leads to acidosis and free radical formation,

which are well known triggers for primary afferents to cause severe painful sensations. In CRPS patients, experimentally induced tissue acidosis increased pain.[89] However, blood oxygen was not deprived as was demonstrated with capillary blood gas analysis.[90]

Hypoxia in CRPS has been proposed to be caused by extreme vasoconstriction, either sympathetically thriven (see before) or resulting from a local dysbalance between endothelial factors.[91] In the latter case, nitric oxide (NO) and endothelin (ET-1) are opposite mediators, whereas NO induces vasorelaxation and ET-1 vasoconstriction. In CRPS patients, venous ET-1 levels were found equal between the affected and unaffected sides[10], but in blister fluid ET-1 levels were increased at the affected side, while NO levels were decreased.[11] Since this was observed in chronic cold CRPS patients, these findings suggest a role of endothelial dysfunction induced hypoxia in ongoing CRPS, for which consequently NO donating agents have been proposed as therapeutics.[92]

In addition to sustaining chronic cold CRPS, acute hypoxic injury might also contribute to CRPS induction. In an animal model for CRPS, called the chronic post ischemia pain (CPIP) model, rats develop CRPS like symptoms, including swelling, hyperaemia and allodynia, after three hours of ischemia caused by tourniquet binding of the hindlimb, followed by rapid reperfusion.[93] In the model, neuropathic pain like symptoms develop without microscopic demonstrable nerve damage, similar to CRPS-I in human. The authors have proposed triggering of afferents and initiation of inflammatory responses by free radicals, which are formed under oxidative stress. Although the model could not be fully reproduced by another research group that used an alternative method for ischemia induction[94], the involvement of free radicals is likely in view of the positive outcomes of randomized clinical trials in human CRPS wherein scavengers, such as dimethylsufoxide[95], N-acetylcysteine[95] and vitamine C[96], were effective in the early treatment.

Psychological factors

Psychological factors and behavioural aspects are thought to contribute to chronic pain disorders in general and to CRPS in particular.[97,98] In an extreme view CRPS has been characterised as '*a pseudoneurological disease, i.e. that many features of CRPS are manifestations of somatoform disorders, malingering, and psychiatric pathology'*.[99] Although '*this view is now generally disregarded'* [100], experts do emphasize the need for psychological and behavioural therapy as part of optimal treatment programs for CRPS patients.[101] Still, the actual association between psychological factors and CRPS remains controversial due to the lack of methodological high quality studies. Most studies do not include prospectively collected data, have limited patient numbers, and lack a proper control group.[102,103] Few of the studies that addressed psychological factors in the etiology of CRPS have reported increased incidences of stressful life events in relation to CRPS onset.[104-106] More studies have addressed psychological factors and personality in the *maintenance* (instead of onset) of CRPS, whereby occasionally associations with anxiety, depression, somatisation, and hypochondria, were observed. However, the majority of studies showed no relation between psychological factors and CRPS[105-107], not even in particular subgroups.[108]

A psycho-physiological mechanism that possibly affects chronic pain in CRPS patients is the mode of anger expression. Anger-out, meaning the tendency to express

anger overtly through verbal or physical means, was related with increasing pain in the affected extremity of CRPS patients but not in controls.[109] Anger-out influences pain intensity in other chronic pain disorders, and is assumed to act through reactive muscle activity and by inhibition of the endogenous opioid antinociceptive systems activation. Another physio-psychological mechanism in CRPS may be the stress induced release of catecholamines, which have been observed as systemically elevated in CRPS patients.[110,111] In a vicious circle, emotional distress can sustain the pain, but be a consequence of it as well. Affective distress indeed has been demonstrated a predictor for the near future pain intensity in CRPS patients.[112]

Extreme fear for pain can lead to disuse of the affected extremity, thereby making a feasible contributor to the disease course of CRPS. Prolonged immobilisation can cause a decreased active range of motion, diminished nutritive blood flow, and trophic alterations.[113] Graded activity physical therapy, conducted at overcoming movement anxiety is considered of great therapeutic value[114], since it stimulates desensitization for mechanical allodynia and prevents against accumulation of catecholamines, neuropeptides and inflammatory mediators.[101]

Risk factors and determinants

Demographics

Population based incidence rates have been investigated in one North American[2] and one European[1] study. The US study reported an incidence rate of 5.5 per 100.000 person years, while the European reported 26.2. The striking difference between both studies most likely results from differences in case validation methodology and reflects the importance of uniform diagnostic assessment and nomenclature. However, despite the varying incidence rates, some demographic characteristics were generally similar in both studies. CRPS affected women predominantly with a ratio approximating 3.5. Its onset ranges from childhood through old age, but most cases were seen between their fifties and seventies. It is generally believed that CRPS occurs mainly in Caucasian and Japanese people.[115]

Injury related factors

Wrist fractures are considered the typical initiating trauma for CRPS, but the reported incidences after a wrist fracture vary broadly between 1 and 37%.[116,117] Incidences after other precipitating events vary as well, for example between 0.7 and 21% after total knee prosthesis surgery[118,119] and between 1.6 and 48.8% after a stroke.[120,121] The estimated incidence rates appear highly dependent on the applied diagnostic criteria as well as the time between trauma and assessment of CRPS.[119]

Case reports describe the onset of CRPS after a wide subset of events, including any type of common or iatrogenic injury, cardiovascular events, cancer, infections, and medications.[122] Spontaneous CRPS is rare[1,122], but occurs. In general, fractures are the most common precipitating events and the upper extremity is more frequently involved than the lower.[1,118,119] Severity of the physical injury is not related to the risk of CRPS[97,123,124], although in two studies CRPS patients more often had an intra-articular localization of the fracture.[125,126] Fracture repositions and external fixation are not associated with CRPS

occurence.[123,124] During cast immobilization, increased pressure and early complaints of tightness are predictive factors for the onset of CRPS.[125,127]

Genetics

The risk of CRPS may depend on the susceptibility for exaggeration of the underlying mechanisms of disease, such as inflammation and sensitization. In line with this, the presence of a specific genetic profile in CRPS patients has been suggested. Based on the biological plausibility, polymorphisms have been studied of genes that code for potential mediators of inflammation in CRPS, including TNFα (increased in blisters from CRPS patients)[57,60] and angiotensin converting enzyme (ACE, modulator of SP and bradykinin)[128,129]. Primarily warm CRPS was associated with a polymorphism in one of the TNFα promoter genes.[130] The ACE I/D polymorphism was increased in a small population of Japanese CRPS patients (n=14)[131], but not in a larger, but still relatively small European study (n=60).[132] Polymorphisms in the human leukocyte antigen system (HLA) have also been studied and loci from all three HLA classes have been reported associated with CRPS onset[133,134], treatment resistance[135] or related dystonia.[136] The associations with the HLA-DR and HLA-DQ polymorphisms were particularly remarkable in view of the similar findings in patients with multiple sclerosis and narcolepsy.[133-135]

Antecedent infections

Since CRPS is suggested to be (partly) the consequence of an exaggerated inflammatory response, an autoimmune approach of CRPS becomes feasible. The many existing case reports of CRPS onset in patients with an autoimmune disease underline this idea.[137-143] Bearing in mind the mechanisms of infection induced cross reactivity against auto-antigens, the prevalence of antecedent viral infections in CRPS patients has been investigated. Two reports revealed increased seroprevalences of Parvo B19 IgG antibodies compared to healthy controls[144,145], while one report revealed increased levels of IgG antibodies against herpes simplex viruses.[146] Increased immunoreactivity against Campylobacter Jejuni was observed in CRPS patients with recent onset.[147] CRPS after lyme boreliosis has been described[148,149], as well as after spirochetal infection.[150] Finally, Rubella and hepatitis B vaccination have been noted in case reports to precede CRPS.[151,152] Recently, two studies have demonstrated the presence of autoantibodies against neuronal structures in CRPS patients[147,153], but their actual contribution is not yet elucidated.

Related disorders

The co-occurrence of CRPS with other diseases is mainly described in case reports. Patients with bone metabolism disorders, including osteoporosis[154], osteomalacia[155] and osteogenesis imperfecta[156,157] have been suggested as more susceptible for CRPS. However, they are prone for fractures and probably thereby for CRPS. Other case reports describe CRPS in patients suffering from chronic inflammatory disorders, such as rheumatic diseases.[137-143] CRPS as paraneoplastic phenomenon has been suggested twice.[158,159] Additionally, two publications report CRPS in a patient with amyotrophic lateral sclerosis[160,161] and one in patients with Ehlers-Danlos syndrome.[162]

A limited number of studies on CRPS related disorders have been conducted in relatively small study populations. One retrospective case-control study using

questionnaires revealed headaches to be more than twice as common in CRPS patients, even before the actual onset of CRPS.[163] Furthermore, associations between psychiatric disorders and CRPS have been studied, although most of these studies are considered methodologically poor and address the influence on sustaining CRPS rather that the etiological contribution[102,103]. The results are contradictive, but associations between CRPS and depression, anxiety and somatoform disorders have occasionally been described.[102,103,105,106,164]

Discussion

Several distinct pathogenic mechanisms may contribute to the clinical syndrome that is currently named complex regional pain syndrome (CRPS). Alternating in time, CRPS has been regarded a disorder that was mainly based on a neurological, inflammatory or psychosomatic etiology. In the past decade significant progress has been made in the understanding of the separate disease mechanisms. Although sometimes speculative, paths for interaction between the different mechanisms can be hypothesized (figure 1):

1. Hypoxia may trigger inflammatory responses.[93] *(Interaction: hypoxia ↔ inflammation (classic and neurogenic))*.
2. Continuous nociceptive input by hypoxia[89,91], inflammation[19,62] or sympathetic stimulation[6,14,19,25] may lead to sensitization and alterations in cortical organization of sensory and motor units.[165] (*Interaction: hypoxia, inflammation and autonomic dysfunction ↔ somatic neuronal dysfunction (sensitization and cortical reorganization))*.
3. Neuropeptides (SP) released in the dorsal horn may facilitate sensitization trough interaction with NK-1 and NMDA receptors.[8,70,166] *(Interaction: inflammation (neurogenic) ↔ somatic neuronal dysfunction (sensitization))*.
4. Sympathetic dysfunction (either central sympathetic hyperactivity or increased peripheral adrenergic receptor hypersensitivity) may cause hypoxia due to impaired nutritive blood flow.[8] *(Interaction: autonomic dysfunction ↔ hypoxia)*.
5. Adrenergic receptors can be expressed on immune cells and catecholamines can modulate cellular immunity[8,111,167], while it also has been speculated that inflammation may change sensitivity or expression of α-adrenergic receptors on nociceptive fibres.[168] *(autonomic dysfunction ↔ inflammation (classic))*.
6. Cytokines influence the NO/endothelin balance.[169] *(Interaction (classic) inflammation ↔ hypoxia)*.
7. Psychological distress may influence sympathetic outflow and levels of catecholamines.[21,110,111] *(Interaction: psychological factors ↔ autonomic dysfunction)*.
8. Severe chronic pain and disability may cause psychological distress. *(Interaction: somatic neuronal dysfunction (sensitization) ↔ psychological factors)*.
9. Fear of movement may result in the accumulation of inflammatory mediators and free radicals, and prevent desensitization.[101] *(Interaction: psychological factors ↔ inflammation and hypoxia and somatic neuronal dysfunction)*.

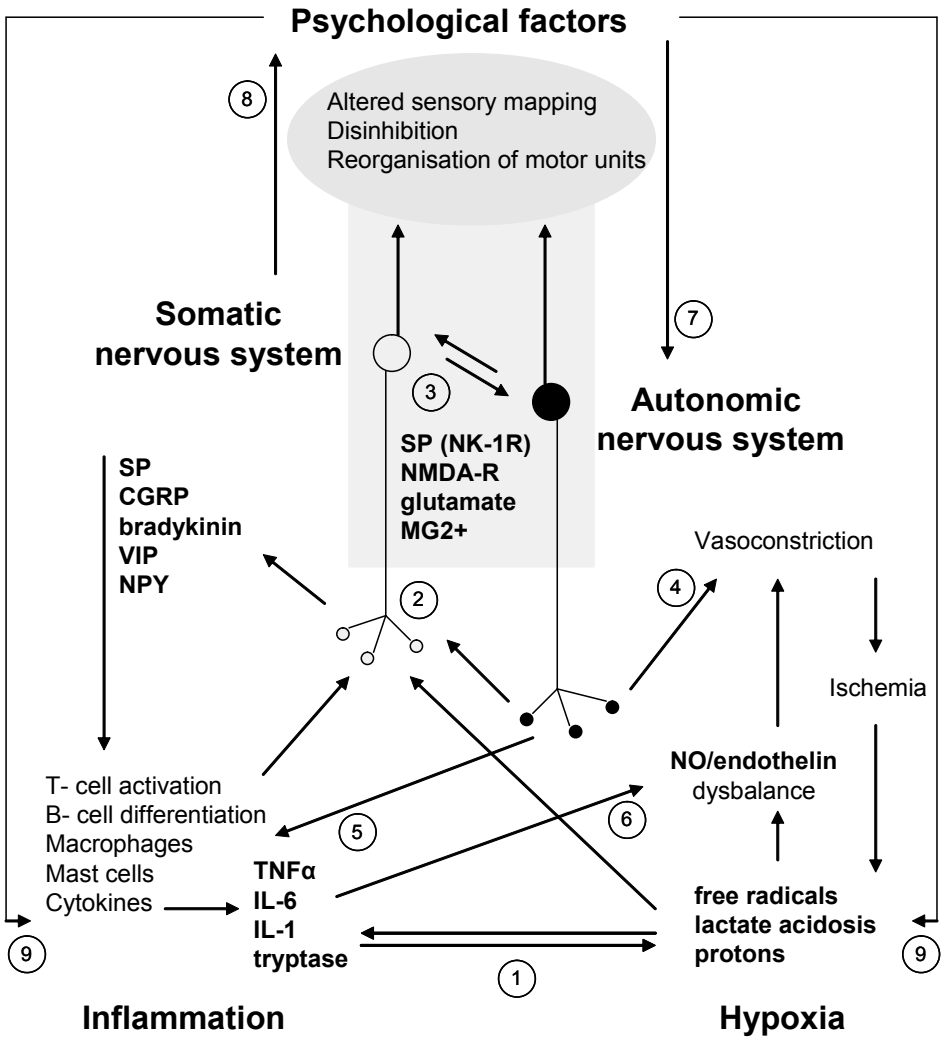

Figure 1. Interaction between the pathogenic mechanisms underlying CRPS. For explanation of the numbers see the discussion section on the opposite page.

The representation of CRPS as the sum, or even more than the sum, of several involved disease mechanisms makes sensible why CRPS is a disorder with varying clinical presentations and disease severity. However, while separate mechanisms may keep each other ongoing, the origin remains unclear. Are there premorbid alterations in the central nervous system that (genetically) predispose a person for CRPS at the moment of a trauma? Does the disease start with minimal peripheral nerve injury, making CRPS a specific kind of a neuropathic pain syndrome? Are the neuropathic pain symptoms secondary to ischemic injury or an exaggerated immunological response, perhaps subsequent to a viral infection or related to autoimmunity? Or is CRPS a somatoform disorder in emotionally unstable patients? Finally, is the initiating or predominant underlying mechanism the same for every patient, or does CRPS consist of many subtypes, all with different etiology and pathogenesis, but with a clinical presentation that much similar that they are all gathered under the same painful syndrome?

Apart from these questions addressing etiology, two other clinically relevant issues stand up. First, which patients develop CRPS? In order to answer this question determinants and risk factors associated with CRPS need to be assessed. Until today, sound epidemiological studies that compare pre-morbid characteristics in a large cohort of CRPS patients to a valid control group, have been performed scarcely. Of course such studies will encounter many challenges, for example related to the relative low prevalence of CRPS and its poor diagnostic definition. Nonetheless such efforts may provide very interesting new information that can lead to new insights in CRPS.

The second relevant issue addresses the lack of effectiveness of most current treatment strategies, despite their interference with plausible underlying disease theories. Considering the complex, multifactor pathogenesis of CRPS, it is understandable that not one single therapeutic modality is sufficient to attenuate all ongoing processes together. Additionally, it is well possible that not all disease mechanisms are equally prominent in each single patient. Finding a common factor that is relevant in all CRPS patients might be a challenging effort, but would be highly interesting. If a candidate could be identified, it would likely be a general down stream compound in a biochemical pathway. It might not be specific for CRPS and targeting it would interfere with important physiological processes and provoke unpleasant or even serious side effects. Nonetheless, if such a common factor should be found, the balance between therapeutic and unwanted effects of targeting this factor needs to be investigated. For severe CRPS patients side effects, safety provided, will outweigh their pain and disability.

Conclusion

Based on the results from fundamental and clinical studies, it is reasonable to assume that mediators of different disease mechanisms are involved in a complex network of interactions, resulting in the painful and impairing disorder CRPS. Although ongoing fundamental research is needed to further elucidate the molecular background, from both a clinical and etiological perspective it is also of high importance to investigate determinants and risk factors for CRPS in epidemiological studies. Increased insight in predisposing factors may facilitate early diagnosis and improve the chance of good outcome, but also may provide new clues for potential underlying mechanisms. The elucidation of a single biochemical factor common in all potential underlying disease mechanisms would offer a highly interesting target for pharmacotherapy.

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Chapter 2

Descriptions

2.1 | The Incidence of Complex Regional Pain Syndrome: a Population Based Study

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Abstract

Introduction: The Complex Regional Pain Syndrome (CRPS) is a painful disorder that can occur in an extremity after any type of injury, or even spontaneously. Data on the incidence of CRPS are scarce and mostly hospital based. Therefore the size of the problem and its burden on health care and society are unknown. The objective of the present study was to estimate the incidence of CRPS in the general population.

Methods: A retrospective cohort study was conducted during 1996-2005 in the Integrated Primary Care Information (IPCI) project, a general practice research database with electronic patient record data from 600,000 patients throughout the Netherlands. Potential CRPS cases were identified by a sensitive search algorithm including synonyms and abbreviations for CRPS. Subsequently, cases were validated by electronic record review, supplemented with original specialist letters and information from an enquiry of general practitioners.

Results: The estimated overall incidence rate of CRPS was 26.2 per 100,000 person years (95% CI: 23.0-29.7). Females were affected at least three times more often than males (ratio: 3.4). The highest incidence occurred in females in the age category of 61-70 years. The upper extremity was affected more frequently than the lower extremity and a fracture was the most common precipitating event (44%).

Discussion: The observed incidence rate of CRPS is more as four times higher than the incidence rate observed in the only other population based study, performed in Olmsted County, USA. Postmenopausal woman appeared to be at the highest risk for the development of CRPS.

Introduction

Complex Regional Pain Syndrome (CRPS), formerly known as Sudecks dystrophy or reflex sympathetic dystrophy, is a painful disease with clinical features that include pain, sensory, sudo- and vasomotor disturbances, trophic changes and impaired motor function.[1] The disease course varies from relatively mild and self-limiting to chronic disease with a high impact on daily functioning and quality of life.[2] Usually, symptoms appear in one extremity after even a relatively mild trauma, for example a fracture, contusion or surgery, but symptoms have also been described after varicella zoster infection and myocardial infarction.[3] The diagnosis is based on the findings during the history and physical examination, for which several diagnostic criteria sets have been developed. The most well known are the IASP (International Association for the Study of Pain) criteria, that where established during a consensus meeting of experts in 1994.[4] The pathogenesis and etiology may involve both neurological and inflammatory disorders, but remain to be completely unraveled.[5,6]

Due to its complexity and broad spectrum of symptoms, CRPS patients are treated by physicians from different clinical backgrounds, including anesthesiologists, (orthopedic) surgeons, neurologists, rheumatologists and rehabilitation doctors. The incidence of CRPS has been studied retrospectively and prospectively in clinical settings after a certain precipitating event, most frequently after a distal radius fracture.[7-11] Sandroni and colleagues have been the only ones so far to assess the incidence of CRPS in the general population (Olmsted County, USA) and they reported an incidence rate of 5.46 per100,000 person years (PY).[12]

In our study, the objective was to assess the incidence of CRPS in the general population in the Netherlands. Moreover, we classified cases according to different diagnostic criteria and described the precipitating events of CRPS.

Methods

Setting

The Integrated Primary Care Information Project (IPCI) is a longitudinal observational database including electronic patient's records of more than 600,000 patients from more than 150 general practitioners (GPs). The patient population is representative of the Dutch population regarding age and sex.[13,14]

In the Dutch Health Care System, all persons need to be registered with a GP who acts as a gatekeeper for further medical care. The electronic records store information on demographics, signs and symptoms (using the International Classification for Primary Care (ICPC) codes (a classification system for primary care[15]) and narratives), diagnoses (using ICPC and narratives), clinical findings, specialist referrals, laboratory findings, hospitalizations, and drug prescriptions. Summaries of the hospital discharge letters and information and letters from specialists are entered in a free text format and hard copies of original letters can be provided upon request. To maximize completeness of

the data, GPs who participate in the IPCI project are not allowed to use paper-based records. The system complies with the European Union guidelines on the use of medical data for medical research and has been proven valid for (pharmaco)-epidemiological studies.[16] The Scientific and Ethical Advisory Group of the IPCI project approved the study (projectnumber 04/70)

Source Population

The source population comprised all persons of all ages, with at least 1 year of valid history in the IPCI database during the study period (January 1996-June 2005). This meant that the practice had been contributing data to the IPCI database for at least one year and that the patient had been registered with the GP for at least one year. This one year period was required to have sufficient background information on all subjects. Follow-up started at the beginning of the study period or on the date that one year of valid history was available, whichever date was latest. Follow-up was terminated when the person transferred out of the practice, on the date of last data supply by the GP, death, diagnosis of CRPS or at the end of the study period, whichever came first.

Since additional data collection was required for validation of CRPS, the source population was restricted to all practices that were still active in the IPCI database in 2006 and provided additional information. For clarification of the data collection procedures see also figure 1.

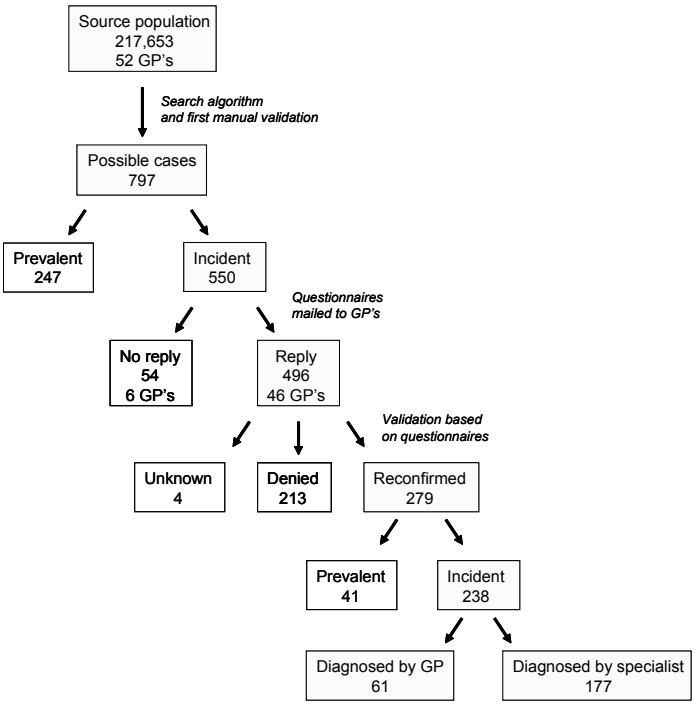

Figure 1. The process of identification of CRPS cases in the Integrated Primary Care Information (IPCI) database.

Case Definition

Potential cases of CRPS were identified in the IPCI database using an extensive string search including an exhaustive list of synonyms and abbreviations for CRPS (for example complex regional pain syndrome, Sudecks dystrophy, reflex sympathetic dystrophy, posttraumatic dystrophy, CRPS, RSD, PTD, etc.), plus prescriptions of dimethyl sulfoxide (DMSO). In the Netherlands, DMSO is exclusively prescribed for CRPS. In a first validation step, performed by a medical doctor with clinical experience in CRPS, all potential cases were manually evaluated by reading the patients records in order to eliminate obvious non cases and to classify possible cases as incident or prevalent. A possible case was defined as each patient for whom CRPS was suggested or diagnosed in the patient record. A possible case was considered incident when the first occurrence fell within the follow-up time of that person.

To further validate the diagnosis of CRPS in the incident possible cases, a short questionnaire was mailed to the GPs. The questionnaire was used to confirm whether the person, according to the GP's judgment, indeed suffered from CRPS and whether the patient had been seen and diagnosed by a specialist. Copies of all specialist letters were requested. Specialist letters usually provide information about history and physical examination of the patient. That information was used to verify the fulfillment of diagnostic criteria for CRPS according to the IASP criteria[4], the Bruehl criteria[17,18], and the Veldman criteria[9] (see the legend of table 1b for a description of the criteria sets). The choice for these sets of criteria was based on international acceptance of the IASP criteria, high specificity of the Bruehl criteria, and national acceptance of the Veldman criteria. These criteria sets differ from each other in the types and the number of symptoms and signs that have to be present in order to establish the diagnosis CRPS. The IASP criteria are regarded as very sensitive, whereas the Bruehl criteria have lower sensitivity, but are highly specific. The Veldman criteria are the only ones that theoretically allow a diagnosis of CRPS in the absence of pain.

The fulfillment of the diagnosis according to the different criteria sets was judged independently by two physicians who are familiar with CRPS. In case of discrepancies between their judgments, consensus was reached by discussion. Where pain and an increase of the symptoms after use of the affected limb (an obligatory feature according to the Veldman criteria) were not mentioned in the letters, it was reasonable to assume that they were present in most of the cases. Therefore, the criteria were first applied both in a strict sense, and subsequently without taking into account the presence of pain and increase after use of the affected limb. Information on precipitating events and referrals were derived from the electronic medical records and the specialist letters.

Analysis

The incidence rate of CRPS was calculated by dividing the number of incident cases (as established by reconfirmation of the diagnosis) (numerator), by the total number of accrued person years in the population (denominator). Incidence rates (IR) were calculated per calendar year, sex, and age category. Calculations were stratified for CRPS following a fracture and CRPS following other precipitating events. 95% Confidence intervals were constructed around the rates based on the Poisson distribution.

In addition to the incidence rate based on the reconfirmed diagnoses, we also calculated an incidence rate based on cases that fulfilled the IASP criteria. However, the IASP criteria could only be applied in a subset of the specialist diagnosed cases (for whom specialist letters with diagnostic information were available). Therefore, the percentage of fulfillment was extrapolated only within the specialist diagnosed case group. Cases that were diagnosed by the GP alone were excluded from this analysis.

In order to be able to compare our results with the incidence found by Sandroni and colleagues, we calculated standardized morbidity ratios (SMR), using the method of indirect standardization on age and gender, as described by Rothman.[19]

Standard descriptive statistics were used to compare categorical variables (Chi-square test, univariate logistic regression), or means (Student's t-test). Kappa statistics were calculated to judge interrater agreement for the diagnostic criteria. The Statistical Package for Social Sciences (SPSS) 12.0 for Windows was used for all statistical tests.

Results

In the initial source population of 217,653 persons registered with at least one year of valid history at one of the 52 active practices in the IPCI database, 238 incident cases of CRPS could be identified after finalization of the validation process (figure 1). The response rate for the short questionnaires amongst GP's was 88%. Only the populations from the practices that responded were included in the source population for calculations of the IR. This source population comprised 190,902 persons from 46 practices, and concerned the mentioned 238 cases.

For 95 (54%) of the 177 cases that were diagnosed by a specialist, letters with information on anamnesis and physical examination were available. Structured extraction of data (table 1a) allowed for classification of the cases according to different CRPS criteria sets (table 1b). 86% of these specialist diagnosed cases fulfilled the strictly applied IASP criteria for CRPS. If pain and an increase in pain after use of the body part were assumed to be present (even if not mentioned in the letter), 93% of the cases fulfilled the IASP criteria, 47% the Bruehl criteria and 58% the Veldman criteria (table 1b). The interrater agreement varied between the different criteria sets, with the lowest $\kappa = 0.43$ (moderate) for the IASP criteria with pain assumed to be present, and the highest $\kappa = 0.78$ (good) for the Veldman criteria. In the available specialist letters the presence or absence of vaso- and sudomotor and motor-trophic signs and symptoms were reported more frequently than the presence or absence of sensory and neurological signs and symptoms.

Table 1a. Relevant characteristics in anamnesis and physical examination, described in specialist letters regarding the CRPS patients.

N=95	Anamnesis						Physical examination					
	present		absent		not mentioned		present		absent		not mentioned	
Symptoms/signs	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Sensory												
Spontaneous pain	81	85.3%	0	0%	14	14.7%	37	38.9%	1	1.1%	57	60.0%
Hyperesthesia	4	4.2%	0	0%	91	95.8%	5	5.3%	5	5.3%	85	89.5%
Hyperalgesia	0	0%	0	0%	95	100%	2	2.1%	3	3.2%	90	94.7%
Allodynia	9	9.5%	0	0%	86	90.5%	11	11.6%	4	4.2%	80	84.2%
Hyperpathy	2	2.1%	0	0%	93	97.9%	6	6.3%	4	4.2%	85	89.5%
Paraesthesias	9	9.5%	0	0%	86	90.5%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hypoesthesia	4	4.2%	0	0%	91	95.8%	8	8.4%	0	0%	87	91.6%
Vasomotor												
Temperature asymm.	56	58.9%	2	2.1%	37	41.1%	44	46.3%	8	9.2%	43	45.3%
Colour asymmetry	51	52.6%	2	2.1%	42	44.2%	43	45.3%	9	9.5%	43	45.3%
Sudo-motor												
Swelling/oedema	53	55.8%	0	0%	42	44.2%	55	57.9%	5	5.3%	35	36.8%
Sweating asymmetry	23	24.2%	6	6.3%	66	69.5%	29	30.5%	11	11.5%	55	57.9%
Motor-trophic												
Limited range of motion	20	21.1%	2	2.1%	73	76.8%	51	53.7%	3	3.2%	41	43.2%
Paresis	7	7.4%	0	0%	88	92.6%	15	15.8%	6	6.3%	74	77.9%
Dystonia	4	4.2%	0	0%	91	95.8%	5	5.3%	0	0%	90	94.7%
Altered hair growth	2	2.1%	6	6.3%	87	91.5%	5	5.3%	15	15.7%	75	78.9%
Altered nail growth	4	4.4%	3	3.2%	88	92.6%	4	4.3%	10	10.5%	81	85.3%
Skin atrophy			0	0%	95	100%	2	2.1%	0	0%	93	97.9%
Neurologic												
Disturbed coordination	2	2.1%	0	0%	93	97.9%	1	1.1%	1	1.1%	93	97.9%
Tremor	0	0%	0	0%	95	100%	1	1.1%	0	0%	94	98.9%
Involuntary movements	1	1.1%	0	0%	94	98.9%	0	0%	1	1.1%	94	98.9%
Paralysis	0	0%	0	0%	95	100%	1	1.1%	0	0%	94	98.9%
Muscle atrophy	0	0%	0	0%	95	100%	7	7.4%	1	1.1%	87	91.6%
Various												
Increase after use	26	27.4%	0	0%	69	72.6%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Alternative diagnosis	6	6.3%	0	0%	89	93.7%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table 1b. Fullfillment of the GP confirmed CRPS patients according to the different diagnostic criteria sets.

N=95	Strict		If presence of pain is assumed		If increase of pain after use of affected body part is assumed		Interobserver agreement
	n	%	n	%	n	%	κ
IASP[A]	82	86.3%	88	92.6%	n.a.	n.a.	0.43 - 0.66 (moderate - good)
Bruehl[B]	41	43.2%	45	47.4%	n.a.	n.a.	0.66 - 0.69 (good)
Veldman[C]	17	17.8%	17	17.8%	55	57.9%	0.63 - 0.78 (good)

A. IASP citeria: 1. Develops after an initiating noxious event (type I) or after a nerve injury (type II)
2. Spontaneous pain or allodynia/hyperalgesia that is not limited to the territory of a single peripheral nerve and is disproportionate to the inciting event.
3. There is or has been evidence of edema, skin blood flow abnormality, or abnormal sudomotor activity in the region of the pain since the inciting event.
4. This diagnosis is excluded by the existence of conditions that would otherwise account for the degree of pain and dysfunction

B. Bruehl criteria: 1. Continuing pain which is disproportionate to any inciting event.
2. Must report at least one symptom (history) in each of the following categories. Must display at least one sign (physical examination) in two or more of the following categories.
Sensory: hyperesthesia, hyperalgesia (to pinprick) and/or allodynia (to light touch)
V*asomotor*: temperature asymmetry and/or skin color changes and/or skin color asymmetry
Sudomotor/edema: edema and/or sweating changes and/or sweating asymmetry
Motor/trophic: decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin).

C. Veldman criteria: 1. 4 or 5 of: Unexplained diffuse pain; Difference in skin color relative to other limb; Diffuse edema; Difference in skin temperature relative to other limb; Limited active range of motion
2. Occurrence or increase of above signs and symptoms after use.
3. Above signs are present in an area larger than the area of primary injury or operation and including the area distal to the primary injury.

Characteristics of the cases are displayed in table 2. The mean age at CRPS diagnosis was 52.7 ± 2.20 (range: 7-90) years in the total group. The mean age for males was 51.1 ± 4.2 years and for females 53.0 ± 2.6 years. The age at diagnosis did not differ between males and females (p = 0.404). The most common precipitating event for CRPS was a fracture, followed by a contusion/sprain. In more than ten percent of the cases no precipitating event was reported or could be identified in the medical record. Upper extremities were more often affected than lower extremities (59.2% versus 39.1%, p <0.001), whereas the right side and left side of the body were affected with the same frequency (p=0.464). Patients who were diagnosed only by GPs were significantly older than patients who were also referred to and diagnosed by a specialist (p=0.039). Anesthesiologists were the most frequently involved specialists and usually one type of specialist was seen by the patient.

Table 2. Characteristics of CRPS patients.

Characteristics	Total, N=238		By GP only, N=61		By specialist, N=177		p-value
	n	%	n	%	n	%	
mean age at onset (std)	52.7 (17.31)		56.6 (19.63)		51.3 (16.27)		0.039*
Female	184	77.3%	52	85.2%	132	74.6%	0.086
CRPS II	7	2.9%	1	1.6%	6	3.4%	0.485
Precipitating event							
None	25	10.8%	8	13.1%	17	9.6%	0.941
Fracture	105	44.1%	28	45.9%	77	43.5%	0.790
Sprain	42	17.6%	13	21.3%	29	16.4%	0.953
Elective surgery	29	12.2%	5	8.2%	24	13.6%	0.617
CTS	8	3.4%	1	1.6%	7	4.0%	0.441
Dupytren	6	2.5%	1	1.6%	5	2.8%	0.577
Tendon injury	13	5.5%	2	3.3%	11	6.2%	0.484
Others	21	8.8%	4	6.6%	17	9.6%	0.575
unknown	3	1.3%	1	1.6%	2	1.1%	
Localization at diagnosis							
Right side of the body	114	47.9%	31	50.8%	83	46.8%	0.279
Left side of the body	115	48.3%	26	42.6%	89	50.2%	0.154
Unknown	9	3.8%	4	6.6%	5	2.9%	
Upper extremity	141	59.2%	40	65.6%	101	57.1%	0.243
Lower extremity	93	39.1%	19	32.2%	74	41.8%	0.141
Unknown	4	1.7%	2	3.2%	2	1.1%	
Type of specialist to whom patient is referred (more than one possible)							
Anesthesiologist					87	49.7%	
Rehabilitation doctor					52	29.4%	
Orthopedic Surgeon					49	27.7%	
Surgeon					45	25.4%	
Neurologist					19	10.7%	
Rheumatologist					8	4.5%	
Plastic Surgeon					5	2.8%	
Other					6	3.4%	
No. of specialists seen							
1					105	59.3%	
2					54	30.5%	
3					14	7.9%	
4					4	2.3%	

* significance at $p<0.05$, X^2 test, given for differences in characteristics between patients diagnosed by the GP alone and patients additionally confirmed by a specialist.

The incidence rate of CRPS in the Netherlands was 26.2 per 100,000 (PY) (95% CI: 23.0-29.7) (table 3). The standardized morbidity ratio (SMR) was calculated as 4.2, meaning that, after standardization for age and gender to the source population of Sandroni and colleagues, we found a 4.2 times higher incidence rate than described in their study. If only specialist confirmed cases were considered, the incidence rate was 19.5 per 100,000 PY (95%CI: 16.8-22.5). The incidence rate based on specialist diagnosed cases that fulfilled the IASP criteria was 16.8 per 100,000 PY (95%CI: 14.7-19.2). The SMR compared to the results of Sandroni was 2.7.

Gender-specific incidence rates, based on the reconfirmed diagnoses, for females and males were 40.4 (95% CI: 34.8-46.5) and 11.9 (95% CI: 9.0-15.4) per 100,000 PY, respectively. The incidence of CRPS was more than threefold higher in females than in males (RR: 3.4, 95%CI: 2.9-3.9). The incidence rate of CRPS did not change significantly over time between 1996 and 2005 (figure 2). The confidence intervals in 1996 and 2005 were relatively wide due to the low number of person years by left censoring in 1996 (early stage of the IPCI database) and a high degree of right censoring in 2005 (data available only until June). The incidence varied profoundly with age, the highest incidence rate was observed in the group 61-70 years of age (figure 3). The age and sex distribution pattern was similar in a subgroup including only the cases with another precipitating event than a fracture.

Discussion

In this study, we demonstrated that the population-based incidence of CRPS in the Netherlands is 26.2 per 100,000 PY, with a peak incidence at 61-70 years of age. Fracture was the most common precipitating event accounting for 44% of the CRPS cases. The upper extremities were more often affected than the lower extremities with no preference for either left or right side. A wide variety of specialists was involved in the diagnosis and treatment of CRPS patients.

The incidence rate in this study is more than four times higher than the population-based incidence rate that was reported by Sandroni and colleagues in Olmsted County[12]. The difference sustained even after standardization (IR: 22.8 per 100,000 PY) and when we included only specialist-diagnosed cases in our calculations (IR: 19.0 per 100,000 PY). Possibly, differences in population characteristics such as ethnicity, socio-economic factors and incidence of fractures can explain the observed difference. More likely, however, it is secondary to the difference in case definitions and validation. The study of Sandroni and colleagues used the IASP criteria, which were applied retrospectively to information from electronical medical records. We also used a retrospective approach and used both electronical medical records as well as information from GP questionnaires and specialist letters for the diagnosis. In contrast to Sandroni we did not require that all cases should fulfill diagnostic criteria; we retained all cases on the basis of a reconfirmed diagnosis of CRPS by the GP or specialist.

Criteria sets were also applied on a subset for which detailed diagnostic data were available, but were used merely to see differences in criteria sets. However, an incidence rate based on the strictly applied IASP criteria (IR: 16.8 per 100,000 PY), as done in the Sandroni study, was calculated and was still almost three times higher in our study

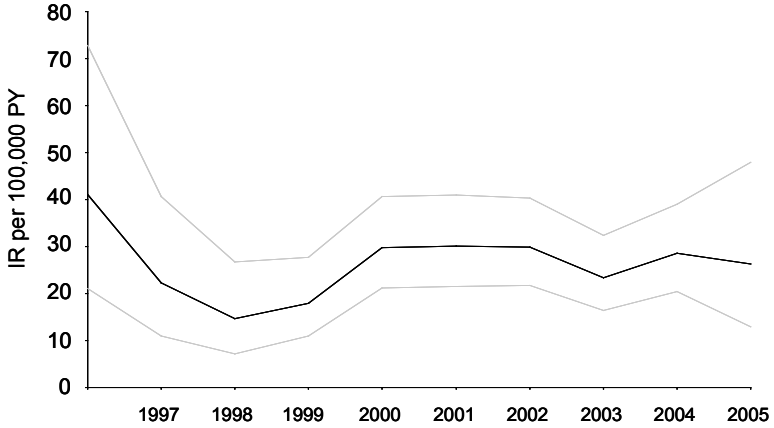

Figure 2. Incidence rates (with 95% confidence lines) of CRPS in the Netherlands per calender year.

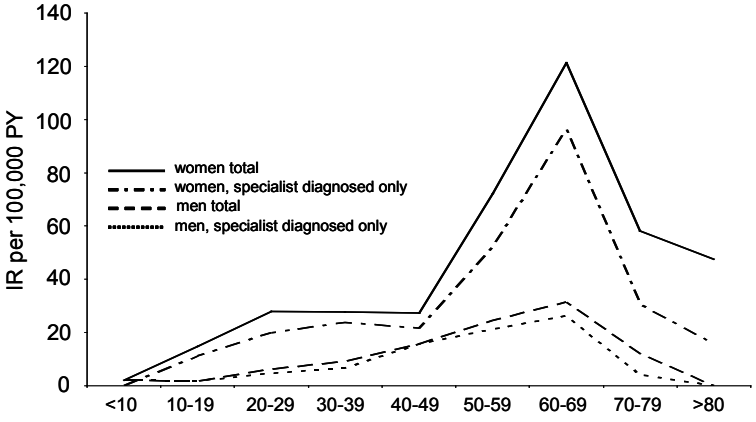

Figure 3. Incidence rates of CRPS in the Netherlands per age catgory, stratified by gender and confirmation level (diagnosed by GP alone or additionally confirmed by a specialist).

Table 3. Incidence of CRPS in the Netherlands per calenderyear and per age category.

	Males			**Females**			**Total**		
	Cases	**PY at risk**	**IR per 100.000 PY**	**Cases**	**PY at risk**	**IR per 100.000 PY**	**Cases**	**PY at risk**	**IR per 100.000 PY**
Year									
1996	2	11874	16.8	8	12484	64.1	10	24358	41.1
1997	0	19800	0.0	9	20663	43.6	9	40464	22.2
1998	1	30296	3.3	8	31231	25.6	9	61528	14.6
1999	2	50098	3.9	16	50335	31.8	18	100433	17.9
2000	12	60441	19.9	24	60619	39.6	36	121060	29.7
2001	8	61394	13.0	29	61529	47.1	37	122923	30.1
2002	12	66799	18.0	28	66888	41.9	40	133687	29.9
2003	10	70710	14.1	23	70679	32.6	33	141390	23.3
2004	6	64790	9.3	31	64524	48.0	37	129314	28.6
2005	1	17220	5.8	8	17063	46.9	9	34283	26.3
Age group									
<10	1	51252	2.0	1	49182	2.0	2	100434	2.0
10-19	1	56063	1.8	8	53639	14.9	9	109702	8.2
20-29	4	64319	6.2	17	60723	28.0	21	125042	16.8
30-39	7	77401	9.0	20	72058	27.7	27	149459	18.1
40-49	11	70805	15.5	19	69640	27.2	30	140445	21.4
50-59	15	61482	24.4	43	59597	72.1	58	121080	47.9
60-69	12	38206	31.4	48	39554	121.3	60	77760	77.2
70-79	3	24582	12.2	19	32695	58.1	22	57277	38.4
>80	0	9313	0.0	9	18931	47.5	9	28245	31.9
Total	54	453425	**11.9 (9.0 - 15.4)**	184	456018	**40.4 (34.8 - 46.8)**	238	909443	**26.2 (23.0 - 29.7)**

as the incidence rate found in Olmsted County (SMR=2.7). Remarkable is, that in our subset of specialist diagnosed cases 86% fulfilled the IASP criteria, compared to 19% of the cases in the Sandroni study. The supposedly high rate of incorrectness of the CRPS diagnosis (81%) in the Sandroni study has been questioned by others before[20], and suggests that the retrospective application of the IASP criteria to information on electronic charts might have been overly strict. The IASP criteria are considered highly sensitive and incidence rates based on this should be comparable with incidence rates based on specialist's diagnoses.

The highest incidence rate in our study was observed in the age group of 61-70 years and the mean age at diagnosis was 52.7 years. This age peak is higher than is generally expected and observed in some non-population based investigations[9]. However, other clinical studies show high average ages of the included CRPS patients, in line with our observation.[7,10,11] It could be suggested that the increasing incidence of CRPS with age is due to a higher occurrence of fractures at older age. However, the same age distribution pattern was observed in the group of patients with another precipitating event than a fracture. From our findings, it can be concluded that the majority of the CRPS cases in females occur in the post-menopausal stage of life. This was noted before by Zollinger et al.[10] The age and sex distribution pattern suggests that hormonal etiological factors may be involved in the pathogenesis of CRPS.

Noteworthy is that less than half of the CRPS cases have a fracture as precipitating event, similar to the observations of Sandroni and colleagues. Fracture is often regarded as the primary precipitating event and the incidence of CRPS after a fracture has been studied prospectively.[8,9] Hence, other precipitating events, such as surgery and tendon rupture may be worthwhile including in prospective research. Additional findings of interest were the fact that patients who were diagnosed only by the GP without referral to a specialist, tended to be older.

Limitations in our study are related to the absence of a gold standard for the diagnosis of CRPS. As observed in the specialist letters, physicians focused on vaso- and sudomotor and motor/trophic signs, whereas the presence or absence of sensory and neurological symptoms was not frequently reported. Van de Beek and colleagues have stated that general dissatisfaction with the available criteria has resulted in the use of personally favoured criteria.[21] As a consequence of this, descriptions of the patients differ and lack detail, which has complicated uniform classification of the cases in our study. However, if detailed information for the application of diagnostic criteria would have been available as in a prospective study, the choice for one specific criteria set for validation of the diagnosis above the others would also have been disputable, since none of them is definitely superior.[21] For this reason we decided that the most reliable incidence rate calculations would be based on reconfirmed clinical diagnoses. However, the problems regarding case definition that were encountered during our study emphasize again the need for validated and well documented diagnostic criteria, that can be applied more in primary and secondary care and in prospective and retrospective studies.

Despite the above described, we do not believe that the inability to uniformly classify all patients has resulted in an overestimation of the incidence rate. We sought reconfirmation of all cases and allowed GPs to reconsider whether an initial diagnosis actually was correct. In addition, almost three quarters of the patients were referred to a

medical specialist who reconfirmed the diagnosis as well, after exclusion of alternative diagnoses. The actual incidence rates varied with the choice to base the validation of the CRPS diagnosis on GP or specialist confirmed cases. However, the pattern of the incidence rate (sex and age distribution) was similar for both groups. On the contrary, we consider it more likely that our incidence rates are an underestimation of the reality. Although we have used a sensitive search algorithm for identification of the CRPS cases, we might have missed cases that had symptoms but were not diagnosed as such in the medical record because of unfamiliarity of the GP with the syndrome and its nomenclature. Mainly the relative mild and self limiting cases of CRPS might not always have been recorded as such in the medical journal and were therefore not included in our calculations.

In conclusion, we estimated an incidence of CRPS in the general population of the Netherlands of 26.2 per 100,000 PY, which is much higher than previously described. Postmenopausal women appeared to be at an increased risk for the development of the disease. Uniform use of more generally accepted diagnostic criteria would improve the quality of epidemiological and clinical studies concerning CRPS in the future.

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2.2 | Disease Outcome of Complex Regional Pain Syndrome

Submitted

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Abstract

Introduction: The outcome of complex regional pain syndrome (CRPS) is relatively unknown. High disease resolution rates have been reported, but also long-lasting impairments in many patients. The present study aims to assess CRPS outcome in a population based cohort of CRPS patients.

Methods: CRPS patients were retrospectively identified (1996-2005) in a Dutch GP database, the Integrated Primary Care Information project, and included if at onset (i.e. in the past) they had complied with IASP diagnostic criteria. The disease status at minimal two years since onset was assessed during visits using questionnaires, interviews and physical examination. Symptoms (subjective) and signs (objective) were compared to reference patients with an identical past injury but without CRPS. Actual fulfillment of the IASP criteria, treatment status, self-reported recovery, and working status were recorded. Moreover, to identify potential prognostic factors, baseline patient characteristics were compared across subgroups according to CRPS outcome. These subgroups were derived by cluster analysis on actual symptoms and signs.

Results: Hundred-and-two CRPS patients were assessed at on average 5.8 years (range 2.1-10.8) since onset. CRPS patients displayed still higher symptom and sign prevalences in all categories (sensory, vasomotor, sudomotor, motor/trophic) than references. Sixteen percent (95%CI: 9-22) reported the CRPS as still progressive, while 31% (95%CI: 19-43) was incapable to work. Patients in the poorest outcome cluster had more often their upper extremity affected, another event than a fracture, and cold CRPS.

Discussion: Severe CRPS outcome is rare, but a majority of patients has persistent impairments at two or more years since onset.

Introduction

Complex Regional Pain Syndrome is a painful complication of a fracture, surgery or other type of injury. The diagnosis is mainly based on the presence of symptoms (subjective) and signs (objective). Several sets of diagnostic criteria have been proposed, of which the criteria from the International Association for the Study of Pain (IASP) are the most widely accepted for clinical use.[1,2] The criteria proposed by Harden and Bruehl are considered the most specific and have been recommended for research purposes.[2,3]

Insight into the disease course and outcome of CRPS is limited. Various studies have been performed, often in small or selected populations and without a reference group. Regarding the course of CRPS, Bonica et al. classically described the disease as one with sequential stages, wherein the initial sensory disturbances diminish over time and the motor/trophic abnormalities increase.[4] Bruehl et al. tried to actually demonstrate these stages in a group of CRPS patients by conducting a cluster analysis based on clinical features (symptoms and signs). However, the three distinct clusters that were formed were not associated with CRPS duration and therefore the results argued against sequential staging of CRPS.[5]

Regarding the outcome of CRPS, contradicting reports have been published. For example Sandroni et al. performed a population based study and mentioned complete symptom resolution in 74% of the patients within one year after CRPS onset[6], based on medical chart review. On the contrary, also after one year or more, Veldman et al. and Galer et al. objectively assessed persistent disturbances in most of their patients.[7,8] In addition both Geertzen et al. and Vaneker et al. reported impairments and disability in patients whom they assessed at three to nine years since CRPS onset.[9,10] The variability in results may reflect either the assessment methods (chart review versus actual assessments) or the setting (population based versus hospital based). Prognostic factors for a good or poor outcome of CRPS are not known, although coldness of the affected limb has been associated with longer disease duration[11] and worse functional outcome.[12]

The aim of the present study was to get more insight into the general disease outcome of CRPS, using a population-based retrospective comparative cohort study. CRPS patients and reference (no CRPS) patients had experienced a similar past physical trauma at least two years prior to the assessment date.

Methods

Setting

All patients were selected from the Integrated Primary Care Information (IPCI) project, a longitudinal General Practitioners (GP) database maintained by the Department of Medical Informatics of the Erasmus Medical Center in Rotterdam. Presently, the database stores the electronic patient records of more than 800,000 patients. The population in the database reflects the general population of the Netherlands regarding age and sex distribution. Details of the project have been described elsewhere.[13] In the Dutch health

care system all persons have to be registered with one GP, who acts as a gatekeeper and receiver for secondary care. GPs who participate in the IPCI project are not allowed to use paper-based patient records, in order to maximize completeness of the electronic data. The IPCI project complies with the European Union guidelines on the use of medical data for medical research and has been proven valid for (pharmaco-epidemiological) research.[14] The study has been approved by the Scientific and Ethical Advisory Group of the Project (project number 04/70) and by the Medical Ethical Board of the Erasmus Medical Center (Protocol number 2006-099).

Source population

The source population was restricted to all practices that were still active in the IPCI database in 2006 and were able to provide additional information upon request. From these, all persons in database follow-up between January 1996 and June 2005 were included. To be able to characterize patients, a minimum of one year of valid history was required for each person before follow up was started, which meant that the GP practice had been participating in IPCI for at least one year and that the patient had been registered with that GP for at least one year.

CRPS cohort

Identification of possible CRPS cases was performed using a sensitive string search algorithm in the IPCI database, followed by a first validation step in which GPs were asked to reconfirm the CRPS diagnosis in a short questionnaire. The index date was chosen as the date of the precipitating injury, or in cases with spontaneous CRPS at the date of first symptoms in the GP records. This part of the case identification procedure is explained in detail in our study on the incidence of CRPS in the Netherlands.[15]

The confirmed cases were invited via the GP to participate in the present study. In the invitation letter we clearly explained that the time load of participation would be less than three hours in total, visits could occur either at the GP or at home, and that early morning/late evening visits were possible to avoid interference with daily (work) activities. Additionally we stressed that participation was relevant regardless if the CRPS was still active or not.

Patients who provided informed consent were visited once by the primary investigator (MM), who is a physician with clinical experience in diagnosing CRPS, together with a research assistant (MHB). Due to the retrospective nature of the study these visits occurred at a minimum of two years after CRPS onset (date of precipitating injury) for all subjects. Prior to the visit participants received a questionnaire about their CRPS related complaints, precipitating injury, disease course, referrals, therapy, impairments, and ability to work. These questionnaires were completed by the patients in advance, and were discussed briefly during the visit in order to avoid incompleteness or misinterpretations. Subsequently, during the visit a history focused on CRPS related symptoms was taken, as well as physical examination of the affected and contralateral unaffected limb. Symptoms (subjective) and signs (objective) relevant for the diagnosis of CRPS were collected in a standard assessment form, as is used within TREND (Trauma Related Neuronal Dysfunction), the Dutch scientific knowledge consortium in which the study

was embedded.[16] Time since injury was categorized as 2-5 years; 5-7.5 years or >7.5 years.

Based on self-reported predominant temperature characteristics of the affected limb(s) patients were classified as warm or cold CRPS. Patients were included in the study if at the moment of CRPS onset (i.e. in the past, not per definition by the time of visit) they had fulfilled the IASP criteria for CRPS, based on the combined information from the visits (symptoms and signs history), the GP records and specialist letters.

Reference cohort

Reference patients were randomly selected from the IPCI database and matched to the CRPS patients on age, gender, calendar time (two year band) and specific type of injury that precipitated CRPS (fracture, surgery, soft tissue injury, etc.). This meant that each CRPS case patient was compared to a reference patient with an identical injury at a similar location (upper or lower extremity). If the case had no specific injury preceding CRPS onset, the reference patient was allowed to be free of injury either. For each CRPS patient two reference patients were asked to participate. If both refused, up to six additional persons were invited. Reference patients who provided informed consent received a questionnaire similar to the one for CRPS patients, without the parts specifically addressing CRPS. Reference patients were visited, during which a history taking and physical exam was performed of the past injured extremity, as well as the contralateral extremity, using the same standardized assessment form as for CRPS patients. Time since injury was categorized as 2-5 years; 5-7.5 years or >7.5 years.

Outcome parameters

In this descriptive study we used three different ways to investigate the long-term outcome of CRPS. First, we compared the actual prevalence rates of specific symptoms (subjective) and signs (objective) as assessed during the visit between CRPS patients and the reference group.

Second, within the group of CRPS patients alone we categorized patients according to the following classifications of disease outcome: 1. fulfillment of IASP criteria; 2. continuing CRPS treatment 3. self-reported recovery (completely recovered, stabilized or progressive disease); 4. employment status (completely resumed former work activities, working with adaptations or complete incapability to work).

Third, within the group of CRPS patients, we also defined subgroups according to CRPS outcome status by using a cluster analysis method. Patients were grouped in one cluster if they resembled each other with respect to long-term persisting symptoms and signs. We compared baseline patient characteristics between these subgroups (clusters) with the purpose to identify prognostic factors of CRPS outcome.

Statistics

Standard descriptive statistics were used to compare means (one way analysis of variances (ANOVA)) and frequencies (Pearson chi square) across subgroups. The symptom and sign prevalence rates at the moment of visit were compared between CRPS cases and reference patients using a McNemar test for matched observations.

To better identify subgroups of CRPS outcome, a K-means cluster analysis was

performed in a similar manner as done before by Bruehl et al.[5] The K-means cluster analysis is a non-hierarchical clustering method to derive homogeneous subgroups of patients based on selected characteristics, for which we choose the symptoms and signs of CRPS at the moment of visit. First, for each patient we defined the number of features that was still present at the moment of visit within the following classes of signs/symptoms: sensory, vasomotor, sudomotor/edema and motor/trophic[17] (appendix 1). A total of eight scores were derived per patient. Since the maximum number of features differs across the categories we converted the raw scores into standardized scores (Z-scores) using the formula $(X-\mu)/\sigma$, wherein X is the raw score, μ is the mean of the population, and σ is the standard deviation. Finally, the individual patient Z-scores were imported as dependent variables in the K-means cluster analysis. We pre-specified to search for three clusters in order to investigate the intuitive existence of complete, partially and non-recovered patient subgroups. Additionally, this would make the results comparable to those of Bruehl and colleagues.[5]

As potential prognostic factors for CRPS outcome (categorized in the cluster analysis) we considered: age, gender, precipitating injury (fracture or other), affected extremity (upper or lower), and (self reported) cold/warm CRPS. Migraine, osteoporosis, menstrual cycle related disorders, neuropathies, and hypersensitivity related disorders (especially asthma) were also evaluated, since they were associated with the onset of CRPS in a previous study.[18] Psychological factors were considered as they have often been discussed as involved in CRPS onset and disease course.[19,20] Information on medical history was obtained by systematic review of the complete electronic medical records prior to the index date.

All computerized analyses, including the K-means cluster analysis, were conducted using SPSS, version 12.0. Two-tailed significance was established at $p<0.05$.

Results

Study population

The source population comprised of 204,281 persons from 48 GPs. In this population 259 patients with physician confirmed CRPS were identified, 216 (83%) of them could be contacted for study participation, while the remaining 43 patients were untraceable due to re-identification problems (n=25), transferring out (n=16) or death (n=2). Of the 216 contacted patients, 134 **(**62.0%**)** provided informed consent and completed study assessments. No significant differences in age, gender distribution, CRPS duration, and prevalence of concurrent diseases were observed between participants and non-participants. In participants, the lower extremity was affected more frequently compared to non-participants (45% versus 34%; p=0.04), and participants less often lived in a socio economically deprived area (1% versus 6%; p=0.03).

With the additional information obtained during the visits, eight (6.0%) CRPS patients turned out to be prevalent (onset of CRPS prior to start of the follow-up time) and were excluded. In hindsight, 24 (19%) patients had never fulfilled the IASP criteria, despite their previously physician confirmed diagnosis. Reasons for not fulfilling the criteria were presence of a possible alternative diagnosis (n=18), absence of pain (n=2), and absence of vasomotor or sudomotor disturbances (n=4). In total 102 CRPS patients

remained for inclusion in the cohort. The mean follow-up time since CRPS onset (initial injury) was 5.8 years (median 5.7, range 2.1-10.8). Mean age at CRPS onset was 51 years (range 12-86) and 79% was female. Fracture was the precipitating event in 54%, surgery in 12%, and soft tissue injury in 27% of the CRPS cases. In 3% CRPS had developed spontaneously.

For the 102 included case patients 263 reference patients, matched on age, gender, calendar time, and precipitating injury, were invited to the study. Of these 75 (29%) gave consent for participation, yielding 75 matched case-reference pairs (CRPS patients for whom no matched reference patient participated were excluded from the case-reference comparisons). The mean follow-up time since the precipitating injury in the references was 5.4 years (median 5.4, range 2.1-10.7). Participating and non-participating reference patients were similar regarding age, gender and medical conditions, but participants lived less often in a socio economically deprived area (1% versus 4%; non-significant).

Symptoms and signs prevalence rates in CRPS and reference patients

At the moment of visit CRPS patients still had considerably more symptoms (subjective) and signs (objective) than reference patients who had an identical past injury at a similar point in time (table 1). Differences in prevalences were most pronounced for sensory and motor/trophic signs.

CRPS outcomes

Within the group of CRPS patients, there was no association between symptom/sign prevalences and the duration of CRPS (figure 1).

At the moment of visit 64% (95%CI: 55-73) of the CRPS patients still fulfilled the IASP criteria for CRPS diagnosis (figure 2). According to the self reports 30% (95%CI: 21-39) considered themselves recovered, 54% (95%CI: 44-64) were stable, and 16% (95%CI: 9-22) still suffered from severe progressive disease. Only 27% (95%CI: 18-36) of patients were still actively treated (pharmacological, invasive treatment or physiotherapy). Among the 54 patients who were employed before CRPS onset, 41% (95%CI: 28-54) had resumed their former job completely, 28% (95%CI: 16-40) had resumed their work with adjustments (altered function or decrease in working hours per week), and 31% (95%CI: 19-43) had become completely unable to work.

Table 1. Signs and symptoms at the time of study visit in CRPS patients and their matched (age, gender, time and type of injury) reference patients.

Signs/symptoms categories	Subjective symptoms					Objective signs				
	CRPS cases, N=75		References, N=75		p-value	CRPS cases, N=75		References, N=75		p-value
	n	%	n	%		n	%	n	%	
Sensory	**60**	**80%**	**16**	**21%**	**0.000** *	**44**	**59%**	**5**	**7%**	**0.000** *
Spontaneous pain	24	32%	2	3%	0.002 *	n.a.	n.a.	n.a	n.a	n.a.
Allodynia	13	17%	1	1%	0.002 *	8	11%	0	0%	0.008 *
Hyperesthesia	26	35%	6	8%	0.000 *	17	23%	1	1%	0.000 *
Hypoesthesia	17	23%	2	3%	0.001 *	14	19%	1	1%	0.001 *
Hyperalgesia	38	51%	6	8%	0.000 *	26	35%	3	4%	0.000 *
Hypoalgesia	7	9%	2	3%	0.180	6	8%	0	0%	0.031 *
Vascular	**38**	**52%**	**2**	**3%**	**0.000** *	**22**	**29%**	**2**	**3%**	**0.000** *
Asymmetry in color	26	35%	1	1%	0.000 *	17	24%	1	1%	0.000 *
Asymmetry in temperature	28	37%	2	3%	0.000 *	12	16%	1	1%	0.003 *
Sudomotor	**29**	**39%**	**2**	**3%**	**0.000** *	**14**	**19%**	**2**	**3%**	**0.002** *
Edema	22	29%	2	3%	0.000 *	12	16%	2	3%	0.006 *
Asymmetry in sweating	15	20%	0	0%	0.000 *	2	3%	0	0%	0.500
Mototrophic	**56**	**75%**	**21**	**28%**	**0.000** *	**43**	**57%**	**10**	**13%**	**0.000** *
Decreased range of motion	45	60%	13	17%	0.000 *	33	44%	8	11%	0.000 *
Weakness	44	59%	12	16%	0.000 *	31	41%	4	5%	0.000 *
Dystonia	24	32%	4	5%	0.000 *	6	8%	0	0%	0.031 *
Tremor/myoclonus	7	9%	1	1%	0.070	3	4%	0	0%	0.250
Trophic disturbances	18	24%	0	0%	0.000 *	9	12%	0	0%	0.004 *

* $p<0.05$ in McNemar matched pair analysis.

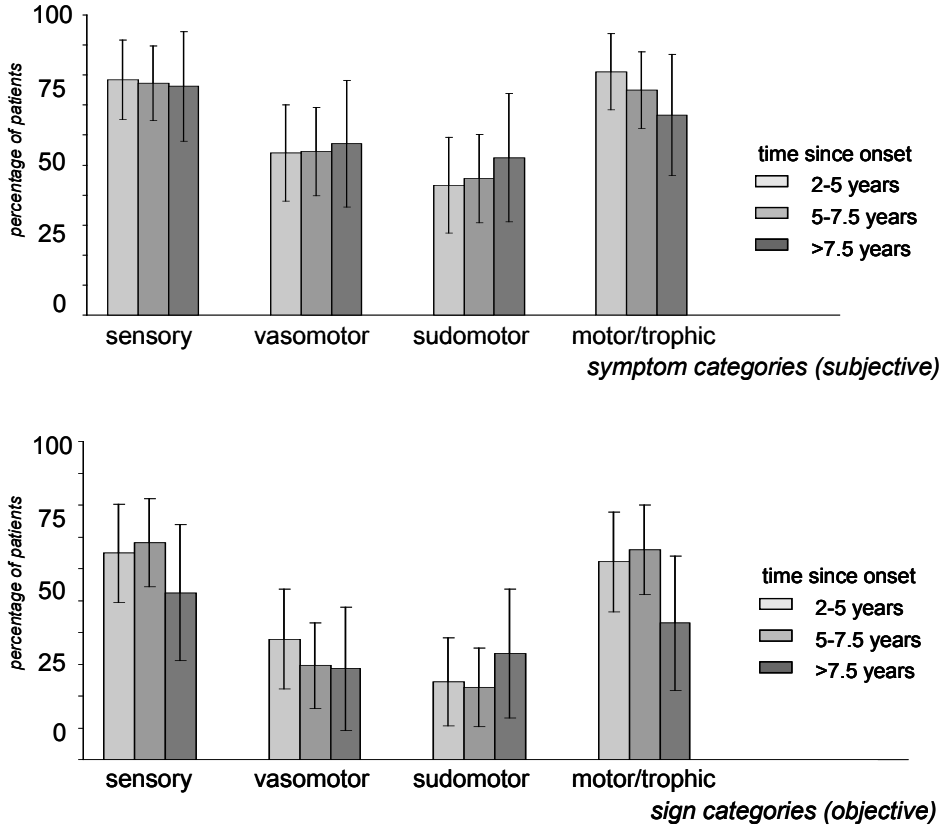

Figure 1. Prevalences of sign/symptom categories in CRPS patients after different time windows since CRPS onset. 2-5 years: n = 37; 5-7.5 years: n=44; > 7.5 years n=21.

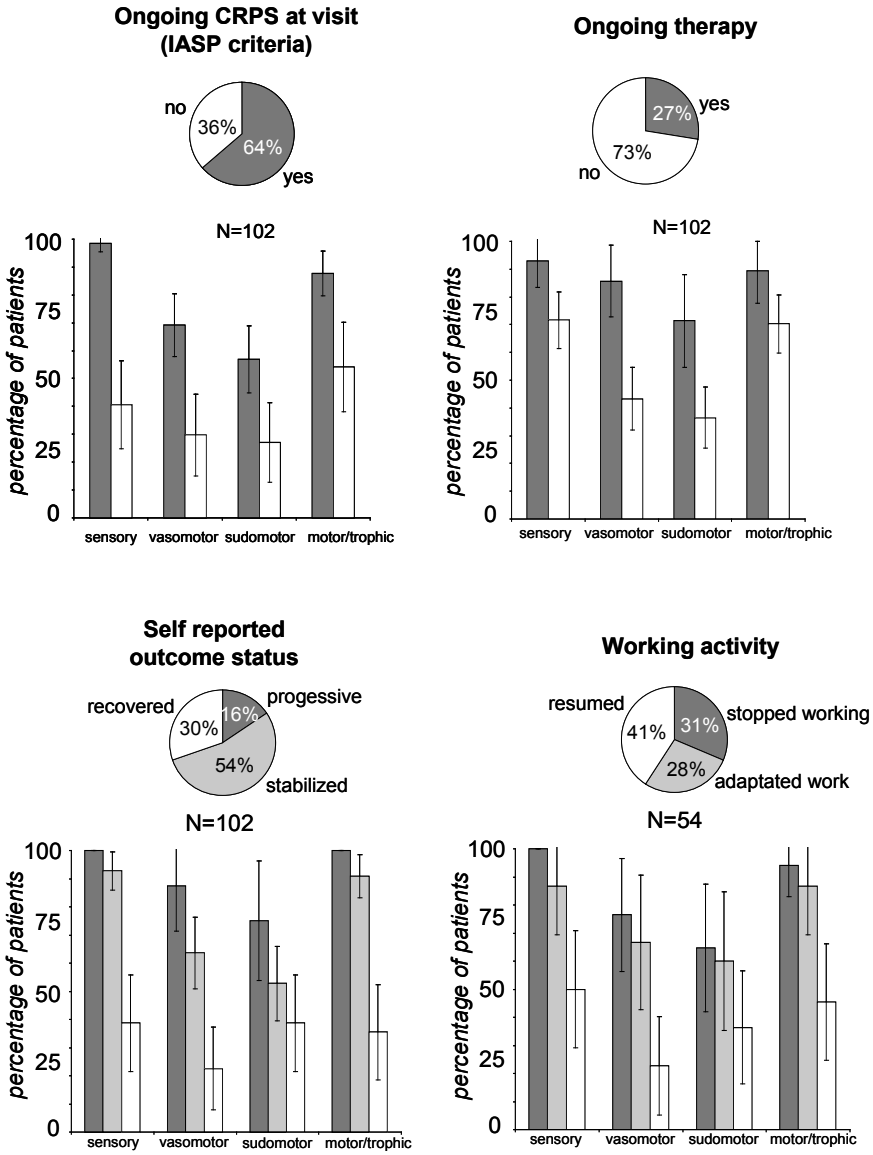

Figure 2. The circle diagrams display the percentage of CRPS patients per outcome status by several parameters (ongoing fulfillment of IASP criteria, ongoing treatment, self reported outcome, working status). The bar diagrams display symptom/sign category prevalences (signs and symptoms taken together) per outcome parameter subgroup. Mean durations since CRPS onset: 5.8 years (range: 2.1-10.8).

Subgroups according to cluster analysis

The K-means cluster analysis on symptoms and signs managed to produce three CRPS patient clusters that were significantly different from each other with regard to almost all symptom and sign categories (table 2). The clusters could be ranked according to disease 'severity', whereby patients in the most severe cluster (cluster 3) also had the worst outcome status if the other outcome measures (IASP criteria, ongoing treatment, self reported outcome status, working status) were compared (table 2). In the poorest outcome cluster all symptoms and signs prevalences were more pronounced than in the other two clusters, except for sudomotor symptoms and signs, which were strongest in

Table 2. Subgroups derived from K-means cluster analyses on signs and symptoms.

	Cluster 1 'best outcome'	**Cluster 2 'moderate outcome'**	**Cluster 3 'poor outcome'**
Number of patients	63	25	14
Symptoms (subjective)	**Mean number (SD)**	**Mean number (SD)**	**Mean number (SD)**
Sensory (n=1)	0.21 (0.41)	0.48 (0.51)	0.86 (0.36)
Vascular (n=2)	0.29 (0.46)	1.44 (0.58)	2.00 (0.0)
Sudomotor (n=2)	0.21 (0.48)	1.24 (0.44)	1.00 (0.68)
Motor/trophic (n=3)	1.03 (0.93)	2.20 (0.87)	2.70 (0.61)
Signs (objective)	**Mean number (SD)**	**Mean number (SD)**	**Mean number (SD)**
Sensory (n=2)	0.22 (0.46)	0.60 (0.71)	1.43 (0.65)
Vascular (n=2)	0.16 (0.37)	0.84 (0.47)	1.93 (0.27)
Sudomotor (n=2)	0.03 (0.18)	0.84 (0.37)	0.21 (0.43)
Motor/trophic (n=3)	0.68 (0.88)	1.56 (1.56)	2.36 (0.74)
Recovery			
Ongoing CRPS (IASP criteria) *no fulfillment at moment of visit* *fulfillment at moment of visit*			
Still receiving therapy *no* *yes*			
Self reported recovery *recovered* *stabilized* *progressive*			
Working activity *former work resumed* *working with adaptations* *stopped working*			

All three of the clusters were significantly different for all sign/symptom categories (one way ANOVA, $p<0.05$), except sudomotor and mototrophic signs between cluster 2 and 3; and sudomotor symptoms between cluster 1 and 3. Patients in cluster 3 had the highest number of signs and symptoms in all categories (except sudomotor). For each of the clusters the distribution of outcome according to other parameters of recovery is displayed in circle diagrams. For all four parameters the patients in cluster 3 had the poorest outcome.

cluster 2 (moderate outcome). Remarkably there was no difference in time since CRPS onset between any of the clusters.

Age at onset was lowest in the patients from the poor outcome cluster (45.8 years compared to 51.3 and 54.1 for cluster 2 and 1 respectively), but the difference was not significant (p=0.279) (table 3). Patients in the worst outcome cluster more often had the upper extremity affected (p=0.017), less often had a fracture as precipitating injury (0.004), and more often had cold CRPS (p=0.000). Medical history prior to CRPS onset was not significantly different between any of the patients clusters, although hypersensitivity related disorders appeared more common in patients with poor outcome (43%), than in patients with a moderate outcome (36%) or best outcome (24%; p=0.260).

Table 2. Patient characteristics per cluster derived from K-means cluster analyses on signs and symptoms.

Characteristics	Clusters according to CRPS 'outcome'						
	Best, N=63		Moderate, N=25		Poor, N=14		p-value
	n	%	n	%	n	%	
Mean disease duration (sd)	5.7 (2.2)		6.2 (2.2)		5.2 (1.7)		0.363
Patient characteristics							
Mean age at onset (sd)	51.3 (15.6)		54.1 (14.3)		45.8 (17.3)		0.279
Female	47	75%	23	92%	11	79%	0.190
Fracture as initiating event	33	52%	19	76%	3	21%	0.004*†
Upper extremity affected	27	43%	8	32%	11	79%	0.017*†
Cold CRPS	19	30%	10	40%	13	93%	0.000*
History prior to CRPS							
Migraine	6	10%	1	4%	2	14%	0.527
Osteoporosis	6	10%	2	8%	1	7%	0.947
Menstrual cycle related dis.	6	10%	3	12%	1	7%	0.881
Neuropathic pain	7	11%	2	8%	1	7%	0.850
Psychiatric disorders	19	30%	7	28%	2	14%	0.482
Hypersensitivity related dis.	15	24%	9	36%	6	43%	0.260
Asthma	4	6%	4	16%	2	14%	0.324

* Significant at $p<0.05$, X^2 test comparing differences between the three clusters.
† There was no difference between the proportion of fractures as initiating event between the upper and lower extremity (48% and 45% respectively).

Discussion

This study has several important findings. First, at an average of 5.8 years after the initiating injury (range 2.1-10.8), CRPS patients still had significantly higher symptom and sign prevalence rates than reference patients with the same precipitating injury. Second, within CRPS patients symptom and sign prevalence rates did not decrease significantly upon longer time since disease onset, and 64% still fulfilled IASP criteria at an average of 5.8 years after the initial injury. Third, the impact of the disease on the ability to work was high: 31% had become permanently incapable to work, while another 28% had to make working adjustments. Fourth, CRPS outcome was worse in patients with involvement of the upper extremity, another precipitating injury than a fracture, and cold CRPS.

To our knowledge, this is the first study that addresses disease status in a population-based cohort of CRPS patients with prolonged disease duration (on average 5.8 years). Our symptom and sign resolution rates are much lower than those in a previous population-based study by Sandroni et al., who reported complete resolution within one year in 74% of the patients.[6] The discrepancy might be explained by the methodology of assessment. Sandroni et al. obtained information on the presence of remaining symptoms/signs from the electronic medical charts while our study involved actual history taking and physical examination. On the other hand, Zyluk et al. also used actual assessments and revealed good resolution of some symptoms, such a pain and swelling. However, that study also revealed a high prevalence of remaining functional impairments.[21]

The study by Sandroni et al. reported that permanent job restrictions related to CRPS occurred in only 11% of the cases[6] while in our population almost half of the patients had a permanent complete or partial job restriction (31% complete inability to work and 28% working with adaptations). Part of this discrepancy may be explained by the difference in social system between the Netherlands and the United States. In the Netherlands people who are unfit for work get financial compensation by the government, while in the US this is less common. One previous hospital based study revealed permanent job restrictions in as much as 90% of the patients[22], however, this might just reflect the severity of CRPS patients selected in hospitals rather than from the general population.

Similar to the findings by Bruehl et al., we observed one patient cluster as clearly poorer than the other two, reflected in a higher frequency of almost all symptoms and signs. In contrast to Bruehl, however, in our study the remaining two clusters also differed from each other with regard to all four sign and symptom categories. Disease duration did not differ across the patient clusters which was in line with the findings of Bruehl et al. Cold CRPS has been suggested to be associated with poor outcome before[11,12] but to our knowledge the association between poor outcome and affected upper extremity has never been previously described. The observation that poor outcome patients had their CRPS more often after an 'atypical' injury (opposite to fracture, which is usually considered a 'typical' injury for CRPS), might suggest that easy triggering of CRPS coincides with a less favorable disease course.

Some limitations of our study should be addressed, which are related to selection, information bias and confounding as in any observational study. First, only 65% of the invited CRPS patients participated in the study. Despite our efforts to reduce time burden

for patients (and reference patients), some selection may have occurred. Patients with full recovery may have been less motivated to participate than those with serious impairments. However, there were no age, gender and disease duration differences between participants and non participants, suggesting that selection was confined. A second limitation is that medical history and physical examination during the visits were performed by one single investigator, allowing the occurrence of systematic error. However, standard forms were used as provided by TREND consortium, which reduced the possibility of deviations in history taking and examination. Finally, the patient cluster with worst outcome CRPS included only 14 patients. This hampered the ability to conduct multivariate modeling for assessment of prognostic factors for poor outcome. Repeated cluster analyses and cluster comparison in a much larger patient cohort is highly recommendable.

A strong feature of our study is the population based setting, providing results representative of the general CRPS patient and not only for (tertiary care) hospital referred patients, who usually suffer more serious disease. Secondly, signs and symptoms were obtained directly from the patients rather than medical charts. Finally, we compared symptom and sign resolution to reference patients with an identical injury as the CRPS patients, showing that the ongoing symptoms and signs are not attributable to the initial injury itself.

In conclusion, severe disease outcome in CRPS is relative rare but incomplete resolution of all signs and symptoms is common and only one third of the patients reaches full recovery according to self-reports. The present findings confirm the existing, but also questioned, ideas that CRPS should be considered a serious condition with a high probability of remaining impairments.

Appendix 1. CRPS diagnostic criteria by Bruehl and Harden (1999).

1. Continuing pain which is disproportionate to any inciting event.
2. Must report at least one symptom (history) in each of the following categories:
   Sensory: hyperesthesia
   V*asomotor*: temperature asymmetry and/or skin color changes and/or skin color asymmetry
   Sudomotor/edema: edema and/or sweating changes and/or sweating asymmetry
   Motor/trophic: 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 (physical examination) in two or more of the following categories:
   Sensory: hyperalgesia (to pinprick) and/or allodynia (to light touch)
   V*asomotor*: temperature asymmetry and/or skin color changes and/or skin color asymmetry
   Sudomotor/edema: edema and/or sweating changes and/or sweating asymmetry
   Motor/trophic: decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)

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2.3 Referral and Treatment Patterns for Complex Regional Pain Syndrome in the Netherlands

Submitted

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Abstract

Introduction: CRPS patients are seen and treated by a variety of physicians. The present study aims to describe referral and treatment patterns for CRPS patients in the Netherlands.

Methods: CRPS patients were selected from 1996 until 2005 in the Integrated Primary Care Information project, a Dutch general practice (GP) database storing electronic medical files. Identified patients were invited for study participation, involving diagnosis verification (International Association for the Study of Pain criteria) and assessment of referrals and treatment through information retrieved from GP journals, patients' questionnaires, pharmacy dispensing lists, and specialist letters if available.

Results: One-hundred-and-two patients were included in the descriptions. Sixty-one percent had presented first at the GP, while 80% subsequently consulted one or more medical specialists, most frequently an anesthetist (55% of the cases) or rehabilitation doctor (41%). Over 90% of the patients received pharmacotherapy, 89% received non invasive therapy (i.e. physiotherapy), and 18% was treated with nerve blocks. Analgesics and free radical scavengers were administered in the early phase of CRPS, while vasodilating drugs and coanalgesics (antidepressants and anti-epileptics) were administered later on. Pharmacotherapy was usually initiated by a medical specialist.

Discussion: Although the Dutch treatment guidelines recommend free radical scavenger prescription (plus physiotherapy) as initial treatment step for CRPS, only half of the cases received a scavenger within the first three months after disease onset. Since two thirds of the CRPS patients present first at the GP, GPs should be encouraged to initiate treatment, while waiting for the results from further specialist consultation.

Introduction

The Complex Regional Pain Syndrome (CRPS) is a painful complication of an injury in the extremities, for example a fracture, which coincides with vasomotor, sudomotor and motor/trophic disturbances.[1] The diagnosis is based on clinical criteria as established by the International Association for the Study of Pain (IASP).[2] The average age of onset lies between the fifth and sixth decade and women are affected more often than men.[3,4] The disease course is unpredictable, but in some cases ongoing discomfort and functional impairment can affect the quality of life and daily activities permanently.[5]

Treatment for CRPS aims at interference with the several presumed underlying mechanisms of disease, including autonomic (sympathetic) dysregulation[6], (neurogenic) inflammation[7,8], ischemic injury[9], impaired sensor/motor processing[10-12], and psychological factors.[13] In the past, sympathetic blocks have been administered with the purpose to relieve vasoconstriction and sympathetically maintained pain.[14] Vasodilating drugs have also been suggested as useful for the improvement of nutritive peripheral blood flow.[15,16] More recently, free radical scavengers have been demonstrated efficacious in prevention and treatment of CRPS[17-19], which is attributed to their attenuation of free radical induced (neurogenic) inflammation.[20] Corticosteroids have been recommended because of their general anti-inflammatory properties[21], as well as bisphosphonates that also reduce accelerated bone remodeling in CRPS[22]. Pain relief is to be obtained by analgesics and anti-neuropathics. The last mentioned group includes antidepressants[16] and anti-epileptics[23] that are commonly applied in the treatment of neuropathic pain. Transcutaneous electronic neurostimulation (TENS) is a non pharmacological method to treat neuropathic pain non invasively.[24] In order to restore motor function and also to diminish pain, physiotherapy is strongly recommended.[25] Muscle relaxants, such as benzodiazepines and baclofen, can be prescribed in patients having dystonia.[26] Psychological/behavioral therapy is usually advised for chronic CRPS patients in order to improve coping skills and pain management.[27]

Beneficial effects have only been demonstrated convincingly in randomised clinical trials for bisphosphonates[28-30], free radical scavengers[17-19], and corticosteroids.[21,31] The evidence for other treatments is scarce, being mostly derived from open label studies or studies without a control group.[16,22,32,33] Based on the available evidence, a treatment guideline for CRPS was released in the Netherlands in 2006. It recommends free radical scavengers combined with analgesics and physiotherapy as the first treatment step for all patients.[34] Dependent on the disease course subsequent treatment steps are advised, for example anti-neuropathics for further pain relief or vasodilating drugs for cold CRPS.

Limited insight exists as to how CRPS patients were actually treated and referred to other disciplines in the Netherlands before issuing of the guideline. This information is useful as a baseline for interpreting the effect of guideline implementation later on. Therefore, we conducted a study to describe treatment (pharmacological and other) and referral patterns in a population based selection of CPRS patients during a study period (1996-2005) prior to issuing of the Dutch evidence based treatment guidelines (2006).

Methods

Setting

The Integrated Primary Care Information Project (IPCI) is a longitudinal observational database maintained by the Medical Informatics department of the Erasmus University Medical Center in Rotterdam, The Netherlands. Currently, the database stores electronic records of more than one million patients of more than 150 general practitioners (GPs). The patient population is representative of the Dutch population regarding age and sex distribution.[35,36] The electronic records comprise anonymous information on demographics, signs and symptoms (using the International Classification for Primary Care (ICPC) codes[37] and narratives), diagnoses (using ICPC codes and narratives), clinical findings, specialist referrals, laboratory findings, hospitalizations, and drug prescriptions. Summaries of the hospital discharge letters and information and letters from specialists are entered in a free text format and hard copies of original letters can be provided upon request. Since in the Dutch health care system the GP acts as a gatekeeper to and from secondary care, the GP is assumed to possess complete medical information regarding the patients.[38] To maximize completeness of the data, GPs who participate in the IPCI project are not allowed to use paper-based records. The system complies with the European Union guidelines on the use of medical data for medical research and has been proven valid for (pharmaco)-epidemiological studies.[39] The Scientific and Ethical Advisory Group of the IPCI project approved the study (project number 04/70), as well as the Medical Ethical Board of the Erasmus Medical Center (protocol number 2006-099).

Source population

The source population comprised all persons with at least 1 year of valid history within the IPCI database during the study period (January 1996-June 2005). Follow-up in the database started on the date that one year of valid history was available and ended upon transferring out of the practice, the date of last data supply by the GP, death, diagnosis of CRPS or at the end of the study period, whichever came first. Since additional data collection was required for validation of the CRPS diagnosis, the source population was restricted to all practices that were still active in the IPCI database in 2006 and were able to provide additional information.

Selection of CRPS patients

Potential incident CRPS cases were identified in the electronic medical records using a sensitive string search algorithm including Dutch synonyms, abbreviations and obvious spelling errors for CRPS. After manual evaluation of the records, GPs were asked to reconfirm or reject the diagnosis in a short questionnaire. The process of case validation until this point has been described in detail in our study on the incidence of CRPS in the Netherlands.[40]

In a second validation step, all identified and GP reconfirmed patients were invited to participate in our study on CRPS per letter through the GP. If informed consent was obtained, patients were visited once by the primary investigator (MM) together with a research assistant (MHB). Prior to this visit, all patients received a questionnaire

addressing CRPS related complaints and impairments, disease course, treatment, general health, quality of life and daily function. Patients completed the questionnaires by themselves and during the visit they were briefly discussed with the investigator to ascertain completeness. In addition, patients underwent a physical examination of the affected and contralateral extremity. Patients were included in the present descriptive study if the visit assessments demonstrated that they (had) fulfilled (in the past) the diagnostic criteria for CRPS as established by the International Association for the Study of Pain (IASP). Since the exact starting date of CRPS complaints and symptoms is difficult to establish in retrospect, the date of CRPS onset was defined as the date of the precipitating injury. In absence of a precipitating injury the index date was set as the date of first symptoms. Based on their own reports patients were classified as initially warm or cold type CRPS.

Referrals

Referrals were studied during the entire disease course. Information on this was derived manually from electronic medical records in the IPCI database, patient reports (in questionnaires) and specialist letters. Referral patterns included the first type of physician that was visited for CRPS, the physician who diagnosed the CRPS and all types of physicians that were consulted for the CRPS during the entire disease course.

Treatment

Treatment patterns were studied during the first two years after the index date (precipitating injury). Information on prescriptions for oral and topical drugs indicated for CRPS was retrieved from the IPCI database and from pharmacy dispensing data that were obtained retrospectively from the pharmacies of the patients. Information on intravenous drugs, for example mannitol (a free radical scavenger), could not be retrieved from these sources, since they are usually administered during hospital admissions. The IPCI database included all prescriptions by the GP, displaying brand name, starting date, quantity, strength, indication, prescribed daily dose and the Anatomical Therapeutical Chemical (ATC) classification code.[41] Pharmacy dispensing data comprised drugs prescribed by both GPs and medical specialists and usually displayed brand name, starting date, stop date, quantity, strength, prescribed daily dose, and type of prescribing physician.

The following oral and topical drugs were considered as study drugs if newly prescribed (i.e. not prescribed in the year prior to CRPS) within two years after CRPS onset: 1. free radical scavengers (topical and systemic), including dimethylsulfoxide cream (50%), N-acetylcysteine and vitamin C; 2. systemic vasodilating drugs, including ketanserin, verapamil and nifedipine; 3. analgesics, including paracetamol, NSAIDS and opiates; 4. coanalgesics, including tricyclic antidepressants and some anti-epileptics (gabapentin, carbamazepine, pregabalin and duloxetin); 5. various other drugs, including capsaicin cream, carnitene, corticosteroids (oral), baclofen (oral), and benzodiazepines. Legend durations for each drug were calculated as the total number of units supplied divided by the number of units taken per day.

Information on intravenous medication, additional treatments (for example physiotherapy), and invasive therapies (for example nerve blocks) was obtained from electronic medical records in the IPCI database, patient reports (in questionnaires) and specialist letters altogether.

Data analysis

The percentages of users of oral and topical CRPS medication were calculated and graphed over time as three months moving averages, meaning that the percentage for each time point is the average of the previous, present and next time month. The median (inter quartile range, IQR) duration of use was calculated as the total number of using days for a specific drug class in the two year follow-up period. Time to treatment (from the index date) was estimated per drug class using Kaplan Meier survival analysis and compared between patients who presented first at GP and patients who presented first at a medical specialist, using a log-rank test. In patients receiving a specific class of drugs the median (IQR) time to treatment for that class was calculated (in days). Treatment patterns were stratified for warm and cold CRPS. All statistics were performed using the Statistical Package for Social Sciences (SPSS) version 12.0 for Windows. Significance was established at a p-value <0.05.

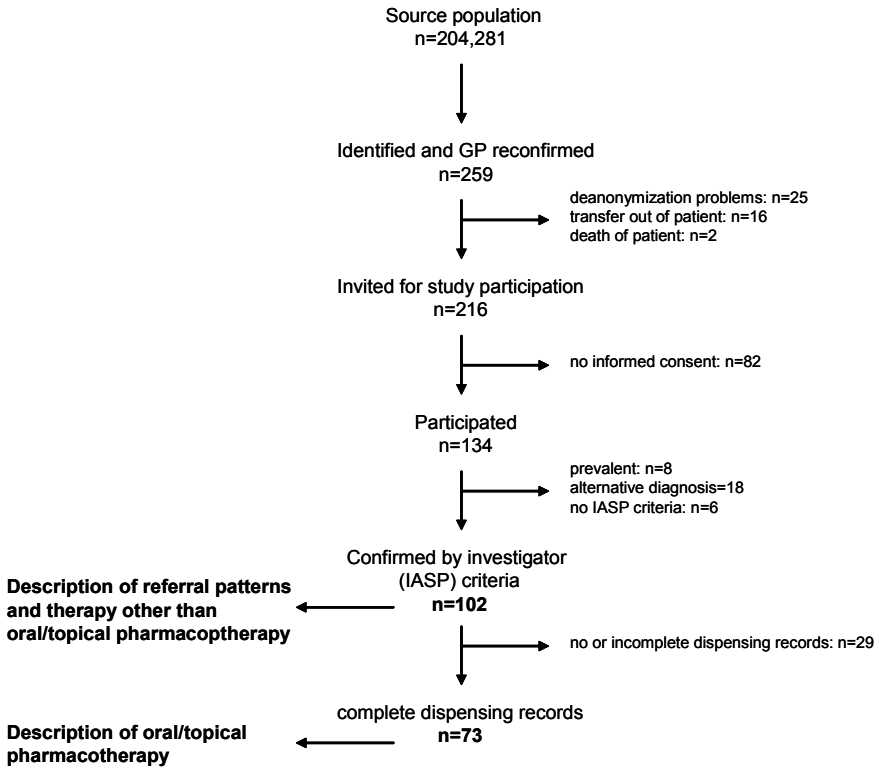

Figure 1. Selection of the study population.

Results

After identification and initial GP reconfirmation, 259 CRPS patients were selected from a source population of 204,281 persons in the IPCI database. Of these 216 (83%) were invited for further study participation, whereas the remaining could not be contacted due to de-anonymization problems (n=25), transferring out of the patient (n=16), or death (n=2). Out of 216 contacted patients, 134 (62%) provided informed consent and were visited in order to fulfill study assessments. Hundred and two (75%) of the visited patients complied or had ever complied with the IASP criteria and were incident during the study period. Among the 32 excluded patients eight appeared prevalent (CRPS onset before study period), 18 had a likely alternative diagnosis for their complaints earlier attributed to CRPS, and six never displayed the signs required to fulfill the IASP criteria (figure 1).

The mean age at CRPS onset was 51 years (range 12-86), whereas 81 patients (79%) were female. Fracture was the precipitating event in 55 cases (54%), while only 3 (3%) had CRPS without a known precipitating event. The average duration since CRPS onset was 5.8 years (median 5.7; range: 2.1-10.8).

Physicians who were consulted by the CRPS patients are displayed in table 1. On average a patient consulted 2.4 different specialties of physicians. The referrals by the first visited physician, the diagnosing physician and by any consulted physician are depicted in figure 2A-C. Especially patients with CRPS following a soft tissue injury presented at the GP first instead of a medical specialist (75% versus 25% respectively, p=0.033), while patients with CRPS after surgery usually first presented at the medical specialist (17% versus 83%, p=0.002). Cases precipitated by a fracture presented themselves at GP and specialist equally often (46% versus 55%, p=0.228). In total 61% of the CPRS patients visited the GP before consulting a medical specialist. In 63% of these cases the GP already suspected or made the CRPS diagnosis. Eventually, more than 80% of the patients visited a medical specialist at some point during their disease course. The anesthetist was the most commonly involved medical specialist, visited by 55% of the CRPS patients, while rehabilitation doctors were the second most common (41%). Medical specialists referred CRPS patients to an anesthetist or rehabilitation doctor in more than half of the cases, while GPs had the tendency to refer more to (orthopedic) surgeons. The most common referral pattern was from GP to (orthopedic) surgeon to anesthetist or rehabilitation doctor. In most cases (over 80%) the CRPS diagnosis had been made before consultation of the anesthetist or rehabilitation doctor.

Table 1. Physicians consulted by CRPS patients.

N=102	First physician		Diagnosing physician		Anytime consulted		Mean order of consultation
Type of physician	**n**	**%**	**n**	**%**	**n**	**%**	
GP	62	61%	39	38%	62	61%	1.0
Surgeon	23	23%	21	21%	35	34%	1.4
Orthopedic surgeon	14	14%	23	23%	38	37%	1.8
Anesthetist	0	0%	12	12%	56	55%	2.4
Rehabilitation doctor	0	0%	2	2%	41	40%	2.9
Neurologist	3	3%	4	4%	15	15%	2.5
Rheumatologist	0	0%	1	1%	5	5%	2.6
Dermatologist	0	0%	0	0	1	1%	2.0

First physician: physician who was first visited for CRPS symptoms; Diagnosing physician: physician who made the CRPS diagnosis; Anytime consulted: any physician consulted for CRPS during complete disease course. Mean order of consultation: the lower this number, the earlier this type of physician is consulted by a patient. For example the GP is usually consulted first and therefore has a mean order of consultation of 1.0.

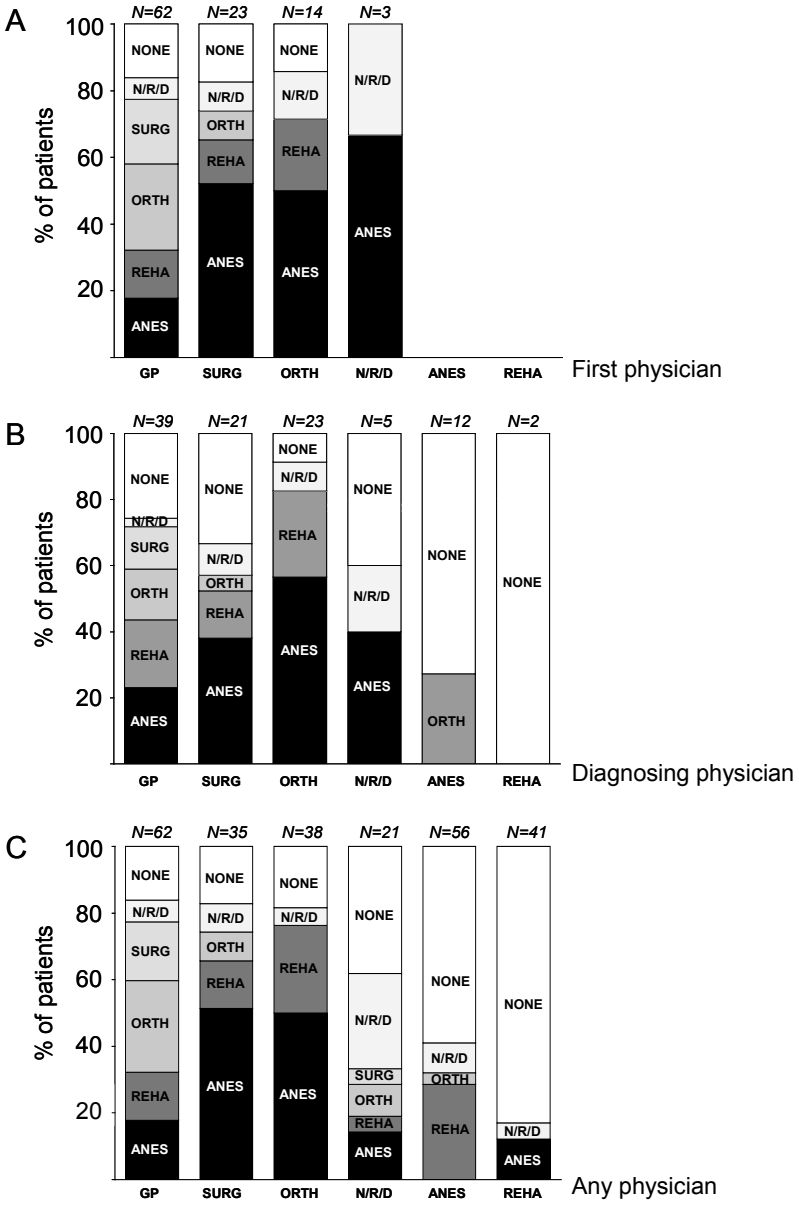

Figure 2. A. Referrals by the first physicians visited by CRPS patients. **B.** Referrals by the physician who diagnosed CRPS. **C.** Referrals by any physician consulted on CRPS. GP = general practitioner; SURG = surgeon; ORTH = orthopedic surgeon; ANES = anesthetist/pain physician; REHA = rehabilitation physician; N/R/D = neurologist/rheumatologist/dermatologist

Pharmacy dispensing records that covered complete follow-up time from the index date until the moment of visit were only available for 73 (72%) patients. For other patients the pharmacy data did not cover the onset of CRPS since it was too long ago. These patients were excluded from the analyses regarding oral or topical administered pharmacotherapy. Ninety percent of the GP prescriptions recorded in the IPCI database had also been dispensed.

The use of oral or topical pharmacotherapy in the first two years after onset of CRPS is demonstrated in table 2 and in figure 3A-B. Analgesics and free radical scavengers were mainly used in the early phase of CRPS, while the use of calcium antagonists and co-analgesics increased during later phases. Free radical scavengers (in 79% of the patients) and analgesics (77%) were the most frequently prescribed drugs (table 2). Both drugs were prescribed more in patients with cold type CRPS compared to warm type CRPS (both 87% versus 67% respectively; p-value 0.050). Most of the CRPS specific medications were started by a medical specialist, rather than by the GP (table 2). However, for none of the drug classes the time to treatment was significantly different between patients who had visited the GP first or who had visited a medical specialist first (log rank test). Fifty-two percent of the patients received a scavenger within the first three months after the initial injury. Anti-neuropathics and vasodilating drugs were used for longer periods than analgesics, scavengers and various other drugs (table 2).

Table 2. Oral and topical medication dispensed for CRPS treatment.

N=73	Users	No. of drugs within class	Duration (days) of drug use	Time (days) to treatment (receivers only)	GP first Pre-scriber	Specialist first pre-scriber
Drug classes	%	Mean (range)	Median (IQR)	Median (IQR)	%	%
Scavengers	79%	1.6 (1-3)	122 (67-255)	71 (42-108)	29%	71%
DMSO cream	68%					
N-acetylcysteine	40%					
Vitamin C	19%					
Vasodilators	30%	1.1 (1-2)	328 (93-1257)	126 (62-198)	27%	73%
Verapamil	12%					
Nifedipine	19%					
Ketanserin	1%					
Analgesics	77%	1.5 (1-3)	123 (40-364)	62 (6-186)	52%	48%
Paracetamol	22%					
NSAIDs	77%					
Opiates	21%					
Anti-neuropatics	23%	1.4 (1-3)	232 (93-890)	203 (57-399)	35%	65%
TCAs	19%					
Anti-epileptics	12%					
Other agents	11%	1.6 (1-2)	80 (24-268)	176 (143-470)	50%	50%
Steroids	4%					
Carnitene	3%					
Baclofen	3%					
Benzodiazepines	1%					

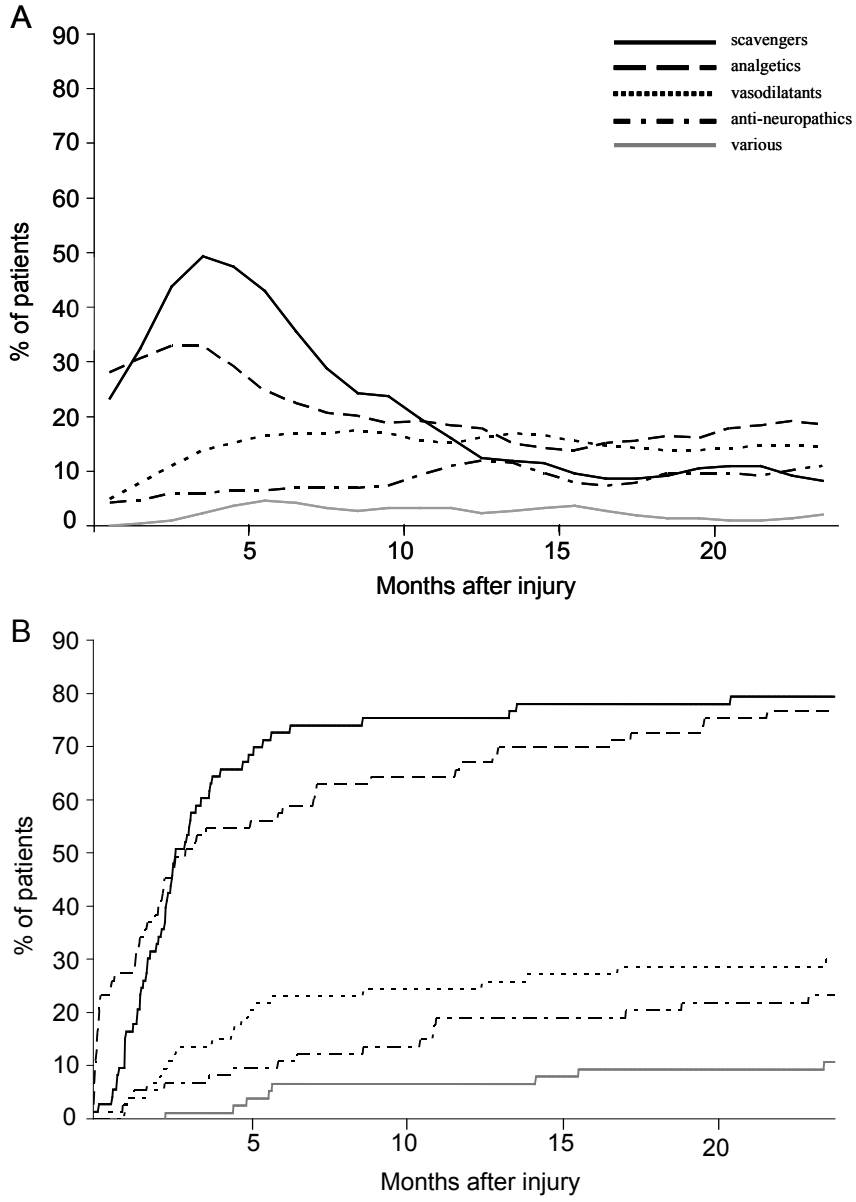

Figure 3. A. Absolute percentage of patients using medication during the first two years after CRPS onset (initial injury). **B.** Cumulative percentage of patients having received medication within the first two years after CRPS onset.

Apart from the drugs included in the four pre-specified classes, a wide variety of other drugs was also reported to have been prescribed for CRPS treatment, including allopurinol, hydrokinine, clonidine, levopromazine, ephedrine, venlafaxine, amantadine, and various types of vitamins (apart from vitamin C) and food supplements. Seven percent of the CRPS patients received no kind of oral or topical pharmacotherapy whatsoever.

The percentages of CRPS patients receiving other treatments than oral or topical pharmacotherapy is displayed in table 3. Non invasive treatments (for example physical therapy) were prescribed to more than 90% of the patients, while invasive nerve blocks were administered only to 18%.

Table 3. Treatment for CRPS other than oral/topical pharmacotherapy.

N=102	**Receiving therapy**		**Beneficial according to patients**
Type of therapy	**n**	**%**	**% of patients receiving therapy**
Non invasive (total)	91	89%	
Physiotherapy	89	87%	76%
Ergonomic therapy	19	19%	68%
Psychological therapy	10	10%	43%
TENS	21	21%	53%
Nerve blocks (total)	18	18%	
Sympathetic block	11	11%	33%
Loco regional pain block	7	7%	25%
Regional intravenous block	1	1 %	100%
Non oral pharmacotherapy (total)	46	45%	
Intravenous mannitol	37	36%	58%
Intravenous other drugs	10	10%	80%
Iontoforesis	15	15%	57%

Discussion

In the presented study, we describe referral and treatment patterns in 102 CRPS patients selected from a GP database in the Netherlands in a study period from 1996 until 2005, which is before the issuing of the Dutch evidence based treatment guideline for CRPS in 2006. The majority of the patients first presented with CRPS at the GP, before consulting a medical specialist, especially cases following a soft tissue injury or fracture. Free radical scavengers were the most frequently administered drugs, prescribed in 79% of the patients. However, only in half of the patients scavenger treatment was commenced within the first three months after the initiating injury.

High quality trials showing effectiveness of CRPS treatment have hardly been performed.[32,42] The observation that free radical scavengers are prescribed in a majority of the CRPS patients is in line with available evidence in the literature. Scavengers have been demonstrated beneficial in randomized trials[17,18] and the high prescription frequency in practice suggests good clinical experience with these drugs as well. On the contrary, corticosteroids have also been demonstrated as beneficial in randomized trials[21,31], but are rarely prescribed in practice, probably out of caution for the notorious adverse effects on energy metabolism and the immune system.

Anti-neuropathics were used by less than a quarter of the patients, despite the proven effectiveness (in particular gabapentin) in one randomized trial.[23] The evidence for oral vasodilating drugs is limited (no randomized trials), but oral vasodilating drugs were prescribed in almost one third of the patients and the use was quite prolonged. Common analgesics were prescribed widely in our study population, despite a complete lack of evidence in the literature for benefit from these drugs in CRPS. However, the prescription of an analgesic is likely a natural response to a patient who presents with pain in an early phase of CRPS. Remarkable are the reports of intravenous mannitol administration in one third of the patients, in the absence of trials that have proven its benefit.[43] Since hospital admission is usually needed, mannitol is an expensive treatment option, with a risk for renal failure in patients with pre-existent renal dysfunction.[44]

Most CRPS patients presented at the GP first, but GPs prescribe predominantely analgesics. Due to the relatively low incidence of CRPS[40], GPs may be relatively unfamiliar with the disease spectrum and treatment opportunities. However, early commencement of therapy may be of relevance since this may prevent potential long term damage by the ongoing underlying disease processes. The Dutch evidence based treatment guideline for CRPS, which came out in 2006, presents a schematic display of suggested therapeutic strategies, which starts with free radical scavenger prescription combined with analgesics and physiotherapy.[34] Although before guideline appearance a majority of patients was already treated with a scavenger at some point in time, a treatment delay of more than three months was observed in half of the cases. Realizing this, CRPS treatment can be improved by earlier commencement of therapy. Especially GPs should be informed and encouraged to do this, as they are usually the first physician to whom CRPS patients present and they often suspect the diagnosis already. The straightforward general first treatment step from the guideline can easily be initiated by the GP (or any other physician who is confronted with the first presentation of CRPS), while waiting for further evaluation by a consulted CRPS specialist.

Some limitations bound to this descriptive study need to be addressed. First, the number of patients for whom sufficiently complete information could be collected was low, especially regarding dispensing patterns of oral and topical pharmacotherapy. Therefore, we were not able to compare treatment strategies across subtypes of patients and types of medical specialist. Additionally, while we had detailed time information for oral and topical pharmacotherapy from pharmacy delivery lists, this was lacking for other types of treatment, for which the information had to be obtained by questionnaires. Therefore, prescription patterns and delays of such treatments, for example physiotherapy, could not be described and we had to restrict to simple summarization of treatment percentages. Finally, the results are reflecting the Dutch situation and may not be generalizable to other geographic areas. Although no reports exist that argue in favor of or against international variability, we have the impression that free radical scavengers may be prescribed particularly in the Netherlands, since most of the trials regarding these drugs have a Dutch origin.

A strong feature of our study is the fact that it was performed in a population based setting, preventing selection of a subset of patients with high disease severity. We believe that by using pharmacy dispensing lists we had accurate information on prescribed oral and topical pharmacotherapy in CPRS patients, considering a retrieval rate of 90% of the prescriptions from the GP database in the pharmacy lists. For all other data we also used several sources of information, including GP journals, specialist letters if available, and patient reports.

In conclusion, we observed that GPs are frequently the first physicians with whom CRPS patients present, but that therapy is often initiated by a medical specialist upon referral. Therefore, especially GPs should be encouraged to initiate CRPS treatment according to the first steps of the guidelines. Early commencement of CRPS treatment may eventually improve CRPS disease course and outcome, an assumption which may be investigated if the period past guideline implementation is sufficiently long.

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Chapter 3

Risk Factors

and

Leads to Etiology

3.1 | Medial History and the Onset of Complex Regional Pain Syndrome

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Abstract

Introduction: Knowledge concerning the medical history prior to the onset of complex regional pain syndrome (CRPS) might provide insight into its risk factors and potential underlying disease mechanisms.

Methods: To evaluate prior to CRPS medical conditions, a case-control study was conducted in the Integrated Primary Care Information (IPCI) project, a general practice (GP) database in the Netherlands. CRPS patients were identified from the records and validated through examination by the investigator (IASP criteria) or through specialist confirmation. Cases were matched to controls on age, gender and injury type. All diagnoses prior to the index date were assessed by manual review of the medical records. Some pre-specified medical conditions were studied for their association with CRPS, whereas all other diagnoses, grouped by pathogenesis, were tested in a hypothesis generating approach.

Results: Of the identified 259 CRPS patients, 186 cases (697 controls) were included, based on validation by the investigator during a visit (102 of 134 visited patients) or on specialist confirmation (84 of 125 unvisited patients). A medical history of migraine (OR: 2.43, 95%CI: 1.18-5.02) and osteoporosis (OR: 2.44, 95% CI: 1.17-5.14) was associated with CRPS. In recent history (one year before CRPS), cases had more menstrual cycle related problems (OR: 2.60, 95% CI: 1.16-5.83) and neuropathies (OR: 5.7; 95% CI: 1.8-18.7). In a sensitivity analysis, including only visited cases, asthma (OR: 3.0; 95%CI: 1.3-6.9) and CRPS were related. Psychological factors were not associated with CRPS onset.

Discussion: Because of the hypothesis-generating character of this study, findings should be confirmed by other studies.

Introduction

The complex regional pain syndrome (CRPS) can be a painful disorder affecting one or more extremities. It usually occurs following a physical injury, for example a fracture or surgery, but spontaneous onset may occur as well.[1] The diagnosis is based on its clinical presentation, whereby the diagnostic criteria as developed by the International Association for the Study of Pain (IASP) are the most widely accepted.2 Apart from pain and sensory disturbances, these criteria demand the presence of edema, skin blood flow abnormalities or abnormal sudomotor activity. Functionality of the affected limb is often impaired[1,3] and ongoing pain and dysfunction can leave patients severely disabled.[4] The peak incidence of CRPS lies between 50 and 70 years of age and women are affected more frequently than men.[5,6]

In the past decade, insights into the mechanisms underlying CRPS have gradually increased. The role of inflammation is endorsed by the demonstration of inflammatory mediators in serum[7], blister fluid[8], and spinal fluid[9] from CRPS patients. Additionally, abnormal vasoconstriction patterns, either sympathetic nerve system driven[10] or due to local factors[11], can result in blood flow disturbances. These peripheral disease mechanisms may precede and sustain the sensitization[12] and altered sensory processing at spinal and supraspinal levels[13,14], that lead to pain of neuropathic nature. Sympathetically maintained pain, probably caused by sympathetic-afferent coupling.[15] occurs in a subset of patients.[2]

To date it is unknown whether other diseases that also involve inflammation, impaired (micro-) circulation, or neuropathic pain lead to an increased risk of CRPS. However, studies on the potential co-occurrence of these disorders with CRPS can be informative, since they might give clues to potential shared pathogenic or etiologic factors, as well as reveal risk factors for CRPS. The aim of the present investigation was to identify whether and which medical conditions or categories of medical conditions are associated with the occurrence of CRPS.

Methods

Setting

A retrospective case-control design was used to compare disease history prior to the onset of CRPS between patients and controls from the general population. The study was nested in the Integrated Primary Care Information (IPCI) database, which is a general practice (GP) database of longitudinal electronic medical records of around 800,000 patients.[16, 17] The project was initiated in 1992 and new practices have started to contribute data ever since. The IPCI population is considered representative of the general population in the Netherlands regarding age and gender distribution. The Dutch Health Care System requires all persons to be registered with one GP (even if they are healthy), who acts as a gatekeeper for further medical care and who receives and files all health care information. Therefore, the electronic records contain virtually complete

medical information concerning each patient.[18] To optimize completeness of the data, GPs participating in the IPCI project do not keep additional paper records (except for specialist and discharge letters). The present study has been approved by the Scientific and Ethical Advisory Group of the Project and by the Medical Ethical Board of the Erasmus Medical Center (protocol number 2006-099).

Source population

The dynamic source population comprised all persons of all ages with at least 1 year of valid history in the IPCI database during the study period (January 1996 - June 2005). Observation time in the database started on the date that one year of valid history was available and ended upon transferring out of the practice, the date of last data supply by the GP, death, diagnosis of CRPS or at the end of the study period, whichever came first. Since additional data collection was required for validation of the CRPS diagnosis, the source population was restricted to all practices that were still active in the IPCI database in 2006 and were able to provide additional information.

Cases

Details on the case identification procedure in the IPCI database have been described in our incidence study on CRPS in the Netherlands.[5] Briefly, potential incident CRPS cases were identified using a search algorithm including synonyms and abbreviations for CRPS in codes and narratives. Subsequently, GPs were asked to reconfirm or reject the CRPS diagnosis in a short questionnaire. Information regarding the precipitating injury for the CRPS was extracted from the GP journals and from specialist letters when provided by the GP. Injury categories included fracture, soft tissue injury, surgery, tendon injury and various other types of injuries (including nerve injury).

For this specific study, all previously identified and GP reconfirmed cases were contacted to ask them to participate in a nationwide study on CRPS. Patients who provided written informed consent were visited by the primary investigator of this study (MM), a physician with clinical experience in diagnosing CRPS. A physical examination was performed of the affected and contralateral extremity, and self-administered questionnaires were used to gain information on present and past symptoms of CRPS. Patients were included into the main analyses of this study if during the visit the CRPS diagnosis was verified by the investigator using the IASP criteria.[2] Additionally, cases were included who could not be visited, but of whom the CRPS diagnosis had been confirmed by a medical specialist (in a letter to the GP). The index date was chosen as the date of the precipitating injury, or the date of first symptoms in cases with spontaneous CRPS. Since some previous studies have revealed different results in 'warm' and 'cold' type CRPS patients[19-21], suggesting these to be distinct subtypes, cases were divided into subgroups based on their own reports of predominant temperature characteristics (visited patients only).

Controls

First, for each CRPS case all other persons in the IPCI database with similar age (year of birth) and gender were selected as potential controls. Subsequently, from these, up to four controls per case were chosen who had experienced an injury *identical* to the injury that precipitated the CRPS in the case (within a two year band of calendar time). This

meant that cases with CRPS after a fracture were matched to up to four controls with a fracture, cases with a surgery to controls with a surgery, cases with a nerve injury to controls with a nerve injury, etc. Similar to cases, the index date in controls was defined as the date of the injury. When CRPS had occurred in a case without precipitating injury, controls were not required to have an injury either and the index date was chosen as the date of first symptoms in the case.

Medical history

The medical history until the index date was extracted from the electronic medical records during the observation time in the database. While being blinded to the case or control status of the patient, the entire medical record was evaluated and all GP contacts were classified into episodes of medical problems.

The association between CRPS and medical conditions prior to CRPS onset was studied in three different ways. First, in a hypothesis generating approach, all medical conditions were categorized based on pathogenesis and each category was examined for its association with CRPS. The chosen pathogenic categories were mutually exclusive and included the following (see also supplemental appendix): *anatomic, trauma-induced, degenerative, hormonal, metabolic, neoplasm, infections, inflammation, psychological, no disease* and *miscellaneous*. Sometimes, a patient had contacted the GP for complaints or symptoms for which no explanatory cause had been provided. These episodes were categorized as 'unexplained complaints' (including subjective complaints such as nausea) or 'unexplained symptoms' (including objective symptoms such as vomiting). The main pathogenic categories were divided further into mutually exclusive subcategories (see also supplemental appendix 1). Initiating injuries for CRPS (fracture, sprain, nerve injury, etc.) were matching factors and were excluded from this categorization. The assignment of the medical conditions to a specific disease category was performed independently by two physicians (MM, JSHAK) and kappa statistics were calculated to assess interrater agreement.

Second, and apart from our hypothesis-generating approach, we tested a priori hypotheses about associations between CRPS and a few medical conditions that had occasionally had been suggested associated with CRPS (in one or more previously published studies). These conditions included headaches[22], osteoporosis[23], psychological factors[24,25] and fibromyalgia.[26]

Third, we explored the association between CRPS and groups of medical conditions that might hypothetically be related to CRPS, based on similarities in the assumed underlying disease mechanisms. These groups of medical conditions involved hypersensitivity/exaggerated inflammation related disorders (asthma, autoimmune disorders, allergies), disorders caused by impaired circulation (cardiovascular diseases, raynaud's syndrome, chilblains, etc.), and preexisting disorders marked by sensory disturbances (neuropathies).

Data Analysis

Standard comparative statistics were used to compare frequencies (Chi-square test), or means (Student's t-test and Mann Whitney-U test). Associations between prior to CRPS medical history and CRPS were investigated by conditional logistic regression, adjusted

for observation time in the database. Medical history was studied in two exposure windows: 1) the complete database history of patients, and 2) the one year before the index date. Subgroup analyses were performed for 'warm' and 'cold type' CRPS. Sensitivity analyses were performed to inspect misclassification of outcome (CRPS) and determinants (diagnoses prior to CRPS). Misclassification of CRPS was limited in sensitivity analyses including only cases that could be visited and verified by the investigator (IASP criteria). Misclassification of diagnoses prior to CRPS was inspected by considering a diagnosis valid only when specific treatment was given (for example inhalator medication for asthma, anti-migraine drugs for migraine, etc.). Finally, in order to evaluate presence of Berkson's bias (patients who visit the physician more often may also be diagnosed with diseases earlier), we adjusted for the number of GP contacts. SPSS 12.0 was used for all statistical tests. Significance was established at $p<0.05$.

Results

In the source population, comprising 204,281 persons, 259 CRPS patients were identified and reconfirmed by the GP. Some of these cases were untraceable for further contact due to retirement of the GP or software changes at the practice (n=25 patients), patients having left the practice (n=16) or death of patients (n=2). In total 216 (83.4%) cases could be contacted for study participation. Of these, 134 **(**62.0%**)** provided informed consent and completed study assessments. No significant differences in age, gender distribution and disease prevalences were observed between participants and non-participants.

During the study visit, eight (6.0%) cases turned out to be prevalent (the patient already had CRPS at the start of observation time in the database) and were therefore excluded from the final study population. Hundred-and-two (82.3%) of the remaining 126 incident CRPS cases had fulfilled the IASP criteria according to the investigator. Reasons for not fulfilling the IASP criteria were the presence of a possible alternative diagnosis (n=18), the absence of pain (n=2), and the absence of vasomotor or sudomotor disturbances (n=4). Verification of the CRPS diagnosis using the additional information obtained during visits yielded a false positive rate of 19% for all patients who were initially reconfirmed by the GP and 13% for the patients with an additional medical specialist diagnosis.

A total of 186 CRPS cases were finally included in the main analyses (102 cases validated during a visit plus 84 unvisited cases with a specialist confirmed diagnosis). A total of 697 controls matched to a case on age, gender and type of injury were selected from the source population in the database. The mean age at onset in the study population was 51 years and 77% was female. A fracture was the most common precipitating injury in 91 (49%) of the cases, while for 15 (8%) no precipitating event could be extracted from the records (similar percentages for the matched controls). Other initiating events included soft tissue injury (20%), surgeries (11%), tendon injuries (6%), and various others (6%). The summed observation time until the index date was 529 years for cases (mean: 2.8; range: 0.9-8) and 1,994 years for controls (mean: 2.9; range: 1-10). The mean number of GP contacts and medical episodes per year did not differ significantly between cases (contacts: 4.3; episodes: 3.8) and controls (contacts: 3.8; episodes: 3.3; $p=0.107$ and $p=0.093$ respectively). However, cases had more medication prescriptions per year than controls (8.9 versus 6.4 respectively; $p=0.009$).

The interrater agreement for categorization of disease episodes into pathogenic groups varied per group, but was very good (kappa > 0.80) for all groups except for metabolic disorders and miscellaneous disorders, for which the interrater agreement was good (kappa > 0.60).

Results of the analysis that comprised all medical conditions prior to CRPS are demonstrated in table 1. The odds ratio's (OR) are displayed for all main pathogenic categories and for subgroups only if significant. The association between menstrual cycle related disorders and CRPS (OR: 2.60, 95%CI: 1.16-5.83) in the year before the index date was the only new observation, that was not hypothesized a priori. In the one year window also the main groups of trauma induced conditions (excluding known initiating events for CRPS), infections and the number of unexplained complaints were associated with CRPS, but none of its subgroups were significant.

From the medical conditions that have occasionally been associated with CRPS before, migraine (OR: 2.43, 95%CI: 1.18-5.02) and osteoporosis (OR: 2.44, 95%CI: 1.17-5.14) were significantly associated in our study (table 2). No association was observed between psychological factors and CRPS (OR: 1.17, 95%CI: 0.83-1.67). The prevalence of fibromyalgia was too low to perform any meaningful analyses.

From the categories of diseases that were hypothesized to be related with CRPS based on presumed pathogenic similarities, asthma (OR: 3.0; 95%CI: 1.3-6.9) was associated with CRPS, but only in the sensitivity analysis including visited cases that fulfilled the IASP criteria (table 3). Preexisting neuropathies (OR: 5.7; 95%CI: 1.8-18.7) were also more frequent in CRPS patients in the one year window.

In the subgroup with self reported 'warm' CRPS (cases=48, matched controls=182), all previously mentioned associations decreased and became non significant. In the subgroup with self reported 'cold' CRPS (cases=42, matched controls=156) associations increased for asthma (OR: 10.6, 95%CI: 2.1-53.5), migraine (OR: 4.6, 95%CI: 1.1-20.5), and for osteoporosis (OR: 5.8, 95%CI: 1.0-34.8), but not so much for menstrual cycle related morbidity (OR: 2.8, 95%CI: 1.0-8.0). However, power in these analyses was minimal due to low numbers.

If specific disorders in the medical history were considered only when treatment was given (for example treated asthma or migraine, osteoporosis) an increasing OR was observed for migraine, menstrual cycle related disorders, and neuropathies; an unchanged OR for osteoporosis; and a lower OR for asthma (table 4). Adjustment for the contact frequency at the GP did not affect the OR substantially (table 4).

Table 1. Associations between CRPS and prior to CRPS medical conditions, categorized by pathogenesis.

Pathogenesis Category	Full journal prior to index date				Last year prior to index date			
	Main analysis†			Sens. Analysis‡	Main analysis†			Sens. Analysis‡
	Cases N=186	Controls N=697	$OR^{matched}$ (95% CI)	$OR^{matched}$ (95%CI)	Cases N=186	Controls N=697	$OR^{matched}$ (95% CI)	$OR^{matched}$ (95%CI)
	n	n			n	n		
Anatomic	38	131	1.22 (0.73-1.73)	1.3 (0.8-2.4)	20	3	1.14 (0.65-2.00)	1.6 (0.8-3.3)
Traumatic	35	119	1.19 (0.78-1.83)	1.4 (0.9-2.5)	19	56	1.43 (0.80-2.55)	**2.3 (1.1-4.8)**
Degenerative	36	135	1.00 (0.63-1.62)	1.3 (0.7-2.4)	21	78	1.11 (0.63-1.94)	1.8 (0.9-3.5)
Metabolic	38	134	1.03 (0.66-1.62)	1.0 (0.5-1.9)	28	104	1.02 (0.61-1.72)	1.0 (0.5-1.9)
Hormonal	44	140	1.36 (0.89-2.18)	1.5 (0.9-2.6)	32	93	1.57 (0.99-2.51)	**2.0 (1.1-3.7)**
Sex hormones	42	124	1.51 (0.98-2.32)	1.6 (0.9-2.7)	30	80	**1.77 (1.09-2.90)**	**2.0 (1.1-3.8)**
Menstrual cycle	14	33	1.81 (0.91-3.61)	2.3 (1.0-5.3)	10	17	**2.60 (1.16-5.83)**	**3.0 (1.1-8.7)**
Neoplasm	37	146	0.94 (0.61-1.44)	0.7 (0.4-1.3)	15	67	0.92-0.51-1.69)	0.9 (0.4-1.9)
Infection	120	431	1.14 (0.78-1.67)	0.7 (0.4-1.3)	84	270	**1.46 (1.03-2.07)**	1.4 (0.9-2.2)
Inflammation	67	258	0.96 (0.67-1.38)	1.4 (0.8-2.2)	46	163	1.19 (0.79-1.77)	1.4 (0.9-2.4)
Hypersensitivity	43	153	1.09 (0.73-1.64)	1.7 (1.0-2.8)	30	99	1.20 (0.70-1.88)	1.8 (1.0-3.1)
Asthma	14	32	1.68 (0.86-3.29)	**3.0 (1.3-6.9)**	11	21	2.05 (0.94-4.50)	**3.0 (1.1-8.0)**
Psychological	64	219	1.17 (0.83-1.67)	0.9 (0.5-1.5)	41	139	1.26 (0.84-1.88)	0.9 (0.5-1.6)
No disease	107	416	0.96 (0.65-1.42)	1.0 (0.6-1.8)	71	287	0.95 (0.66-1.38)	1.0 (0.6-1.5)
Miscellaneous	113	385	1.27 (0.89-1.82)	1.2 (0.8-2.0)	80	278	1.15 (0.82-1.62)	1.1 (0.6-1.7)
Mean no. of unexplained complaints	4.45	3.10	1.03 (1.00-1.05)	1.0 (1.0-1.1)	1.47	1.05	**1.11 (1.04-1.21)**	1.0 (1.0-1.2)
Mean no. of unexplained symptoms	1.20	0.86	1.08 (1.00-1.16)	1.1 (1.0-1.2)	0.39	0.29	1.18 (0.97-1.43)	1.2 (0.9-1.5)

† Including cases verified during a visit (IASP criteria, N=102) + not visited cases diagnosed by a specialist (N=84), total 186 cases

‡ Including only cases verified during a visit (IASP criteria, N=102), total 102 cases and 381 controls

^{matched} OR calculated by conditional logistic regression, cases and controls matched for age, gender, type of injury and calendar time (2 year band), corrected for observation time.

Table 2. Associations between CRPS and previous suggested associated disorders.

Prespecified disorders based on previous literature	Full journal prior to index date				Last year prior to index date			
	Main analysis†			Sens. Analysis‡	Main analysis†			Sens. Analysis‡
	Cases N=186	Controls N=697	OR[matched] (95% CI)	OR[matched] (95%CI)	Cases N=186	Controls N=697	OR[matched] (95%CI)	OR[matched] (95%CI)
	n	n			n	n		
Headache	25	66	1.54 (0.94-2.53)	1.6 (0.8-3.0)	15	31	**2.02 (1.06-3.87)**	**2.3 (1.1-5.5)**
Migraine	13	22	**2.43 (1.18-5.02)**	**2.6 (1.1-6.5)**	8	12	**2.67 (1.08-6.62)**	2.6 (0.9-8.1)
Other headache	13	48	1.06 (0.56-2.0)	1.0 (0.5-2.4)	8	20	1.63 (0.69-3.81)	2.2 (0.8-5.9)
Osteoporosis	13	24	**2.44 (1.17-5.14)**	**3.9 (1.4-10.8)**	12	21	**2.84 (1.28-6.32)**	**5.6 (1.8-17.1)**
Fibromyalgia	0	3	n.a.	n.a.	0	3	n.a.	n.a.
Psychologic. factors	64	219	1.17 (0.83-1.67)	0.9 (0.5-1.5)	41	139	1.26 (0.84-1.88)	0.9 (0.5-1.6)
Anxiety	27	78	1.31 (0.80-2.13)	0.6 (0.2-1.7)	16	50	1.23 (0.68-2.24)	0.9 (0.4-2.4)
Depression	14	55	0.95 (0.51-1.77)	1.0 (0.5-2.0)	8	42	0.82 (0.37-1.82)	0.2 (0.0-1.5)
Psychosocial prob.	8	33	0.96 (0.43-2.15)	1.2 (0.4-3.4)	7	21	1.31 (0.52-3.30)	1.4 (0.4-4.9)
Stress	32	105	1.25 (0.80-1.97)	0.9 (0.5-1.7)	13	43	1.22 (0.63-2.34)	1.2 (0.5-2.8)

† Including cases verified during a visit (IASP criteria, N=102) + not visited cases diagnosed by a specialist (N=84), total 186 cases

‡ Including only cases verified during a visit (IASP criteria, N=102), total 102 cases and 381 controls

[matched] OR calculated by conditional logistic regression, cases and controls matched for age, gender, type of injury and calendar time (2 year band), corrected for observation time.

Table 3. Associations between CRPS and disorders with potentially similar pathogenesis/etiology.

Prespecified disease classes based on underlying mechanism	Full journal prior to index date				Last year prior to index date			
	Main analysis†			Sens. Analysis‡	Main analysis†			Sens. Analysis‡
	Cases N=186	Controls N=697	OR^{matched} (95% CI)	OR^{matched} (95%CI)	Cases N=186	Controls N=697	OR^{matched} (95%CI)	OR^{matched} (95%CI)
	n	n			n	n		
Hypersensitivity[a]	43	153	1.09 (0.73-1.64)	1.7 (1.0-2.8)	30	99	1.20 (0.70-1.88)	1.8 (1.0-3.1)
Autoimmunity	7	27	1.04 (0.45-2.41)	1.2 (0.4-3.2)	4	21	0.82 (0.28-2.46)	0.6 (0.2-2.6)
Asthma	14	32	1.68 (0.86-3.29)	**3.0 (1.3-6.9)**	11	21	2.05 (0.94-4.50)	**3.0 (1.1-8.0)**
Allergy	33	126	1.02 (0.65-1.60)	1.6 (0.9-2.8)	23	73	1.31 (0.79-2.21)	1.8 (0.9-3.5)
Preexist. neuropathies[b]	14	37	1.55 (0.81-2.97)	1.9 (0.8-4.2)	7	13	2.01 (0.77-5.29)	**5.7 (1.8-18.7)**
Impaired microcirc.[c]	1	9	0.44 (0.06-3.50)	n.a.	0	3	n.a.	n.a.
Impaired macrocirc.[d]	43	160	0.94 (0.60-1.47)	1.0 (0.6-1.9)	35	137	0.93 (0.58-1.48)	0.7 (0.4-1.4)

† Including cases verified during a visit (IASP criteria, N=102) + not visited cases diagnosed by a specialist (N=84), total 186 cases

‡ Including only cases verified during a visit (IASP criteria, N=102), total 102 cases and 381 controls

matched OR calculated by conditional logistic regression, cases and controls matched for age, gender, type of injury and calendar time (2 year band), corrected for observation time.

[a] Hypersensitivity: see supplementary appendix.

[b] Preexisting neuropathies: including nerve entrapement, radicular syndromes, polyneuropathy, phantom pain, neuralgias, neuropathies, paresthesias and pain syndromes.

[c] Impaired microcirculation: including Raynauds syndrome, chilblains and syndrome of Klippel-Trenaunay.

[d] Impaired microcirculation: including cardiovascular disorders and venous insufficiencies.

Table 4. Sensitivity analyses regarding diseases associated with CRPS.

	Main analysis† Cases: N=186; Controls: N=697			Sens. Analysis‡ Cases: N=102; Controls: N=381		
	$OR^{matched}$ (95%CI)	**OR^{adj} (95%CI)**	**OR^{sens} (95%CI)**	**$OR^{matched}$ (95%CI)**	**OR^{adj} (95%CI)**	**OR^{sens} (95%CI)**
Full journal						
Migraine	**2.4 (1.2-5.0)**	**2.4 (1.2-5.0)**	**2.8 (1.2-6.1)**	**2.6 (1.1-6.5)**	**2.5 (1.0-6.2)**	**3.0 (1.2-8.0)**
Osteoporosis	**2.4 (1.2-5.1)**	**2.4 (1.1-5.1)**	**2.5 (1.2-4.1)**	**3.9 (1.4-10.8)**	**3.6 (1.3-10.1)**	**3.9 (1.4-10.8)**
Asthma	1.7 (0.9-3.3)	1.8 (0.9-3.5)	1.6 (0.7-3.4)	**3.0 (1.3-6.9)**	**3.1 (1.3-7.3)**	2.4 (0.9-6.3)
One year window						
Menstrual cycle related	**2.6 (1.2-5.8)**	**2.3 (1.0-5.1)**	**3.5 (1.3-9.2)**	**3.0 (1.1-8.7)**	2.6 (0.9-7.8)	**4.0 (1.0-16.2)**
Preexisting Neuropathies	1.6 (0.8-3.0)	2.0 (0.8-5.2)	2.9 (0.8-10.6)	**5.7 (1.8-18.7)**	**5.2 (1.6-16.5)**	**6.4 (1.3-31.8)**

† Including cases verified during a visit (IASP criteria, N=102) + not visited cases diagnosed by a specialist (N=84), total 186 cases

‡ Including only cases verified during a visit (IASP criteria, N=102), total 102 cases and 381 controls

matched OR calculated by conditional logistic regression, cases and controls matched for age, gender, type of injury and calendar time (2 year band), corrected for observation time.

Adj Analysis additionally adjusted for the contact frequency at the GP (to correct for potential Berkson's bias).

Sens Analysis in which the determinant (prior to CRPS diagnosis) is considered only when specific treatment is given (to correct for potential misclassification of the determinants).

Discussion

In this study, we systematically investigated associations between medical history and CRPS occurrence with the purpose to find potential risk factors and leads towards disease mechanisms underlying CRPS. While the study confirmed previously reported associations between CRPS and osteoporosis and headaches[22,23], the increased prior to CRPS presences of asthma and menstrual cycle related disorders were new. In addition, we did not find evidence that psychological factors were related to CRPS. Although a few have reported otherwise[24,25], this is in line with the conclusions of several other studies on psychological factors and CRPS.[27-29] Subgroup analyses in 'warm' and 'cold type' CRPS patients revealed that the observed high OR for migraine, osteoporosis, and asthma were mainly attributable to 'cold type' CRPS.

CRPS shares pathogenic mediators with both migraine and asthma. First, neurogenic inflammation, marked by neuropeptides such as gene related peptide (CGRP) and Substance P (SP), is likely to play a role in all three disorders.[30-33] Asthma patients show hyper-responsiveness to SP[34], while migraine and other headache patients have increased serum levels of both SP and CGRP.[35,36] In CRPS patients CGRP was systemically elevated and SP release was facilitated.[37,38] Second, in both asthma[39] and migraine[40] mast cells are involved, while tryptase (a product released by mast cells) is elevated in blister fluids[41] of CRPS patients. Finally, another common mediator in asthma and migraine is the nuclear factor kappa B[42,43], a transcription factor involved in inflammation and apoptosis, that was recently hypothesized to be of importance in CRPS based on the results of automated information retrieval from Medline.[44]

The strong association between CRPS and osteoporosis and menstrual cycle related disorders needs further exploration. Interestingly, osteoporosis has been considered as a consequence of CRPS rather than as a risk factor. Although one could argue that osteoporosis predisposes to fractures and thereby to CRPS, this can not explain the observations of the present study, since controls were matched to cases on injury type (for example fracture). Inflammatory mediators in CRPS, such as IL-1 and TNFα, have also been suggested to be increased in post-menopausal osteoporosis, but a definite role was never established.[45,46] Remarkably, bisphosphonates, frequently used in the treatment of osteoporosis, have been proven effective in CRPS treatment.[47] Sex hormones, such as estrogens, are of interest with regard to CRPS, due to its high incidence in women and at postmenopausal age. The observations in the present study warrant further investigation regarding hormonal factors in CRPS.

The increased presence of preexisting neuropathies (that included mainly radicular syndromes and polyneuropathies) in the year before CRPS suggests that existing sensitization predisposes to new sensitization. It is unlikely that the reported neuropathies were early unrecognized symptoms of CRPS, since they were reported prior to the CRPS precipitating injury. However, since the actual numbers are very small the results should be interpreted with caution.

In the past, it has been suggested that CRPS is a (partially) psychosomatic disorder and associations with several psychological factors have occasionally been described.[48,49] However, methodology of these studies was considered as poor.[24,25] and results were not confirmed in several other studies.[27-29] In the present study, where psychological factors

were registered in the records prospectively by the GP before the onset of CRPS, no association was observed between any type of psychological factors and CRPS. Also, in contrast to previous suggestions[50], there was no strong indication for a general tendency of somatisation in CRPS patients. We found no significant increase in prior to CRPS GP contact frequency in cases compared to controls, although our study may have lacked power to demonstrate minor differences. Such an eventual small increase can be explained as a consequence of more pre-existing somatic illness. A marginal, however significant, increase in unexplained complaints was observed in CRPS patients in the last year before the index date alone, but this was not strengthened in a sensitivity analysis.

Being observational, this study should be interpreted in the light of its limitations. Major threats to the validity of this study are selection bias and misclassification of the outcome (CRPS) or determinants (prior to CRPS medical conditions).

Selection of the study population was limited by still including part of cases that could not be visited (refusers and untraceable patients), namely the specialist diagnosed cases. Additionally, no significant differences in age and gender distribution or prevalences of medical conditions were observed between participants and non-participants.

Misclassification of the outcome (CRPS) was reduced due to an extensive case validation procedure and by performing sensitivity analyses including only cases that could be verified by the investigator during a visit. Despite this, some misclassification may have remained since ascertainment of CRPS had to be done retrospectively, combining patient-reported symptoms and disease course with GP reconfirmation and specialist letters if available. However, at the time of acute CRPS, the diagnosis was always assessed clinically by a physician based on symptoms and signs at that moment, and only cases that had been reconfirmed afterwards by the GP were included. The additional validation step that implied visiting the cases that were identified during the previous described CRPS incidence study[5], yielded a false positive CRPS diagnosis for 19% of the cases and a 6% misclassification of the date of onset. This suggests an overestimation of the incidence rate calculated using the reported methods in that study (validation by GP reconfirmation only). A revised incidence calculation after the additional validation step (in which we were rather strict in excluding cases in order prevent misclassification) would roughly provide an estimate of 20 incident cases per 100,000 person years (instead of the previously reported 26). This incidence rate is still much higher than previously mentioned.[6]

Misclassification of the determinants (medical conditions prior to CRPS) may have occurred. The dynamic character of the IPCI database has lead to variation in length of observation time within the study population, being shorter in patients who entered the database early during the study period or who got CRPS soon after the start of their observation period in the database. To deal with this, a minimum observation time of one year before CRPS onset was required for each patient, while also in the analyses we adjusted for observation time, thereby preventing against confounding by the length of the observation period. Diagnoses could have been missed if they occurred before start of observation in the database, although we suspect that serious ongoing medical conditions will have been noted again during the actual observation period. Moreover, diagnoses mentioned in the journal may be false positive since for efficiency reasons in general practice they are not always validated with complementary research. However, any

misclassification resulting was likely indifferent between cases and controls and would therefore only have leaded to an underestimation of associations. The actual observed associations increased in sensitivity analyses, wherein the diagnoses (determinants) were strengthened by requiring specific treatment.

The fact that all medical conditions have been assessed and registered in patients before the onset of CRPS, meaning that both doctor and patient did not know that the patient was susceptible to CRPS, is a strong feature of the study. Recall bias and differential information bias are therefore not an issue. A second advantage is the availability of a relatively large population based control group that was matched to the cases on the same type of injury preceding CRPS (additional to age and gender). As CRPS generally occurs after an injury, matching on this risk factor is important if additional risk factors are sought. For 8% of the CRPS patients no precipitating injury could be extracted from the journal, which is in line with previously reported percentages of spontaneous CRPS.[1] Finally, due to the gatekeeper role of the GP in the Netherlands, it was possible to study the medical history of the patient from the electronic medical records, which is likely to be more accurate than a self report by the patient.

In conclusion, a medical history of asthma, migraine, osteoporosis and a recent history of menstrual cycle related problems and preexisting neuropathies were associated with CRPS. Since the etiologies of some of these diseases are better understood they may give leads to potential disease mechanisms underlying CRPS. The association with asthma and migraine favors the existing ideas of neurogenic inflammation involvement in CRPS.

Supplementary data associated with this article can be found in the online version at doi:10.1016/529 j.pain.2008.07.002.

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3.2 The Association between ACE inhibitors and Complex Regional Pain Syndrome

Submitted

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Abstract

Introduction: Antihypertensive drugs interact with mediators that are also involved in the complex regional pain syndrome (CRPS), such a neuropeptides, adrenergic receptors and vascular tone modulators. Therefore we aimed to study the association between the use of antihypertensive drugs and CRPS onset.

Methods: A population based case-control study was conducted in the Integrated Primary Care Information (IPCI) database in the Netherlands. Cases were identified from electronic records (1996-2005) and included if they were confirmed during an expert visit (using IASP criteria), or if they had been diagnosed by a medical specialist. Up to four controls per cases were selected, matched on gender, age, calendar time and injury. Exposure to angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, β-blockers, calcium channel blockers, and diuretics was assessed from the automated prescription records. Data were analyzed using multivariable conditional logistic regression.

Results: A total of 186 cases were matched to 697 controls, (102 confirmed during an expert visit plus 84 with a specialist diagnosis). Current use of ACE inhibitors was associated with an increased risk of CRPS ($OR^{adjusted}$: 2.7, 95%CI: 1.1-6.8). The association was stronger if ACE inhibitors were used for a longer time period ($OR^{adjusted}$: 3.0, 95%CI: 1.1-8.1) and in higher dosages ($OR^{adjusted}$: 4.3, 95%CI: 1.4-13.7). None of the other antihypertensive drug classes was significantly associated with CRPS.

Discussion: ACE inhibitors use was associated with CRPS onset. We hypothesize that ACE inhibitors influence the neuro-inflammatory mechanisms underlying CRPS by their interaction with the catabolism of substance P and bradykinin.

Introduction

The complex regional pain syndrome (CRPS) can occur in one or more extremities as painful complication from a fracture, surgery or any other type of physical injury.[1] Its incidence in the Netherlands is estimated at 26 per 100,000 person years, while women are affected 3.4 times more frequently than men.[2] In the absence of a sensitive and specific biomarker the diagnosis is based on clinical criteria as established by the International Association for the Study of Pain (IASP).[3] The mechanisms underlying CRPS have been studied increasingly over the past decade and parts of the pathogenesis become slowly unraveled. Both inflammatory and neurogenic (autonomic and somatic) disturbances contribute to CRPS and are represented in the clinical presentation. Most patients display classic inflammatory signs like pain, swelling, redness, and warmth in the initial phase of the disease.[1] Autonomic disturbances, neuropathic pain, and motor impairment may follow, causing ongoing discomfort and functional disability.[4]

Mediators of inflammation in CRPS include classic pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα, which are elevated in blister fluid and spinal liquor of CRPS patients.[5-7] Additionally, neuropeptides such as calcitonin gene related protein (CGRP) and substance P (SP) play a role in CRPS[8,9], contributing to vasodilatation, long lasting erythema and plasma protein extravasation.[10-13] Substance P also stimulates other immunological responses[14] and, when released by terminal nerve endings in the dorsal horn, it mediates central sensitization.[15] Moreover, bradykinin, which is involved in acute and chronic inflammatory responses[16] and in peripheral nociceptor sensitization[17] is systematically elevated in CRPS patients.[9]

In addition to inflammatory markers CRPS patients also have elevated systemic levels of catecholamines[18] whereas local levels are decreased.[19] Catecholamines are considered to be mediators in sympathetic hyperactivity, to which many signs of CRPS have been attributed. Under certain circumstances, catecholamines can also induce proinflammatory responses, mediated through the interaction with α-receptors expressed on immune cells.[20,21]

Some of the above mentioned inflammatory mediators that are elevated in CRPS patients also play a role in the mechanism of action of antihypertensive drugs. In particular ACE inhibitors are of interest, as they might block the ACE dependent degradation of substance P and bradykinin.[22,23] Therefore ACE inhibitors could be hypothesized to increase the risk of CRPS onset. On the other hand, β-blockers attenuate the effects that are mediated by catecholamines, and therefore may decrease the symptoms of CRPS. Calcium channel blockers cause vasodilatation, thereby improving peripheral blood circulation and counteracting potential CRPS symptoms. Based on these mechanisms of action, we hypothesized that antihypertensive drugs could influence the occurrence of CRPS. This study aims therefore to investigate whether antihypertensive drugs, and in particular ACE inhibitors, are associated with the risk of developing CRPS.

Methods

Design and setting

A retrospective case-control design was used, comparing antihypertensive drug use in CRPS patients to that in controls selected from the same general population. The study was nested in the Integrated Primary Care Information (IPCI) database, which is a longitudinal general practice (GP) database that currently contains the electronic records of more than 800,000 persons in the Netherlands. The IPCI population reflects the age and gender distribution of the general Dutch population. In the Dutch Health Care System all persons are registered with one GP independent of their health status. The GP acts as a gatekeeper for further medical care and as a central receiver of information about secondary care. Therefore, the electronic records can be assumed to contain complete medical information of each patient.[24] GPs participating in the IPCI project do not keep additional paper records, except for specialist and discharge letters. Details on the database have been described previously.[25]

The IPCI project complies with the European Union guidelines on the use of medical data for medical research and has been proven valid for (pharmaco-)epidemiological research.[26] The present study has been approved by the Scientific and Ethical Advisory Group of IPCI and by the Medical Ethical Board of the Erasmus Medical Center (protocol number 2006-099).

Source population

The source population comprised all persons with at least 1 year of valid history in the IPCI database during the study period (January 1996-June 2005) to ensure sufficient baseline information on all subjects. This meant that the practice had been contributing data to the IPCI database for at least one year and that the patient had been registered with the GP for at least one year. Follow-up started on the first of January 1996 or on the date that one year of valid history was available, whichever date was latest. Follow-up was ended upon transferring out of the practice, the date of last data supply by the GP, death, diagnosis of CRPS or at the end of the study period, whichever came first. The source population was restricted to all practices that were still active in the IPCI database in 2006.

Cases

Potential incident CRPS cases were identified in the database using a sensitive string search algorithm. Subsequently, short questionnaires were mailed to the GPs, in which they were asked to reconfirm whether the person indeed had suffered from CRPS and to provide copies of all available specialist letters. A more detailed description of the case identification and validation process up to this point has been described in our study on the incidence of CRPS in the Netherlands.[2]

In a subsequent step all confirmed cases were invited to our study. Cases who consented to participate in our study were visited by the primary investigator, a physician with clinical experience in diagnosing CRPS (MM). Preceding this visit, patients received an extensive questionnaire concerning their CRPS related complaints and symptoms, and disease course. Patients completed the questionnaire by themselves and during the visit

the investigator and patient solved incomplete answers together. A physical examination of the affected and contra-lateral unaffected extremity was also performed.

Patients were included as cases in the analysis if they were judged to have ever fulfilled the diagnostic criteria for CRPS as established by the International Association for the Study of Pain (IASP)[3], using the combined information from the visit, electronic journal and specialist letters (if available). Patients who could not be visited but who had a specialist diagnosis of CRPS were included as a case in the primary analysis, but excluded in a secondary (sensitivity) analysis. The index date was chosen as the date on which CRPS was first mentioned in the medical records.

Controls

Per case, up to four age (year of birth) and gender matched controls were selected from the IPCI database, with the requirement that each control had encountered a similar type of injury as its matching case within the two years prior to the date of CRPS diagnosis of the case. This meant that cases with CRPS following a fracture were matched to controls with a fracture, cases with CRPS following a soft tissue injury were matched to controls with a soft tissue injury, etc. If the CRPS had occurred in a case spontaneously (no initiating injury) the control was not required to have had an injury either. For each control the index date was established as the date of the injury plus the time between injury and CRPS onset in its matched case.

Use of antihypertensive drugs

Drug prescriptions were retrieved from the IPCI database. The available data comprised Anatomical Therapeutical Chemical (ATC) classification code, prescription start date, quantity, strength, indication and prescribed daily dose. The following groups of antihypertensives were included[27]: ACE inhibitors, angiotensin II (AT2) receptor antagonists, β-blockers, calcium channel blockers and diuretics (alone or in combination with other antihypertensives). AT2 receptor antagonists were analyzed separately from the ACE inhibitors since they do not affect SP and bradykinin degradation and therefore were not suspected of affecting CRPS occurrence. The duration of a prescription was calculated as the quantity of prescribed units (mostly tablets) divided by the daily intake of units. Episodes of use per drug were created by combining consecutive prescriptions and correcting for overlap. A person was classified as a current user of a certain drug if the duration of the most recent prescription plus seven days covered the index date. If the last prescription ended more than seven days prior to the index date, persons were classified as past users. In current users we assessed the duration of use as the number of days that the drug was used during the year prior to the index date. The prescribed daily dose was expressed as the number of dose equivalents of the defined daily dose (DDD) for the last prescription[28]. In order to evaluate the accuracy of the GP prescription data pharmacy delivery lists were requested for a subset of patients and compared to the prescription data.

Covariables

The medical history prior to the onset of CRPS was extracted from the electronic medical records. Smoking and disorders related to antihypertensive drug use, including hypertension, hypercholesterolemia, cardiovascular diseases (for example angina pectoris and myocardial infarction) and diabetes mellitus (DM) type II were considered as potential confounders. Additionally, diseases that were found to be associated with CRPS onset in a previous study conducted within the IPCI database were taken into account, namely osteoporosis, migraine, asthma and menstrual cycle related disorders.[29] Current use of drugs that might be associated with either antihypertensive use or CRPS was also evaluated as potential confounder (NSAIDs, corticosteroids, statins, SSRIs, tricyclic antidepressants, anti-epileptics, and drugs for migraine (ATC: N02C)).

Statistical analyses

Conditional logistic regression analyses were performed to study the association between current and past use of antihypertensive drugs use and CRPS onset, calculating crude and adjusted odds ratios (OR) with 95% confidence intervals (CI). Since two or even three antihypertensive drugs are frequently co-prescribed, antihypertensive drug monotherapy was analyzed separately, whereby (concomitant) users of other antihypertensives than the one of interest were classified into a separate category. Covariates were included in the model if they altered the OR for current use of ACE inhibitors by more than 10%. Sensitivity analyses were conducted to estimate the effect of outcome misclassification, including only the cases that were validated by a visit (using IASP criteria), thereby excluding the unvisited cases which were diagnosed by specialist. To investigate effect modification by gender stratified analyses were performed and interaction terms for gender and antihypertensive drug use were tested in the regression model.

Results

Two-hundred-and-fifty-nine cases were identified and reconfirmed by the GP in the source population of 204,281 persons: 191 (74%) with a specialist diagnosis and 68 (26%) with only a GP diagnosis. The procedure for final case inclusion in the main and sensitivity analyses is presented in figure 1. The final case set comprised cases that were validated upon a visit (n=102) supplemented with the specialist diagnosed cases from non visited patients (n=84). Thus, the total study population comprised 883 patients, including 186 cases and 697 age, gender and injury matched controls (up to four per case).

Characteristics of the study population are displayed in table 1. The mean age on the index date in the study population was 51 years, 77% was female, and CRPS patients had more often migraine and osteoporosis in their medical history and they were more frequently current users of NSAID's.

For 166 patients (19% of the total study population) complete pharmacy delivery lists were available for comparison with GP prescription records. The sensitivity for current use of an antihypertensive in the GP records was 82%, while the specificity was 99%. This was non-differential between cases and controls.

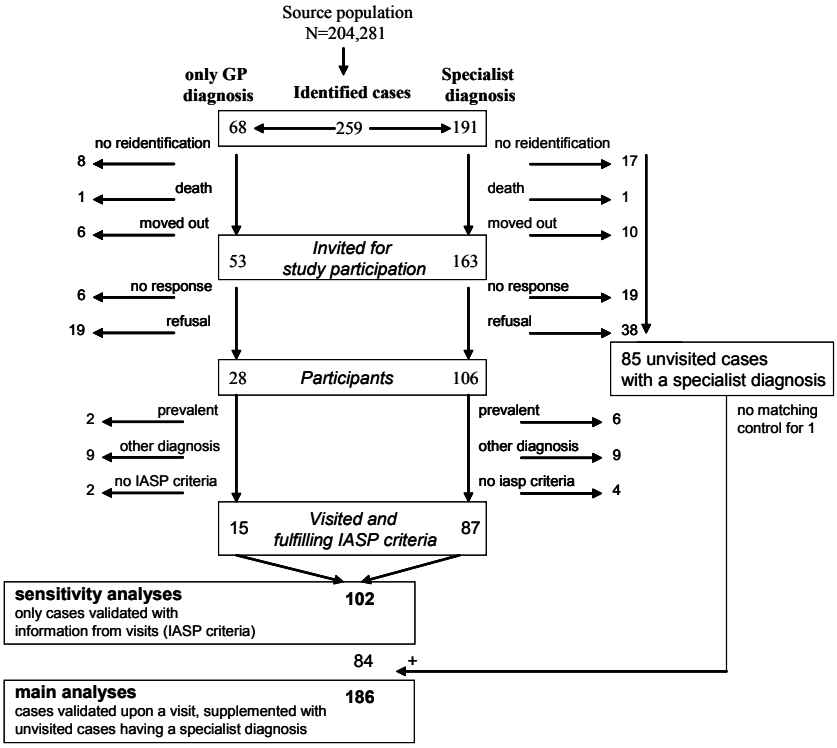

Figure 1. Inclusion of cases for main and sensitivity analyses. Re-identifiction means the process of decoding the patient number in the IPCI database. The first decoding step is performed by the IPCI gatekeeper, who subsequently contacts the GP. Only the GP can retrieve the patient names and addresses for contact purposes and all patient contact is mediated through the GP. Reasons for failure of re-identification are changes in software systems or retirement of the GP. In prevalent cases CRPS onset occurred before start of follow up time in the database. Since we were interested in drug use before CRPS onset these patients were excluded from the analyses.

Table 1. Characteristics of the study population.

Characteristics	Cases N=186		Controls N=697		OR (95%CI)
Age	Mean: 51 year, sd: 16				matched
Gender (female)	677 (77%)				matched
	n	**%**	**n**	**%**	
Smoking	36	36 (19.4)	127	127 (18.2)	1.1 (0.7-1.7)
Co-morbidity					
Hypertension	28	15.1%	119	17.1%	0.8 (0.5-1.3)
Hypercholesterolemia	16	8.6%	58	8.3%	1.1 (0.6-2.0)
Cardiovascular disorders	9	4.8%	25	3.6%	1.3 (0.6-3.0)
Diabetes Mellitus type II	6	3.2%	27	3.9%	0.8 (0.3-2.1)
Heart failure	2	1.1%	3	0.4%	3.2 (0.4-23.4)
Asthma	14	7.5%	32	4.6%	2.0 (0.9-3.3)
Migraine	13	7.0%	22	3.2%	**2.4 (1.2-5.0)**
Osteoporosis	13	7.0%	24	3.4%	**2.4 (1.2-5.1)**
Menstrual cycle related dis.	14	7.5%	33	4.7%	1.8 (0.9-3.6)
Co-medication (current)					
NSAIDs	16	8.6%	23	3.3%	**2.8 (1.4-5.7)**
Corticosteroids	0	0%	2	0%	n.a.
Statins	7	3.8%	35	5.0%	0.8 (0.3-1.8)
SSRIs	5	2.7%	20	2.9%	1.0 (0.4-2.7)
TCAs	2	1.1%	5	0.7%	1.3 (0.2-7.5)
Anti epileptics	1	0.5%	4	0.6%	n.a.
Anti migraine drugs	4	2.2%	4	0.6%	4.0 (1.0-16.0)

NSAID: non steroidal anti-inflammatory drugs, SSRI: selective serotonin reuptake Inhibitor, TCA: tricyclic antidepressant

Associations between antihypertensive drug use and the risk for CRPS are displayed in table 2. No significant associations were observed between CRPS and current use of β-blockers, AT2 antagonists, calcium channel blockers or diuretics. Current use of ACE inhibitors was associated with an increased risk of CRPS (OR: 1.9; 95%CI: 0.9-4.1). This association became stronger upon adjustments for potential confounders (OR: 2.7, 95%CI: 1.1-6.8). The association further strengthened if monotherapy with ACE inhibitors was analyzed separately from combinations of ACE inhibitors and other antihypertensives (OR: 3.3, 95%CI: 1.1-9.8). A stratified analysis by gender displayed an even stronger effect of ACE inhibitor use in women (OR: 4.6, 95%CI: 1.6-13.2 in 143 cases and 534 controls), while in men the association disappeared (OR: 0.5, 95%CI: 0.1-4.9 in 43 cases and 163 controls). However, the multiplicative interaction term in the regression model was non-significant, argueing against significant effect modification. Hypertension was the primary indication for ACE inhibitor prescriptions in 9 of the cases (81.1%) and 17 of the controls (81.0%).

Table 2. Associations between the use of antihypertensive drugs and the risk for CRPS.

Antihypertensive	Cases N=186	Controls N=697	OR[matched] (95%CI)	OR[adj1] (95%CI)	ORadj[adj2] (95%CI)
	n	n			
Ace-inhibitors					
never	171	656	Ref	Ref	Ref
current	11	21	1.9 (0.9-4.1)	**2.8 (1.1-6.8)**	**2.7 (1.1-6.8)**
past	4	20	0.8 (0.3-2.3)	1.2 (0.3-4.2)	1.1 (0.3-4.1)
monotherapy†					
never	137	532	Ref	Ref	Ref
current	7	7	**3.3 (1.1-9.8)**	**5.0 (1.5-16.9)**	**4.7 (1.4-16.3)**
past	1	8	n.a.	n.a	n.a.
concomitant	41	150	1.0 (0.7-1.6)	1.3 (0.8-2.1)	1.2 (0.7-2.1)
AT2 antagonists					
never	183	679	Ref	Ref	Ref
current	2	13	0.6 (0.1-2.6)	0.7 (0.2-3.4)	0.7 (0.1-3.4)
past	1	5	n.a.	n.a.	n.a.
monotherapy†					
never	137	532	Ref	Ref	Ref
current	1	2	n.a.	n.a.	n.a.
past	0	4	n.a.	n.a.	n.a.
concomitant	48	159	1.1 (0.8-1.6)	1.3 (0.8-2.2)	1.3 (0.8-2.2)
B-blockers					
never	160	598	Ref	Ref	Ref
current	8	47	0.7 (0.3-1.5)	0.7 (0.3-1.6)	0.7 (0.3-1.7)
past	18	52	1.3(0.7-2.3)	1.4 (0.7-2.5)	1.4 (0.8-2.7)
monotherapy†					
never	137	532	Ref	Ref	Ref
current	2	27	0.3 (0.1-1.3)	0.3 (0.1-1.4)	0.3 (0.1-1.4)
past	12	33	1.4 (0.7-2.8)	1.6 (0.8-3.4)	1.7 (0.8-3.5)
oncomitant	35	105	1.2 (0.8-1.9)	1.5 (0.8-2.6)	1.4 (0.8-2.5)
Ca antagonists					
never	174	658	Ref	Ref	Ref
current	8	22	1.3 (0.5-3.0)	1.5 (0.6-3.8)	1.4 (0.6-3.8)
past	4	17	0.8 (0.3-2.6)	0.9 (0.3-2.8)	1.0 (0.3-3.1)
monotherapy†					
never	137	532	Ref	Ref	Ref
current	3	6	1.3 (0.3-6.5)	1.6 (0.3-8.7)	1.8 (0.3-9.8)
past	3	5	2.0 (0.5-8.9)	2.1 (0.5-9.6)	2.4 (0.5-11.2)
concomitant	43	154	1.1 (0.7-1.6)	1.3 (0.8-2.2)	1.2 (0.7-2.0)
Diuretics					
never	161	620	Ref	Ref	Ref
current	10	28	1.5 (0.7-3.2)	2.1 (0.9-5.0)	2.0 (0.8-4.8)
past	15	49	1.1 (0.6-2.0)	1.2 (0.6-2.3)	1.1 (0.6-2.1)
monotherapy†					
never	137	532	Ref	Ref	Ref
current	2	6	1.4 (0.3-6.8)	1.6 (0.3-8.2)	1.6 (0.3-8.5)
past	9	27	1.0 (0.4-2.4)	1.1 (0.5-2.6)	0.9 (0.4-2.2)
concomitant	38	132	1.1 (0.7-1.7)	1.4 (0.8-2.5)	1.5 (0.8-2.6)

† Concomitant users of other antihypertensives are classified into a separate group.
[matched] Matched on gender, year of birth, calendar time (2 year band), and type of injury.
[adj1] Adjusted for hypertension, hypercholesterolemia, DM, and cardiovascular disorders.
[adj2] Additionally adjusted for the current use of NSAIDs and statins.

Long term use of ACE inhibitors, as well as a high dose, was associated with a stronger association with CRPS than short term use and a low dose (OR: 3.0, 95%CI: 1.1-8.1 and OR: 4.3, 95%CI: 1.4-13.7 respectively) (Table 3). The association between CRPS and current use of ACE inhibitors remained in a sensitivity analysis that was restricted to the cases that were confirmed CRPS upon expert visitation.

Table 3. Associations between the duration and dosage of ACE inhibitor use and CRPS
In the main analysis and in a sensitivity analysis that excluded unvisited cases.

ACE inhibitors	Main analysis			Sensitivity analysis		
	Cases N=186	Controls N=697	OR^{adj} (95% CI)	Cases N=102	Controls N=381	OR^{adj} (95% CI)
	n	n		n	n	
Duration						
never	171	656	Ref	95	359	Ref
≤180 days	2	3	1.9 (0.3-23.9)	0	0	n.a
>180 days	9	18	**3.0 (1.1-8.1)**	6	11	**4.6 (1.1-19.3)**
past	4	20	1.1 (0.3-4.0)	1	11	0.5 (0.0-5.4)
Dose						
never	171	656	Ref	95	359	Ref
≤ 1DDD	4	12	1.6 (0.4-5.8)	2	7	1.7 (0.2-13.4)
> 1DDD	7	9	**4.3 (1.4-13.7)**	4	4	**9.7 (1.7-54.2)**
past	4	20	1.1 (0.3-4.0)	1	11	0.5 (0.0-5.6)

Main analysis: including cases verified upon a visit (N=102) + unvisited specialist diagnosed cases (N=84), total N=186; **Sensitivity analysis**: including only cases verified upon a visit, total N=102
^{adj} matched on gender, year of birth, calendar year (2 year band), and type of injury; adjusted for hypertension, hypercholesterolemia, cardiovascular diseases, diabetes mellitus, and current use of NSAIDs and statins.

Discussion

In this nested population based case-control study we observed a dose and duration dependent association between use of ACE inhibitors and the risk of CRPS. Other classes of antihypertensive drugs were not associated with either a significant reduced or increased risk of CRPS. B-blockers tended to reduce the risk, but this should be investigated in a larger dataset to obtain sufficient power.

The observed association between ACE inhibitors and CRPS was a-priori hypothesized due to its biological plausibility (figure 2). ACE is one of the most important kininases involved in the inactivation of SP[23] and bradykinin[22] and ACE inhibition therefore would increase the levels of these pro-inflammatory peptides. Both SP and bradykinin are well known mediators in inflammation and sensitization, two important mechanisms underlying CRPS, and both peptides have actually been demonstrated involved in CRPS pathology.[9,30,31]

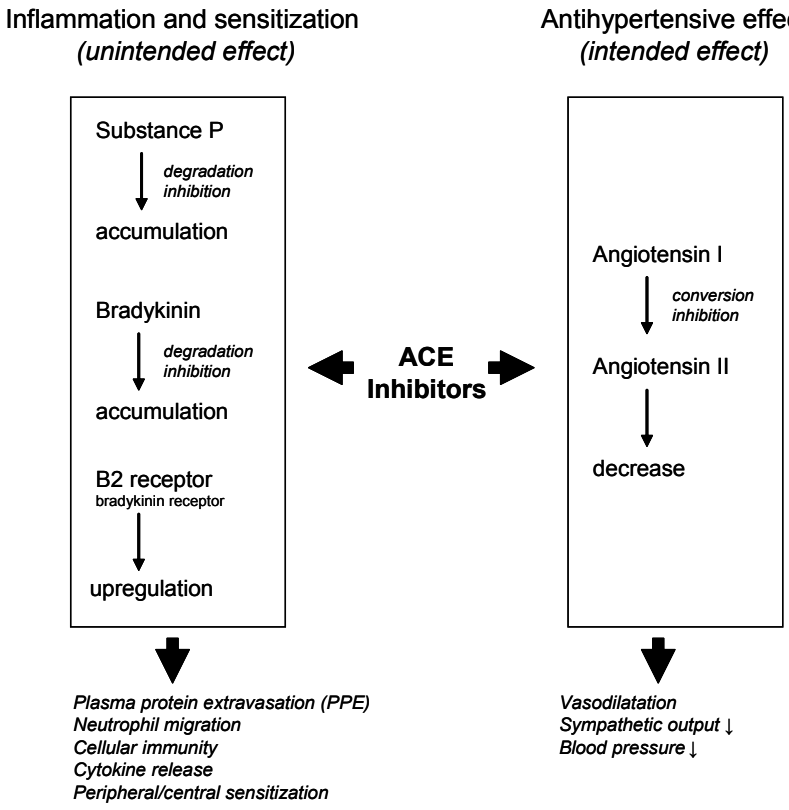

Figure 2. The intended effect of ACE inhibitors is to block the conversion of angiotensin I into angiotensin II in order to decrease blood pressure. However, as an unintended effect, ACE inhibitors prevent the degradation of substance P and bradykinin, causing accumulation of these peptides. In addition to this, ACE inhibitors upregulate the bradykinin (B2) receptor, thereby further potentiating bradykinin activity. Substance P and bradykinin are both important mediators in neuroinflammatory responses and sensitization and probably in the pathogenesis of CRPS.

The role of ACE in CRPS and neurogenic inflammation has been investigated before from a genetic and experimental perspective yielding quite contradictory results. A Japanese study revealed an increased prevalence of the ACE polymorphism DD genotype in 16 CRPS patients compared to the general population (43% versus 20%).[32] The DD genotype however is known to be associated with higher ACE levels and thereby with supposedly lower SP and bradykinin levels. Therefore, the DD genotype would be expected to correlate with a low risk of CRPS, instead of a high risk as was observed in the Japanese study. However, only a small study population (n=16) was included and the findings were not confirmed in a larger German study (48 sporadic and 12 familial patients).[33]

An experimental study in healthy skin showed no facilitation of electrical C-fiber stimulation induced neurogenic inflammation by the ACE inhibitor captopril[34], although this was expected because ACE inhibition would lead to SP and bradykinin accumulation. However, healthy skin is different from the inflamed tissue that is present in the early phase or pre-stadium of CRPS. ACE inhibitors do not facilitate neurogenic inflammation in healthy skin probably because there the levels of its substrates, SP and bradykinin, are low.[34] On the contrary, inflamed skin holds increased levels of SP, released by primary afferents under the influence of cytokines[35] and bradykinin. Normally SP and bradykinin should be degradated by ACE, but ACE inhibitors may block their catabolism, resulting in the further accumulation of these peptides. In this view, ACE inhibitors may not affect the initiation of the neuro-inflammatory response that underlies CRPS, but they can facilitate its progression once it has been triggered by other causes. Eventually this may cause an initially functional inflammatory response to develop towards a point that it becomes pathological, as in CRPS (figure 3).

It has also been shown that under certain circumstances ACE inhibitors facilitate the degradation of CGRP[36], another neuropeptide that has been found to be increased in CRPS patients.[9] As ACE inhibitors may diminish CGRP levels, they could also have been found to protect against CRPS, the opposite to what we observed. However the interaction between ACE inhibitors and CGRP is indirect and occurs only when the main metabolizer of CGRP, an enzyme called neutral endopeptidase (NEP), is blocked.[36] SP and bradykinin are direct substrates for ACE and our observation that ACE inhibitors increase (instead of decrease) the risk of CRPS suggests that SP or bradykinin or both are important mediators in CRPS. CGRP may be important in CRPS as well, but its actual relevance can not be derived from the present study, because the interaction between ACE and CGRP is more complex.

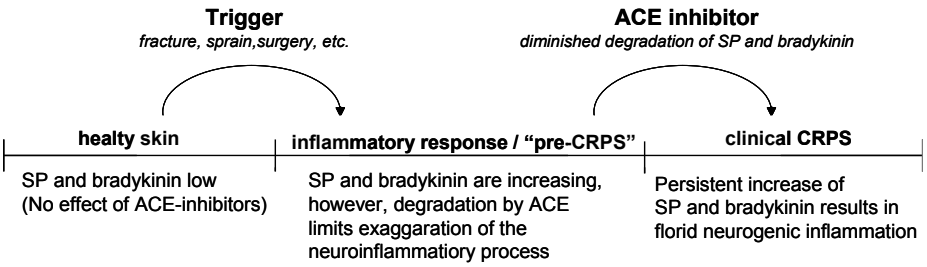

Figure 3. Ace inhibitors do not facilitate neurogenic inflammation in healthy skin (Kramer et. al. 2005), but may enhance an existing neuroinflammatory response that has been initiated by a previous trigger by preventing the degradation of SP and bradykinin. In some cases this may cause a physiological inflammatory reaction ("pre-CRPS") to develop towards a florid neurogenic inflammation, resulting in the clinical picture of CRPS.

Strengths of our study include the unique population based setting and design, wherein controls had an injury similar to the cases. The availability of prescription records with accurate information on prescription dates allowed us to study the role of drugs in the onset of CRPS. However, the study also has some limitations. Misclassification of the CRPS diagnosis, a general problem in CRPS research, may have occurred also in our study, since the final verification of the diagnosis had to be performed retrospectively. Assuming that misclassification of the diagnosis was unrelated to the use of antihypertensive drugs this would only have resulted in an underestimation of the associations of interest. This was confirmed by a sensitivity analysis that was limited to the cases that were verified during a visit (more valid diagnosis) and showed an even stronger association between ACE inhibitor use and CRPS onset. Also, some non-differential misclassification of the exposure (current use of antihypertensives) was present, for example where antihypertensive drugs were prescribed by physicians other than the GP. Potential selection bias was reduced by including the specialist diagnosed cases who were not visited (including refusers and untraceable patients). In addition, antihypertensive drug use did not differ between participants and non-participants (derived from the prescription records in the database). Confounding was addressed as far as possible by the inclusion of various comorbidities and drugs in the model, but residual confounding cannot be ruled out.

In conclusion, we found a positive dose and duration dependent association between the use of ACE inhibitors and the risk of CRPS. This points to the important role of SP or bradykinin or both in the pathogenesis of CRPS, as these inflammatory peptides are metabolized by ACE and increase during ACE inhibition. We hypothesize that ACE is a modulator of the neuro-inflammatory mechanisms that underlie CRPS.

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3.3 | Estrogens and the Risk of Complex Regional Pain Syndrome

Submitted

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Abstract

Introduction: Since the complex regional pain syndrome (CRPS) shows a clear female predominance we investigated the association between the cumulative as well as current exposure to estrogens, and CRPS.

Methods: A population based case-control study was conducted in the Integrated Primary Care Information (IPCI) project in the Netherlands. Cases were identified from electronic records (1996-2005) and included if they were confirmed during a visit (using International Association for the Study of Pain criteria), or had been diagnosed by a specialist. Controls were matched to cases on gender, age, calendar time and injury. Measures of cumulative endogenous estrogen exposure were obtained by questionnaire and included age of menarche and menopause, menstrual life, and cumulative months of pregnancy and breastfeeding. Current estrogen exposure at CRPS onset was retrieved from the electronic medical records and determined by current pregnancy or by the use of oral contraceptive drugs (OC) or hormonal replacement therapy (HRT).

Results: Hundred-and-forty-three female cases (1,493 controls) were included in analyses on drug use and pregnancies, while cumulative endogenous estrogen exposure was studied in 53 cases (58 controls) for whom questionnaire data were available. There was no association between CRPS and either cumulative endogenous estrogen exposure, OC or HRT use. CRPS onset was increased during the first six months after pregnancy (OR: 5.6, 95%CI: 1.0-32.4), although based on small numbers.

Discussion: We did not find an association between CRPS onset and cumulative endogenous estrogen exposure or current OC or HRT use, but more powered studies are needed to exclude potential minor associations.

Introduction

Rehabilitation from a physical trauma, such as a fracture or sprain, can be complicated by the complex regional pain syndrome (CRPS). CRPS is usually located in the distal part of the affected extremity and is marked by pain, vasomotor, sudomotor and motor/trophic disturbances.[1] Its pathogenesis is subject to speculation, but nervous system involvement has since long been acknowledged. However, more recently the interest in inflammatory disease mechanisms underlying CRPS has increased.[2,3] Neurogenic inflammation is mediated by neuropeptides that are secreted by nociceptive nerve endings upon triggering by mechanical, chemical or physical injury.[4] Neuropeptides, such as substance P (SP) and calcitonin gene related protein (CGRP), induce plasma protein extravasation[5], recruitment of immune cells[6] and release of pro-inflammatory cytokines, for example tumor necrosis factor α (TNFα), interleukin (IL)-1 and IL-6.[7,8] Moreover, reactive oxygen species (ROS) have been suggested to be involved in triggering or sustaining CRPS.[9,10]

CRPS has a clear female predominance with a female/male ratio between three and four.[11,12] The peak incidence occurring between the fifth and seventh decade suggests an increasing risk after the menopause, when endogenous estrogen levels drop. Furthermore, a previous study showed an association between CRPS and both menstrual cycle related disorders and osteoporosis.[13] All these observations suggest that sex hormones, in particular estrogens, are of relevance in the pathogenesis of CRPS.

In women serum levels of 17β-estradiol (E2), the primary estrogen during pre-menopausal life, are influenced by menarche, menstrual cycle, pregnancy and lactation, and menopause. Estrogen levels can also be affected by synthetic estrogens or estrogen mimicking drugs that are administrated, such as oral contraceptive drugs (OC) or as hormonal replacement therapy (HRT). The latter is frequently prescribed to women during the peri- or post-menopausal life in order to attenuate the negative effects accompanying decreasing E2 levels, such as vasomotor symptoms, mood changes and loss of bone mineral density. Beyond their role in sexual development and reproduction estrogens play an important role in the cardiovascular system[14] and in bone metabolism.[15] Additionally, estrogens are widely involved in mechanisms of inflammation[16] and may therefore interact with several presumed mediators in the pathogenesis of CRPS. For example, estrogens influence the metabolism of substance P and bradykinin[17] and prevent the formation of reactive oxygen species during ischemia or inflammation.[18] Moreover, NFκB, an important transcription factor in inflammation and probably also in CRPS[19], is inhibited by interaction with estrogen receptors.[20] Overall, these estrogen effects may attenuate the disease mechanisms of CRPS and we therefore hypothesized that high estrogen levels may prevent CRPS onset.

In the present study, we investigated whether exposure to estrogens affects the risk for CRPS onset. We performed a population based case-control study comparing measures of cumulative endogenous estrogen exposure during life, as well as current estrogen exposure at the moment of CRPS onset.

Methods

Design and setting

The case-control study was nested within the integrated primary care information (IPCI) project, a general practitioners (GP) research database in the Netherlands. The database contains the electronic files of over one million patients and is representative of the Dutch population regarding age and gender.[21,22] All inhabitants of the Netherlands are registered with a GP who receives and stores all health care information of their patients.[23] Therefore, GPs can be considered to have complete medical information. GPs who participate in the IPCI project do not keep additional paper records. The electronic records store information on demographics, signs and symptoms (using the International Classification for Primary Care (ICPC)[24] and narratives), diagnoses (using ICPC and narratives), clinical findings, specialist referrals, laboratory findings, hospitalizations, and drug prescriptions. Summaries from medical correspondence with specialists are entered in a free text format and hard copies of original letters can be provided upon request. The IPCI project complies with the European Union guidelines on the use of medical data for medical research and has been proven valid for (pharmaco-)epidemiological research.[25] The present study has been approved by the Scientific and Ethical Advisory Group of the Project (protocol number 04/70) and by the Medical Ethical Board of the Erasmus Medical Center (protocol number 2006-099).

Source population

The source population comprised all persons with at least 1 year of valid history in the IPCI database during the study period (January 1996-June 2005) to ensure sufficient baseline information on all subjects. This meant that the practice had been contributing data for at least one year and that the patient had been registered with the GP for at least one year. Follow-up in the database started on the first of January 1996 or on the date that one year of valid history was available, whichever date was latest. Follow-up was ended upon transferring out of the practice, the date of last data supply by the GP, occurrence of CRPS, or at the end of the study period, whichever came first. Since GP co-operation was needed for additional data collection, the source population was restricted to practices that were still active in the IPCI database in 2006.

Cases

CRPS cases were retrieved from the electronic records using a string search algorithm on narrative journal text, including Dutch synonyms for CRPS, abbreviations, and obvious spelling errors. Initial case validation occurred with a brief questionnaire to the GP to confirm or reject the CRPS diagnosis as mentioned in the records.[11] Specialist correspondence was obtained if available. Subsequently, with mediation of the GP, confirmed cases were invited by mail for further study participation. Patients who provided informed consent were visited once by the primary investigator, a physician with clinical experience in diagnosing CRPS (MM). Prior to the visit participants had filled a questionnaire addressing CRPS complaints, disease course, treatment, medical history, sociodemographics, daily functioning and quality of life. During the visit the investigator and the patient briefly went over the questionnaire together to ascertain

completeness. Additionally, a physical examination of the affected limb and contralateral extremity was performed to assess signs of CRPS.

CRPS patients were included as cases in the study population if they could be confirmed by the investigator, applying the IASP criteria on the symptom and sign assessments during the visits, combined with information from the GP records and specialist letters. CRPS patients who could not be visited, because they were untraceable or refused participation, were included only if the CRPS diagnosis in the past had been confirmed by a medical specialist (instead of by GP only). The index date was chosen as the date on which the CRPS diagnosis was mentioned in the records for the first time. In this specific study only female patients were included.

Controls

Per case, an unrestricted number of age (year of birth) and gender matched controls was selected from the source population. To be included in the study controls were required to have experienced an injury identical to the one that precipitated the CRPS in their matching case, within a two year band of calendar time. This meant that cases with a fracture were matched to controls with a fracture, cases with a soft tissue injury were matched to controls with a soft tissue injury, etc. The index date in controls was chosen as the date of injury plus the delay time between injury and CRPS in the matching case. If a case had spontaneous CRPS (no precipitating injury), controls were not required to have had an injury either. A subset of the controls (1-3 per case) was invited to participate in assessments similar to the cases (self administered questionnaire and physical examination).

Estrogen exposure

The role of estrogens was explored in various ways: 1. cumulative endogenous exposure 2. current endogenous exposure and 3. current exogenous exposure. Information on these determinants was obtained either from questionnaires or from the medical records.

Cumulative endogenous estrogen exposure until the index date was assessed using patient questionnaire data and included the following measures: age of menarche, age of menopause, menstrual life (in years), parity, cumulative months of pregnancy, and cumulative months of breast feeding. Menstrual life was determined as the age of menopause minus the age of menarche (post-menopausal women) or as the index date minus the age of menarche (pre-menopausal women). Cumulative months of pregnancy was calculated by summing the reported months of all pregnancies experienced during life, including full term, preterm and aborted pregnancies. Similarly, the cumulative months of breast feeding was calculated by summing the months of breast feeding for each child. Since all these measures were derived from the patients' questionnaires, they could only be assessed in cases and controls that had participated to the additional data collection during visits.

Current endogenous estrogen exposure included pregnancy close to the index date. These pregnancies were identified in the IPCI database by a string and ICPC code search in free text and in the diagnosis table. Pregnancy start and duration were derived from the records. If this was not well recorded we assumed 280 days for full term deliveries, 245 days for preterm deliveries, 105 days for late miscarriages, 60 days for early miscarriages,

and 70 days for intended abortions. The association between pregnancy and CRPS onset was studied in the three time windows: current pregnancy: CRPS onset (index date) during a pregnancy; recent pregnancy: CRPS onset within six months after delivery; past/no pregnancy: CRPS onset more than six months after delivery.

Current exogenous estrogen exposure at the index date included the use of oral contraceptive drugs (OC) (in pre-menopausal women), and use of hormonal replacement therapy (HRT) (in post-menopausal women). Information about the drug prescriptions was retrieved from the IPCI database. The prescription records comprised the Anatomical Therapeutical Chemical (ATC) classification code, prescription start date, quantity, strength, indication and prescribed daily dose. All estrogen containing OC were included (ATC codes G03AA and G03AB). For each 21 tablets a we took into account a 28 day exposure period, including a pill free week. HRT included all (oral, vaginal, transdermal) estrogen containing drugs intended for prevention or treatment of negative postmenopausal effects (G03C). For HRT we calculated the duration of a prescription as the quantity of prescribed units (mostly tablets) divided by the daily intake of units. Episodes of use were created by combining consecutive prescriptions and correcting for overlap. A person was classified as current user if the legend duration of the most recent prescription ended less than 30 days before the index date. Past users were subjects with the most recent prescription ending between 30 days and one year prior to the index date. Differentiation between pre- and post-menopausal status at the index date was done by imputing the reported average age of menopause in the visited patients into the unvisited patients.

Covariables

Determinants associated with either CRPS onset or with OC or HRT use were considered as potential confounders. Asthma, migraine, menstrual cycle related disorders (Dysmenorrhea, metro/menorragia, poly/oligomenorrhea) and osteoporosis were increased in CRPS patients during a previous study.[13] In addition to these, smoking, hypertension, cardiovascular disorders, climacterial complaints, breast cancer, and cancer of the female reproductive organs were tested since they might be associated with OC or HRT use. All information regarding potential confounders was derived from the electronic journal texts.

Statistical analyses

Standard comparative statistics were used to determine differences in means (paired t-test) and proportions (χ^2-test). Odds ratios (OR) and 95% confidence intervals (CI) for associations between cumulative or current estrogen exposure and CRPS onset were calculated using conditional logistic regression. Measures of cumulative endogenous estrogen exposure were tested both as continuous variables and as categorical variables. Covariables were included in the final model if they were univariately associated with the exposure or the outcome, or if they altered the odds ratio by more than 10%. All analyses were conducted using the Statistical Package for Social Sciences (SPSS) version 12.0 for windows.

Results

The selection of the study population is displayed in figure 1. The response rate for participation concerning further data collection was 64% in CRPS cases and 28% in controls. Participants were not significantly different from non participants regarding age and medical history. The final study population comprised 143 female CRPS patients (81 validated during a visit + 62 non visited patients with a specialist diagnosis) and 1,493 matched controls (median 12 per case, range 2-53). This study population was used to conduct analyses regarding OC and HRT use and actual pregnancies at the moment of CRPS. Since measures of cumulative estrogen exposure could not be retrieved from the IPCI database, but had to be derived from patient questionnaires obtained during the visits, analyses regarding these determinants included only those individuals that had consented for participation in further data collection. For 53 of the participating cases at least one participating matched control was found. Therefore the analyses on cumulative estrogen exposure were conducted in 53 matched pairs (53 cases and 58 controls).

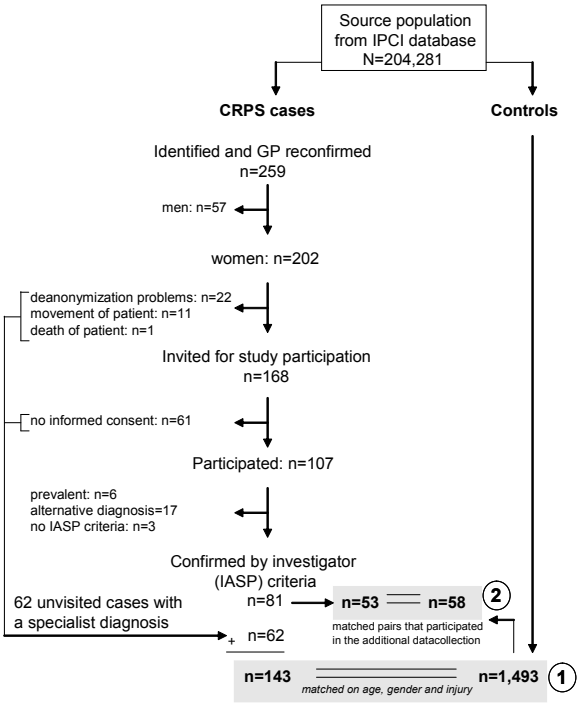

Figure 1. Selection of the study population. **1.**This study population includes matched case-control pairs in the IPCI database. Cases (n=143) were validated during a visit by the investigator (n=81) or were confirmed by a specialist (n=62). Controls (n=1,493) were selected from the database, matched to the cases on age, gender and injury type. This study population was used in the analyses of determinants that were derived from the records in the IPCI database, namely OC and HRT use and pregnancies during follow-up time in the database. **2.**This subset of the study population includes only the matched case-control pairs whereby both the case and at least one control had participated to the additional data collection (visit and questionnaire). This subset was used in the analysis of determinants that were derived from questionnaires, namely cumulative endogenous estrogen exposure.

Characteristics of the study population are displayed in table 1 and measures of cumulative endogenous estrogen exposure in table 2. Within the subset of visited participants (questionnaires available) the average age of menopause onset was 46.6 (sd: 6.6) years for CRPS cases and 47.0 (sd: 6.9) for controls, which was statistically similar. The proportion of post-menopausal patients at the index date was similar between CRPS cases and controls. None of the measures of cumulative endogenous estrogen exposure was associated with CRPS onset, investigated either as continuous or as categorical variables.

Table 1. Characteristics of the study population.

Characteristics	**Cases N=143**		**Controls N=1,493**		**OR^{matched} (95% CI)**
Mean age at CRPS onset (sd)		51 (17)			matched
Female		100%			matched
	n	%	n	%	
Smoking	29	20.3%	249	16.7%	1.3 (0.9-2.1)
Migraine	11	7.7%	54	3.6%	**2.7 (1.3-5.2)**
Asthma	9	6.3%	62	4.2%	1.8 (0.8-3.8)
Osteoporosis	10	7.0%	48	3.2%	**2.5 (1.2-5.3)**
Hypertension	25	17.5%	231	15.7%	1.1 (0.6-1.7)
Cardiovascular disorders	12	8.4%	116	7.8%	1.2 (0.6-2.3)
Venous thrombosis	2	1.4%	3	0.2%	n.a.
Menstrual cycle related disorders	11	7.7%	88	5.9%	1.8 (0.9-3.7)
Climacterial symptoms	13	9.1%	95	6.4%	1.6 (0.9-3.2)
Breast cancer	1	0.7%	13	0.9%	0.7 (0.1-5.7)
Cancer female reproductive organs	0	0%	1	0.1%	n.a.

^{matched} Matched on gender, year of birth, calendar time (2 year band), and type of injury.

Table 2. Cumulative endogenous estrogen exposure in CRPS case patients and controls.

Endogenous estrogen exposure	Cases N=53	Controls N=58	OR[matched] (95% CI)
Post-menopausal at index date	60%	59%	1.3 (0.3-4.8)
Mean age of menarche (sd)	12.7 (2.4)	13.2 (1.7)	0.9 (0.7-1.1)
Categories:			
<12yr	22%	14%	1.9 (0.6-5.8)
12-14yr	40%	47%	reference
>=14yr	38%	40%	1.2 (0.5-2.7)
Mean age of menopause (sd)†	46.6 (6.6)	47.0 (6.9)	1.0 (0.9-1.1)
Categories:			
<40yr	15%	14%	0.9 (0.2-3.8)
40-50yr	47%	45%	reference
>=50yr	37%	41%	0.8 (0.3-2.3)
Mean cumulative years of menstrual life (sd)	30.8 (9.4)	30.1 (9.8)	1.0 (1.0-1.1)
Categories:			
<25yr	23%	24%	0.7 (0.1-3.0)
25-35yr	40%	35%	reference
>=35yr	37%	41%	0.7 (0.3-2.0)
Mean parity (sd)	1.8 (1.3)	1.9 (1.2)	0.9 (0.6-1.3)
Categories:			
0	23%	17%	1.8 (0.5-6.3)
1-2	56%	57%	reference
>2	21%	26%	0.6 (0.2-1.6)
Mean cumulative months pregnancy (sd)	17.6 (14.5)	17.7 (12.4)	1.0 (1.0-1.0)
Categories:			
<12 months	30%	33%	0.8 (0.3-2.0)
12-24 months	49%	38%	reference
>=24 months	21%	29%	0.4 (0.1-1.2)
Mean cumulative months breast feeding (sd)	5.3 (7.8)	3.8 (5.4)	1.0 (0.9-1.1)
Categories:			
0 months	45%	43%	1.5 (0.6-3.3)
1-12 months	34%	45%	reference
>=12 months	21%	12%	2.0 (0.4-8.1)

[matched] Matched on gender, year of birth, calendar time (2 year band), and type of injury.

† Calculated in post menopausal women only (38 cases and 42 controls).

All data on determinants extracted from patient questionnaires, which were obtained only for a small subset of patients that was visited by the investigator.

When extrapolating the mean menopausal age from the questionnaires to the study population in the database, 44 cases (573 controls) were pre-menopausal at the index date and 99 cases (920 controls) were post-menopausal. In pre-menopausal patients neither current (OR: 1.0; 95%CI: 0.5-2.0) nor past (OR: 1.0; 95%CI: 0.3-3.2) use of OC was associated with CRPS onset (table 3). In post-menopausal women current HRT use was less prevalent in CRPS patients than in controls, but this was not statistically significant (OR: 0.5; 95%CI: 0.2-1.8) (table 3).

CRPS did not occur during pregnancy, therefore the association could not be calculated, but comparison with the controls suggests a protective effect. Two of the visited patients had reported in the questionnaire to have become pregnant after CRPS onset: one of them had experienced an improvement of CRPS during the pregnancy, while the other had noted no change whatsoever. The risk of CRPS was increased in the first six months after delivery (OR: 5.6 95%CI: 1.0-32.4), but the actual numbers were low (table 4).

Table 3. Estrogen therapy and the risk of CRPS.

OC use (in pre-menopausal patients)	**Cases N=44**		**Controls N=573**		**OR^{matched} (95% CI)**	**OR^{adj1} (95% CI)**
	n	**%**	**n**	**%**		
No use	24	54.5%	330	57.6%	reference	reference
Ever use (in prior year)	20	45.5%	243	42.4%	1.0 (0.5-1.9)	0.9 (0.5-1.8)
Current use	16	36.4%	189	33.0%	1.0 (0.5-2.0)	0.9 (0.4-1.8)
Past use	4	9.1%	54	9.4%	1.0 (0.3-3.2)	1.0 (0.3-3.2)
HRT use (in post-menopausal patients)	**Cases N=99**		**Controls N=920**		**OR^{matched} (95% CI)**	**OR^{adj2}** (95% CI)
	n	**%**	**n**	**%**		
No use	90	90.0%	827	89.9%	reference	reference
Ever use (in prior year)	9	9.1%	93	10.1%	0.9 (0.4-2.0)	0.9 (0.4-1.8)
Current use	3	3.0%	56	6.1%	0.5 (0.2-1.8)	0.5 (0.1-1.6)
Past use	6	6.1%	37	4.0%	1.6 (0.6-4.2)	1.4 (0.5-3.7)

[matched] Matched on gender, year of birth, calendar time (2 year band), and type of injury.
[adj1] Adjusted for migraine, osteoporosis, smoking and menstrual cycle related disorders.
[adj2] Adjusted for migraine, osteoporosis and hypertension.
OC= oral contraceptive drugs, HRT=hormonal replacement therapy

Table 4. Pregnancy and the risk of CRPS

Time since last pregnancy (in pre-menopausal patients)	Cases N=44		Controls N=573		$OR^{matched}$ (95% CI)
	n	%	n	%	
>6 months or never pregnant	42	95.5%	561	97.9%	reference
Current pregnancy	0	0%	8	1.4%	n.a.
0-6 months since partus	2	4.5%	4	0.7%	**5.6 (1.0-32.4)**

[matched] Matched on gender, year of birth, calendar time (2 year band), and type of injury.

Discussion

We studied the association between estrogen exposure and the risk of CRPS. Cumulative endogenous estrogen exposure was not associated with CRPS onset. In pre-menopausal patients OC use was not associated with CRPS, while in post-menopausal women we observed a non-significant protective effect during current HRT use. Although based on small numbers, the risk for CRPS seemed decreased during pregnancy and was increased during the first six months after delivery.

Measures of cumulative and actual estrogen exposure have to our knowledge not been studied before in relation to CRPS, which makes it impossible to discuss our results in view of previous findings. However, estrogen exposure has been studied in relation to other in inflammatory disorders, for example in rheumatoid arthritis (RA) and multiple sclerosis (MS). In addition to the profound female predominance these disorders share clinical features with CRPS, such as inflammatory signs, pain and functional impairments. Moreover, the CRPS prevalence in a cohort of MS patients was high compared to estimated general population prevalences, suggesting a possible connection between CRPS and MS.[26]

Similar to our present findings for CRPS, studies in RA and MS patients commonly revealed no association with parity[27-30] and menstrual life[27,29], although breast feeding was protective for RA in one study.[29] The association between OC use and both RA[31] and MS[30, 32, 33] is still controversial and due to large heterogeneity between study populations meta-analyses could not provide a final answer.[31] Post-menopausal HRT use was only non- significantly protective for RA.[34] In line with the observations in RA and MS studies, we did not observe a strong association between OC or HRT use and the onset of CRPS either, although due to power limitations we can not exclude a potential mild association. This is especially the case for HRT use, where the non-significant results point into the direction of a protective effect. Regarding OC use, the actual OR for current use is 0.9, which is suggestive of a non-association. It has been suggested before that OC affect

endogenous estrogen serum levels only to a minor extent and therefore can, if any, only play a limited role in the underlying mechanisms of immunological disorders.[16]

During pregnancy the risk for both RA[35,36] and MS[30] is known to decrease, while post partum incidences are increased for both diseases. Hyperprolactinemia[37] and relative hypocortisolism[38] have been hypothesized to (partially) underlie this observation, since they both contribute to a pro-inflammatory immune status. Remarkably, we observed a similar pattern for CRPS, with a lower risk during pregnancy and a higher risk during the first months after, although it has to be noted that this was based on small numbers.

Estrogens interact with many modulators of the immune system, including immune cells, cytokines, growth factors, transcription factors and more.[16] However, the precise role of estrogens is rather complex. Estrogens can induce predominantly anti- or pro-inflammatory responses, dependent of the cell type, kind of trigger, timing, estrogen receptor type and other circumstances.[16] Although the relevance of estrogens in immunologic diseases is reflected in the demographic patterns (female predominance, age distribution), it has been difficult to demonstrate clear associations between such diseases and estrogen exposure parameters. Because of the biologic complexity, high powered prospective studies within homogeneous populations are needed to uncover potential associations. For RA this has already been proven complicated[31], and for a rare disease with a heterogeneous clinical presentation as CRPS it will be an even bigger challenge.

Apart from power issues, other limitations may apply to our study, such as misclassification of the outcome (CRPS diagnosis), the determinants (estrogen exposure), and confounding. Outcome misclassification may have been caused by the retrospective assessment of CRPS, which is usually diagnosed by its clinical presentation (IASP criteria). However, our case validation strategy was extensive and multiple sources for diagnostic information were used. Regarding exposure misclassification, the case/control status is known to potentially influence recall of past events. However, we have no reason to believe that CRPS affects the recall on measures of estrogen exposure as how they were addressed in the questionnaires. Therefore, causing only non differential misclassification, recall problems will not likely have biased our results, although a potential minor association might have been diluted to such an extent that we have missed it. Recall was not an issue in the retrieval of OC and HRT use from the IPCI database, as this had been prospectively registered by the GP, who is the common prescriber for OC and HRT. Confounding may have been caused by factors that are associated with both CRPS and estrogen exposure. For the most common risk factors of CRPS, namely age, gender and injury, we corrected by through matching. Other potential confounders were included as co-variables. However, in the analyses for OC use and HRT we were not able to correct for measures of cumulative estrogen exposure, because these were only available for the small subset of visited patients.

To our knowledge we performed the first study addressing the association between estrogen exposure and CRPS. A strength of our study is that we were able to compare the findings in CRPS patients to a control group that was matched on injury, assuring an equal baseline CRPS risk for cases and controls. This detailed matching procedure was possible due to the large number of available controls in the IPCI database. Another

strong point is that part of the data on determinants (OC, HRT, pregnancy) had been prospectively registered by the GP, making information bias unlikely. Moreover, our study was performed in a population based setting, which means that the results are representative of CRPS patients in general, in contrast to hospital based studies that usually represent a subset of severe patients.

In conclusion, we found no association between cumulative endogenous estrogen exposure and CRPS, and neither between OC or HRT use and CRPS. However, because of limited power, minor associations cannot be excluded and larger, preferably prospective studies are needed to draw more solid conclusions. Based on the small numbers, a decreased risk of CRPS during pregnancy and an increased risk immediately after pregnancy was observed, which is in line with patterns observed in RA and MS.

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Chapter 4

The role of NFκB

4.1 Applied information retrieval and multidisciplinary research

New Mechanistic Hypotheses in Complex Regional Pain Syndrome

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Abstract

Introduction*:* collaborative efforts of physicians and basic scientists are often necessary in the investigation of complex disorders. Difficulties can arise, however, when large amounts of information need to reviewed. Advanced information retrieval can be beneficial in combining and reviewing data obtained from the various scientific fields.

Methods*:* In this paper, a team of investigators with varying backgrounds has applied advanced information retrieval methods, in the form of text mining and entity relationship tools, to review the current literature, with the intention to generate new insights into the molecular mechanisms underlying a complex disorder. As an example of such a disorder the Complex Regional Pain Syndrome (CRPS) was chosen. CRPS is a painful and debilitating syndrome with a complex etiology that is still unraveled for a considerable part, resulting in suboptimal diagnosis and treatment.

Results: a text mining based approach combined with a simple network analysis identified Nuclear Factor kappa B (NFκB) as a possible central mediator in both the initiation and progression of CRPS.

Discussion: the result shows the added value of a multidisciplinary approach combined with information retrieval in hypothesis discovery in biomedical research. The new hypothesis, which was derived in silico, provides a framework for further mechanistic studies into the underlying molecular mechanisms of CRPS and requires evaluation in clinical and epidemiological studies.

Introduction

Early in the history of western medicine, the physician and the basic scientist were one and the same person. However, over the past century, clinical research developed as a separated branch from the basic sciences such as biology, molecular biology, biochemistry, and physiology. The main objective of clinical research is the collection and analysis of clinical data concerning symptoms of the disorders and responses to treatments. Based on these observations new theories about etiology and pathogenesis can be developed. However, detailed information regarding the molecular mechanisms underlying a certain disease process often remains elusive. One avenue into possible mechanisms of complex disorders is through the use of bioinformatics. Bioinformatics applies informatics techniques to organize bio-molecular data on a large scale.[1]

The combination of bioinformatics and biomedical approaches is expected to result in significant advantages in both understanding mechanisms of disorders and individual susceptibility, which in turn will open many possibilities in individualized medical health care.[2] Infobiomed is a Network of Excellence funded by the European Union that aims at enforcing European biomedical informatics as an integrative discipline.[3] One of the main objectives of Infobiomed is to enable pilot applications in several medical fields that demonstrate the benefits of a synergetic approach in biomedical informatics. An example of such a multidisciplinary project is the use of bioinformatics tools in the investigation of the relationship between clinical and molecular data. Of course, the current literature contains information from these different domains. However, the amount of information has become so large that it is very difficult for a single individual to draw conclusions across the various disciplines. Literature based discovery support tools have been developed to bridge these interdisciplinary gaps, and novel scientific hypotheses have been generated and tested.[4,5] This approach, wherein the clinician and the basic scientist collaborate, should be beneficial in the investigation of complex disorders, where clinical research alone is not sufficient to unravel the entire disorder process.

Case studies can be useful in exploring new ways to advance multidisciplinary biomedical research. The Complex Regional Pain Syndrome (CRPS) is an example of a complex disorder from which the etiology and pathogenesis remain unelucidated for a considerable part, despite intensive research in the medical field. For this reason CRPS was chosen as a case study on how text mining techniques could be used in multidisciplinary biomedical focused research. The results should not be regarded as answers to the long unsolved questions regarding CRPS, but rather as hypothetically new insights in the molecular mechanisms underlying the disorder. The main purpose of this exercise was to assess the benefit of a new approach on hypothesis discovery, based on the use of text mining tools by a multidisciplinary team of researchers.

A brief introduction will be provided on the disorder CRPS in the next section, including a short description of the current theories about its pathogenesis.

The Complex Regional Pain Syndrome

CRPS is a painful syndrome affecting one or more extremities of the body, marked by a wide variety of symptoms. The most prominent feature is pain, including spontaneous pain, allodynia, hyperpathia, and hyperalgesia. Additionally, the affected extremity can display changes in color and/or temperature (vasomotor disturbances), edema, alterations in transpiration, hair and nail growth (sudomotor disturbances), and muscular atrophy and/or dysfunction (motortrophic disturbances).[6,7] It is usually described after a specific initiating event, in most cases a trauma or an operation, but sporadically it is observed after a stroke, myocardial infarction, infection or even without an obvious inciting event in a rarity of the cases.[8] The course of the disorder varies from patient to patient, but often ends in diminished function of the affected limb which impacts the quality of life of the patient. In rare cases, the disorder progresses to the point where amputation is necessary.

The pathogenesis of CRPS evolves from disturbances in both the peripheral nervous system (PNS) and the central nervous system (CNS) (figure 1). Regarding the initial phase of the disorder, recently the interest has increased towards the role of inflammatory responses. Inflammatory signs such as swelling, redness, warmth and pain are common features in the early stage of CRPS. Classic inflammation is marked by the presence of proinflammatory cytokines and in CRPS a local increase of the cytokines TNFα, IL-6 and tryptase (a product of mast cell degranulation) was observed in blister fluid derived from the affected extremity. [9,10] IL-1 and IL-6 were also found to be increased in spinal fluid.[11] Additional to classic inflammation, a process called neurogenic inflammation has been demonstrated in CRPS.[12-16] Neurogenic inflammation resembles classic inflammation, but it is initiated by neuropeptides instead of lymphocytes and cytokines.[17] Those neuropeptides include Substance P (SP), Calcitonin Gene Related Protein (CGRP), neuropeptide Y (NPY), Bradykinin (BK) and Vasoactive Intestinal Protein (VIP). Important modulators of neurogenic inflammation are Neutral Endopeptidase (NEP) and Angiotensin Converting Enzyme (ACE).[18]

Endothelial dysfunction, hypoxic changes, and free radical damage have also been suggested as important processes in the pathogenesis of CRPS.[19-23] Ischemia, together with inflammation, can result in the formation of free radicals, lactate acidosis and altered nitric oxide (NO) synthesis. The NO metabolism in its turn has an effect on the microcirculation and thus can influence peripheral oxygen supply.[24] An impaired microcirculation might underlie a cold extremity that is observed in some cases of CRPS.

In time, the peripheral alterations give rise to disturbances at the level of the CNS, and the clinical picture of CRPS evolves more towards that of a neuropathic pain syndrome. The mechanisms behind the development of neuropathic pain have been investigated extensively in animal models.[25,26] The pathogenesis of neuropathic pain is marked by a phenomenon called neural plasticity, which is described by Woolf and Salter as the capacity of central and peripheral neurons to change their function, chemical profile, and their structure in reaction to activation of peripheral afferent nerve endings.[27] This conducts towards a state of hyperexcitability of the peripheral C- and A-fiber transducers, and of the neurons in the dorsal root ganglia (DRG) of the CNS, referred to as peripheral and central sensitization respectively. Sensitization results in a painful response to

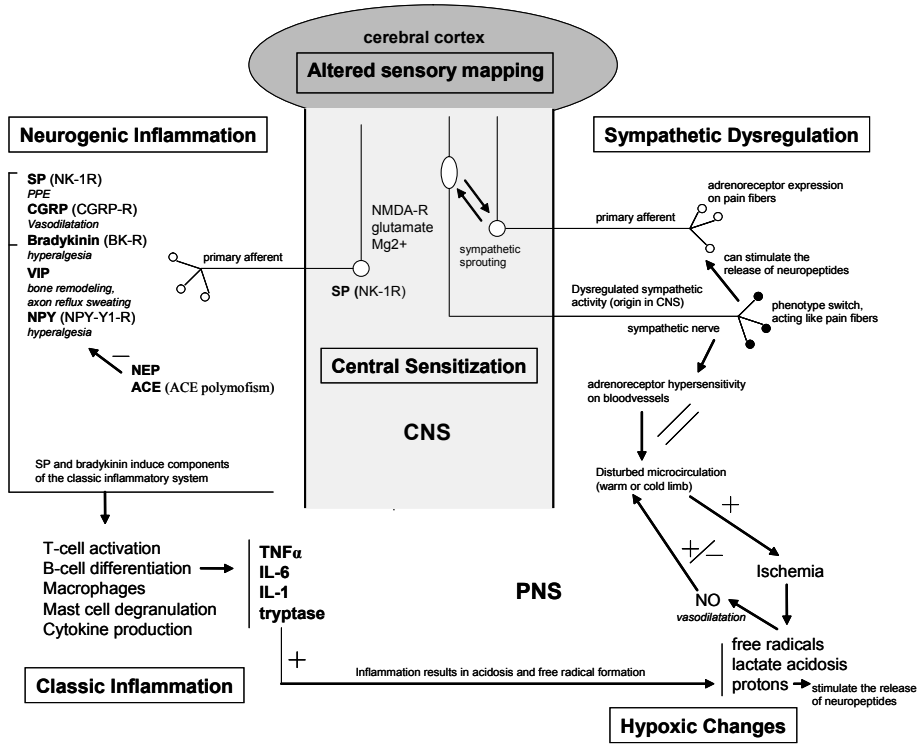

Figure 1. The four pathogenic mechanisms in CRPS and their interactions. SP=substance P, CGRPS=Calcitonin Gene Related Protein, VIP=Vasoactive Intestinal Protein, NPY=Neuropeptide Y, NPY-Y1-R=Neuropeptide Y-Y1 receptor, NEP=Neutral Endopeptidase, ACE=Angiotensin Converting Enzyme, SMP=Sympathetically Maintained Pain, NO=nitric oxide, iNOS=inducible nitric oxidase, ONOO=peroxynitrite, NMDA-R=N-methyl-D-Aspartate receptor, NK1-R=Neurokinin-1 receptor, CNS=central nerve system, PNS=peripheral nerve system.

a normally not painful stimulus, leading to features as allodynia and hyperalgesia. A prominent factor in the evolvement of sensitization is the interaction between glutamate and the N-methyl-D-aspartate (NMDA) receptor, from which functional status Mg2+ is a modulator[27]. Following central sensitization, alterations at the supraspinal level might evolve, resulting in an altered sensory mapping in the cerebral cortex. This pays an additional contribution to the sensational disturbances in chronic CRPS.[28]

Dysregulation of the sympathetic nerve system was classically supposed a main feature of CRPS. This was based on the observation that pain relief could be obtained by performing a sympathectomy, although only in a subset of CRPS patients. Vasomotor disturbances (warm or cold limb)[29] and sympathetically maintained pain (SMP)[30-33] are features that have been ascribed to sympathetic dysregulation. The painful responses may result from the expression of α-adrenoreceptors on sensory fibers.[33] Due to sympathetic

sprouting new communication pathways are formed between sympathetic terminals and sensory neurons[34]. Additionally, the α-adrenoreceptors might have developed a hypersensitivity for normal stimulation by cathecholamines.[29]

The authors have decided in this paper to focus mainly on the peripheral processes in the initial phase of CRPS. The first reason for this is that it is very feasible that the neuropathic pain component of CRPS does not form the initial pathogenesis of the disorder, but that it is preceded and sustained by the presence of the peripheral inflammatory and hypoxic reactions in the affected extremity.[34] The second reason is that, until now, biomolecular research on CRPS in humans and animals concerned mostly the peripheral inflammatory aspect of the disorder. Sensitization and neuronal plasticity have been studied broadly in models for neuropathic pain in general. However, they have never been demonstrated in models for CRPS in particular, although is it highly reasonable to assume that they occur in CRPS in a similar manner.

Methods

The Infobiomed Network of Excellence is organized into various work packages with different aims. The aim of the so called "pilot applications" is to analyse the impact of biomedical informatics in specific fields (Pharmainformatics, Genomics and Microbiology, Genomics and Chronic Inflammation, and Genomics and Cancer). The team of researchers behind the study outlined in this paper was part of the pilot application Pharmainformatics, which aims at assessing the mutual impact of BMI and pharmaceutical research. Research in this area focuses on establishing the information continuum pathology – pathway – target – ligand.

The pathogenesis of CRPS was one of the subjects that were chosen for a case study. The purpose was to investigate how to gain further insight into pathogenesis behind a complex disease and to identify possible pathways, targets and ligands for improving pharmacological therapy, using a multidisciplinary approach. The team of researchers had varying backgrounds mirroring the biomedical informatics research area (one physician with domain expertise on CRPS that was asked to participate in the work package only for this specific case study; one bioinformatician; one specialist in text mining). No formal leader was chosen for the group. All researches provided input based on their background and a plan of action took form by mutual agreement. The physician provided biological and medical concepts that are possibly linked to CRPS, the bioinformatician provided the appropriate software tool to be used in the study, and the text mining expert provided knowledge on how to best perform the extensive literature analysis. The analysis took place in September 2004, at the premises of AstraZeneca in Molndal, Sweden.

The selection of the CRPS related concepts was based on current (but not always objectified) opinions about the pathogenesis and treatment of CRPS and on different pathogenic mechanisms described in a selection of articles concerning the pathogenesis of CRPS.[32,35-38] The focus was on chemical and biochemical identities and mainly, but not completely, on the peripheral components of the disorder. The collection of concepts and their synonyms can be found in Table 1.

The text mining/entity relationship tool PathwayAssis™ (Version 2.5) was used to visualize the connections between the CRPS concepts in a network and to search for new concepts taking part within these relations. PathwayAssist is a software application developed for navigation and analysis of biological pathways, gene regulation networks and protein interaction maps. It has been used before in studies to identify genes that are involved in autism[39] and in regulation of human primordial follicle development.[40] The application has been described in more detail by Nikitin and colleagues.[41] PathwayAssist finds connections between concepts, henceforth referred to as 'nodes', by searching through a database of interactions derived from literature, using the natural language processing (NLP) based software MedScan. MedScan performs a grammatical and semantic analysis of the complete Medline database of life sciences and biomedical bibliographic information. The MedScan software has been described by Novichkova et al.[42] and more recently by Daraselia et al.[43] A brief overview of the technology is presented below.

When parsing a Medline document, a semantic interpreter of the NLP component transforms the syntactic structure into a semantic structure. The syntactic structure and main constituents (surrounded by square brackets) of a sentence can be exemplified using the general sentence *Protein X inhibits protein Y*, which has the syntactic structure of $[[Protein\ X_{[N]}]_{[NP]}\ [inhibits_{[V]}\ Protein\ Y_{[N]}]_{[VP]}]_{[S]}$. The phrasal category is shown immediately following each constituent (**NP** designates noun phrase, **N** designates noun, **V** designates verb, **VP** designates verbal phrase, **S** designates sentence). The syntactic structure will be transformed to a semantic frame of inhibition that has an 'agent' protein X and a 'patient' protein Y. The output of the semantic parse is the input for an ontological analysis that was developed by Daraselia et al.[43] In this an 'entity' is represented either as a protein, a cellular object, a cellular process, or a small molecule and 'controls' describe functional relationships between these entities. Relations between entities are stored in a relational database. These relations can be displayed and explored through a graphical interface.

The list of concepts provided by the physician was regarded as current knowledge about CRPS (table 1) and was used as input for PathwayAssist. PathwayAssist views these concepts as nodes. The option "find only direct interactions between selected nodes" was used to search the underlying interaction database for direct connections between the CRPS concepts. Based on these connections, the system builds a network. This procedure would only detect *direct* relationships between two nodes; mechanisms that require a new intermediate node (a node not present in the original list of nodes that was provided as input) would not be detected. Such intermediate nodes, however, might represent knowledge that the scientists entering the concepts were not aware of at the time of input (such as factors acting as enhancers for a critical step in a pathway, or co-factors needed for a transcription factor to bind to DNA). Therefore, an option was used that incorporates a new node in the network if that node is the intermediate node that allows the creation of a triplet connecting two input nodes. When a new node is added to the network, all links to and from that node to all other nodes in the network are displayed.

Table 1. Concepts used when building the CRPS network in PathwayAssist (description in parenthesis).

Node type	Node name
Complex	Neuronal acetylcholine receptor
Functional Class	NMDA receptor (N-methyl-D-aspartate receptor)
Protein	CALCA (calcitonin/calcitonin-related polypeptide, alpha), IL1A (interleukin 1 alpha), IL6 (interleukin 6), NGFB (nerve growth factor, beta polypeptide), NGFG (nerve growth factor, gamma subunit), NPY (neuropeptide Y), PTGS1 (prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)), TAC1 (tachykinin, precursor 1 (substance K, substance P, neurokinin 1, neurokinin 2, neuromedin L, neurokinin), TACR1 (tachykinin receptor 1), TNF (tumor necrosis factor), BDKRB2 (bradykinin receptor B2), PTGS2 (prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)), NTRK1 (neurotrophic tyrosine kinase, receptor, type 1), TRPV1 (transient receptor potential cation channel, subfamily V, member 1), Ngfa (nerve growth factor, alpha), BDK (Bradykinin), VIP (vasoactive intestinal peptide)
Small Molecule	glutamate, DMSO (Dimethyl sulfoxide), PGE2 (Prostaglandin E2), magnesium, noradrenaline, capsaicin, glucocorticoid, mannitol, pentoxifylline, naproxen, bisphosphonate, verapamil, morphine, ketamine, amitriptyline, clonidine, carbamazepine, nortriptyline, lidocaine, GABA (gamma-aminobutyric acid), ketanserin, infliximab, gabapentin, amantadine, lioresal, benzodiazepine, Baclofen, N-acetyl-cystein

Results

The resulting network based on the imported CRPS concepts is shown in Figure 2. In addition to the original concepts that were provided as input nodes, six new nodes have been added by the algorithm (nodes that were not in the original list of concepts known to be associated with CRPS). Of these six new nodes, the node NFκB was the one connecting to most of the original CRPS concepts and was pivotal in the final network. NFκB appeared as new node because it was part of a triple connection between mannitol and TNFα with the actual sentence being 'High glucose or mannitol also enhanced TNFalpha-stimulated NF-kappaB activity': TNFα stimulates NFκB, and mannitol acts as an enhancer of this process. The sentence describing this particular relationship between mannitol, TNFα, and NFκB had been parsed from the article by Hattori et al.[44]

The transcription factor NFκB is known as a mediator in many different physiological processes.[45,46] It is also related to 7% of all the nodes that exist in PathwayAssist, which mirrors its versatility. However, even though NFκB has a very high connectivity in PathwayAssist and is involved in many physiological processes, the calculated p-value

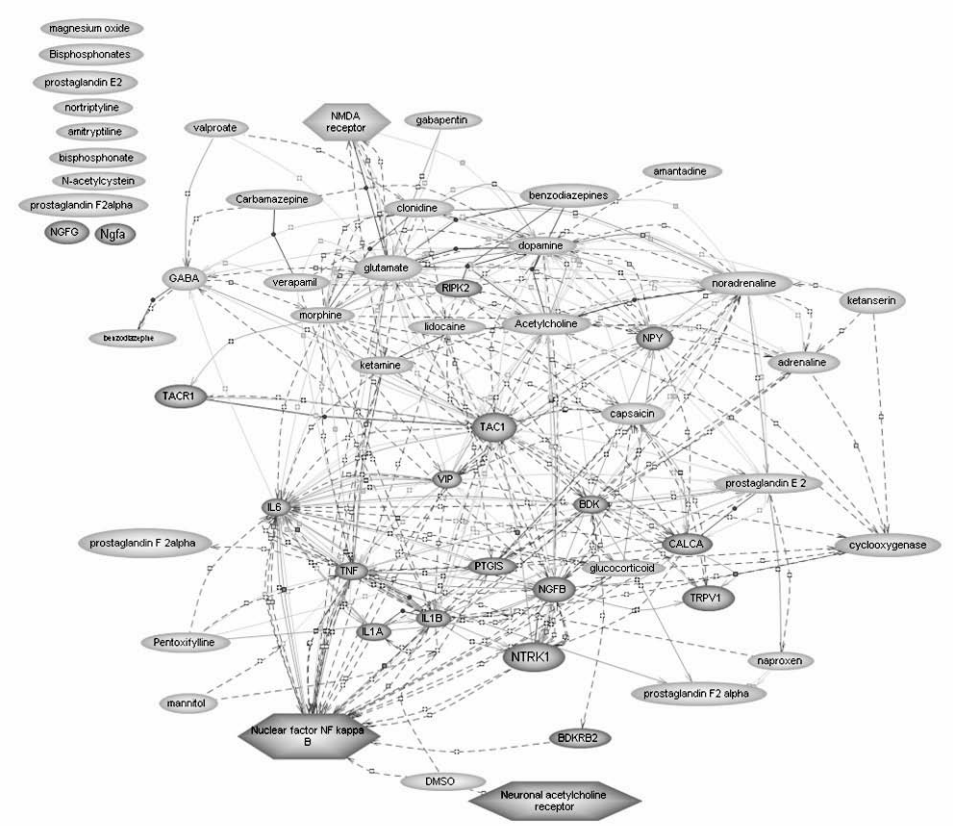

Figure 2. Resulting network of CRPS concepts from PathwayAssist. The PathwayAssist network shows that NFκB is connected with many concepts that are related to CRPS. Red circles denote proteins, red hexagons denote protein complexes, green circles denote small molecules, orange circles denote enzymes, and orange hexagons denote protein functional classes. Concepts for which PathwayAssist could not find a link to any other of the concepts in the network are shown in the upper left.

using a one-tailed Chi-square test with Yates's correction was highly statistically significant (p-value less than 0.0001). This demonstrates the significance of NFκB in the CRPS network in comparison to the whole PathwayAssist database of interactions.

To investigate the possibility that NFκB appeared in the network simply because it is a highly connected node in the database, the in PathwayAssist equally highly connected node "protein tumor protein p53" was manually included into the network and tested for its connectivity with the CRPS concepts. The p-value was found to be equally significant as for NFκB (p-value less than 0.0001). The reason for TP53 not appearing in the expanded CRPS network is related to on the algorithm that was chosen to find new nodes. In order for a new node to be incorporated into the network by PathwayAssist it has to be part of a triplet that connects two input nodes. This was the case for NFκB but not for TP53. There are also other algorithms available in PathwayAssist for incorporating new nodes in a network. These include an algorithm to find the shortest paths between the nodes in the network, an algorithm to find common targets, an algorithm to find common regulators, and also an option to expand the network by finding all nodes in the database that are connected to the nodes in the network. The shortest paths algorithm yielded 20 new nodes, the common target algorithm 46 new nodes and the common regulators algorithm yielded 38 new nodes. The option of finding all nodes connected to the nodes in the CRPS network (the expanded network algorithm) yielded 3872 nodes and it was considered to be practically infeasible to analyze all these nodes separately for their connectivity in the CRPS network. Neither NFκB nor TP53 were found by using any of the other algorithms except for the expand network algorithm. Thus, it might be possible that NFκB emerged in the CRPS network simply because it is a highly connected node in the whole PathwayAssist database. However, NFκB was still connected to more nodes in the CRPS network than the reference node TP53. Therefore it was still regarded as a candidate for a key role in the pathogenesis of CRPS

As far as the authors of this paper know, NFκB has never before been mentioned together with CRPS. For this reason NFκB was considered interesting enough to be explored further by manual research of literature, using advanced PubMed queries concerning the relation between NFκB and the CRPS concepts. Based on the results of this literature research, and on discussions about this topic within the Pharmainformatics group of the Infobiomed network of excellence, theories for the involvement of NFκB in the described pathogenic mechanisms in CRPS were developed. A summary of these theories is described table 2 and further explained below.

Role of NFκB in CRPS related mechanisms

NFκB involvement in neurogenic and classic inflammation

NFκB has been demonstrated to be an essential transcription factor in the mediation of the effects of neuropeptides that are also involved in CRPS: 1. SP induced expression of IL-6 and TNFα (cytokines that are locally increased in CRPS) is regulated by NFκB[47,48]; 2. glutamate, released in the dorsal horn together with SP induces neuronal apoptosis through NFκB induction[49]; 3. expression of CGRP (an important neuropeptide in CRPS) is induced by an NFκB mediated pathway, initiated by IL-1β stimulation[11]; 4. bradykinin activates NFκB and cyclooxygenase-2 via more than one pathway[50,51]; 5. VIP inhibits

Table 2. Key concepts and their relations to CRPS and NFκB.

Key concept	Relation to CRPS	Relation to NFKB
Substance P	Locally elevated upon electrical C-fiber stimulation	Induces NFκB mediated release of IL-6 and TNFα
CGRP	Systemically and locally elevated	Suppresses NFκB activity in thymic cells
Bradykinin	Systemically elevated	Activates NFκB
VIP	Systemically elevated Locally decreased	Inhibits NFκB mediated chemokine production by macrophages. Prevents NFκB binding to promoter site for NO
Neuropeptide Y	Systemically elevated	NPY-Y1-R expression is regulated by NFκB
NEP	Speculated to be decreased	No relation with NFκB described
ACE	Speculated to be decreased Polymorphism in CRPS patients	Reduces NFκB activity
TNFα	Locally increased	Induced by NFκB, Activates NFκB
IL-1β	Locally increased	Stimulates NFκB mediated apoptosis in sympathetic neurons
IL-6	Locally increased	Induced by NFκB
Tryptase	Locally increased	Induced by NFκB
Free radicals	Signs of free radical damage	Second messenger in NFκB activity
Nitric oxide	Elevated after monocyte stimulation	NO reduces NFκB activity, but ONOO induces NFκB
Protons (acidosis)	Lactate increased in skin	Influences NFκB activity
α-receptor	Up-regulated in analgesic skin	Pro-inflammatory responses mediated through NFκB
Sympathetic neuron	Speculated to be damaged in CRPS	NFκB mediates Il-1β induced apoptosis
NMDA receptor	Role in development of central sensitization	NFκB is involved in the up-regulation of some types of NMDA receptors

CGRP=calcitonin gene related protein, VIP=vasoactive intestinal protein, NEP=neutral endopeptidase, ACE=angiotensin converting enzyme, TNF=tumor necrosis factor, IL=interleukine, NMDA=N-methyl-D-aspartate, NO=nitric oxide, NPY-R=neuropeptide Y receptor.

NFκB mediated chemokine production by macrophages[52] and it also prevents NFκB from binding to the promotor site for nitric oxide synthethase iNOS, thereby affecting the microcirculation in CRPS[53]; 6. for the NPY receptor, potential NFκB binding sites have been found in the promoter regions, suggesting a role for NFκB in the expressional regulation of this receptor[54]; 7. the important inflammatory modulator ACE reduces NFκB activity.[55] The overall effect appears to be that NFκB activity is upregulated by the neuropeptides involved in CRPS, resulting in a pro-inflammatory response mediated through NFκB.

NFκB involvement in hypoxic changes

NFκB activity is inhibited by NO.[44,56,57] However, peroxynitrite (ONOO), which is formed from NO after reaction with radical oxygen intermittents, sustains NFκB activity.[44] NFκB also is involved in the expression of inducible nitric oxide synthethase (iNOS) induced by pro-inflammatory cytokines TNFα and IL1-β , which are locally elevated in CRPS.[9,58] Additionally, NFκB activity is involved in and affected by free radical formation[49,59,60] and acidosis.[21,61,62]

NFκB and neuropathic pain

Several animal models demonstrate the role of NFκB in pain induction and pain maintenance in the CNS. For example, NFκB is involved in the upregulation of some types of NMDA receptors which are important mediators in the development of central sensitization.[63] Additionally, NFκB in the CNS mediates IL-1 induced COX-2 up-regulation and prodynorphin expression.[64,65] The derived compound dynorphin is an endogenous opiate that causes hyperalgesia and allodynia in mice. In line with this, intrathecal (in the central spinal fluid) or neuronal injected NFκB inhibitors attenuate proinflammatory cytokine mediated pain in rats.[66,67]

NFκB involvement in sympathetic dysregulation

Pro-inflammatory responses induced by the catecholamines derived from the sympathetic nervous system, which might be dysregulated in CRPS, are mediated by NFκB.[68-70] Additionally, NFκB up-regulates directly the expression of the β-adrenoreceptor on immune cells.[71] Moreover, the expression of the α-receptor might be regulated by NFκB in an indirect way which involves Il-1β mediated apoptosis of sympathetic neurons, leading to up-regulation and/or hypersensitivity of the peripheral α-receptors expressed on blood vessels, immune cells, and nociceptive primary afferents.[72]

Discussion

This paper describes how a multidisciplinary team of investigators applied advanced information retrieval methods, in the form of a text mining/entity relationship tool, with the purpose of discovering new hypotheses concerning the pathogenesis of a complex disorder, exemplified here by CRPS. The exercise should be regarded as a “journey” to discover what benefit could emerge from this kind of collaboration between bioinformatics experts and clinical experts. The purpose was neither to assess the specificity of the used methodology, nor to discover complete underlying biochemical pathways.

The text mining/entity relationship tool PathwayAssist provided a new concept, named NFκB, which is related to a majority of the concepts described to be involved in CRPS. After manual literature search, NFκB appeared to be a link between the various, previously described pathogenic mechanisms and appeared to serve as a mediating component in their connections.

Validity of the method

The MedScan system has been manually validated by the developers through random extraction and analysis of direct physical protein-protein interactions from MEDLINE.[43] From the interactions extracted by MedScan, 91% was correct (precision). Most errors were attributed to the extracted functional interference between proteins rather than physical interaction. Coverage (recall), however, was low: 21%. This is primarily due to the low coverage rate of the NLP component (34%). The concept network on CRPS (figure 2) was validated manually by the authors of this paper by checking all relations leading to or from NFκB. Out of 38 relations, 34 were correct and 4 incorrect.

Naturally, it would be helpful if some sort of independent relevance score could be provided for new hypotheses generated by the method used in this report. In this view, a p-value was calculated for the new node NFκB in the network using a one-tailed Chi-square test with Yates's correction, comparing its node connectivity in the PathwayAssist database to its node connectivity in the CRPS concepts network. In addition, the protein TP53 was chosen as a reference case because of the equally high connectivity in the PathwayAssist database compared to NFκB. Unfortunately, TP53 was found to be equally significant in the CRPS network as NFκB. However, NFκB was connected to more concepts in the original CRPS network than TP53.

An automatic network analysis approach, where a variety of nodes (for example all highly connected proteins in the PathwayAssist database, all nodes generated by the expanded network algorithm, or simply all nodes in the PathwayAssist database) would be tested for positive association to a subnetwork of predefined disease-specific nodes, would be valuable for both hypotheses generation and testing. Unfortunately, there is no such algorithm available in the PathwayAssist version used by the authors of this paper. Furthermore, the necessary data required for carrying out these types of tests outside the framework of the tool (specific connectivity information for each node in the database, i.e. which other nodes it is connected to) are not provided by PathwayAssist. The assessment of the connectivity to the CRPS network for NFκB and TP53 was performed manually, an activity that is very time consuming and not defendable for a larger number of nodes. Despite the limitations regarding the assessment of the specificity of the results, a new node found by one of the network gap analysis algorithms provided by PathwayAssist, which was significantly related to the CRPS network, was considered important enough for further investigation.

One should bear in mind that results from text mining exercises as described in this paper are far from the solution to the medical problem or the complete answer to outstanding questions. The current tools in the biomedical domain are **not** capable of delivering clear and already assessed hypotheses. However, in this case, the results of a simple knowledge gap analysis provided a new idea that, after further manual exploration in literature, appeared very plausible and worthwhile investigating in biological experiments and epidemiological studies.

The generalizability of this method of hypothesis discovery needs to be assessed by repeating the exercise for other complex medical conditions. However, it would be an exhaustive and extremely time consuming task to repeat the exercise for a large group of complex disorders. This is a well-known problem in the evaluation of text mining tools in general.[73]

Testing the new hypothesis

New biological data are needed to verify the in silico derived hypothesis concerning the involvement of NFκB in the pathogenesis of CRPS. These data could be generated through animal models, clinical studies, and epidemiological studies. A rat model for CRPS has been developed by Coderre and colleagues.[74] They named it the chronic post ischemia pain (CPIP) model, since CRPS like symptoms are provoked by ischemia and reperfusion of the hindpaw by binding it temporarily with a tourniquet. This model may prove useful to investigate the role of NFκB in the early development of CRPS, for example by measuring the transcription of NFκB in the affected tissue. Additionally, in this model the effect of administration of NFκB inhibitors could be studied. In the past, NFκB knockout and transgenic animal models have been extensively used to study the NFκB pathway.[75] An increased inflammatory response coupled with an increased susceptibility to opportunistic infections has been recorded[76,77], and a decrease of the electroacupuncture-induced analgesic effects has also been shown.[78] A failure to induce CRPS like symptoms in NFκB knock mice would support the hypothesis of a NFκB as a crucial factor in CRPS.

Clinical and epidemiological studies to test the hypothesis could involve the investigation of determinants of altered NFκB activity and the comparison in this view between CRPS cases and healthy controls. For example it could be tested whether other NFκB related disorders (asthma, autoimmune disorders or atherosclerosis) co-occur with CRPS. Viral infections are known to upregulate NFκB activity. Thus the time relation between CRPS and the occurrence of a viral infection may also be worthwhile investigating. Finally, one could search for features from a genetic origin. The increased prevalence of an ACE polymorphism in CRPS patients was found in a small study.[79] Since ACE is also an inhibitor of NFκB activity, further investigations regarding this polymorphism might be of interest. Certain drugs affect (perhaps unintentionally) the NFκB pathway and could influence the development of CRPS (see also section Targeting the NFκB pathway). Currently, according to the recently developed Dutch evidence based Guideline Complex Regional Pain Syndrome type 1[80], nine drugs have been proven beneficial in the prevention or treatment of CRPS, including ketamine[81], gabapentine[82], DMSO crème[83], N-acetylcysteine[83], corticosteroids[84], bisfosfonates[85], calcium antagonists[86], ketanserine[87], and vitamin C.[88] Interestingly, 126 small molecules are listed as NFκB inhibitors in PathwayAssist, and 5 of these overlap with the list of drugs proven beneficial for preventing or treating CRPS (ketamine[89], DMSO cream[90], N-acetylcysteine[91], corticosteroids[92], and calcium antagonists[93]).

A Dutch cohort of CRPS patients was identified in the Integrated Primary Care Database (IPCI), a database that makes electronic patient records used in routine care available to investigators.[94] This cohort has been used before in research on CRPS[95] and will be used for further testing of these hypotheses within the framework of Infobiomed.

Clinical relevance

NFκB is a transcription factor that is known to be involved in many processes, but its function is best described in inflammation.[45] In the recent past NFκB has been discovered as an important mediator in diseases due to chronic and exaggerated inflammatory responses, including sepsis, asthma, rheumatic disorders, inflammatory bowel disease, and psoriasis. However, in the current available literature NFκB has never before been mentioned in association with CRPS. Therefore, this relation is an interesting new result derived from the text mining exercise. Especially remarkable is that, in the generated network, NFκB is not only linked with the inflammatory concepts related in CRPS, but also to the non-inflammatory concepts in CRPS, such as neuropeptides and catecholamines.

Realizing the possible central role for the NFκB pathway in the mechanisms underlying CRPS (or in the stress response caused by CRPS), unanswered questions concerning the disorder can be reviewed from a new point of view. For instance, when placing NFκB in the centre of the pathogenic process, the wide variety of precipitating events could be explained by the observation that all pathogenic mechanisms in CRPS are related to each other. This relation suggests that an event that specifically triggers one of the four mechanisms induces the entire complex pathogenic process (figure 3A-D). For each mechanism separately the potential triggers are limited, but for the four mechanisms together the possibilities are numerous. With the addition of NFκB, the biochemical picture underlying CRPS becomes one in which several processes can initiate the disorder and in which NFκB can play a key role in its propagation.

Assuming the central unifying role of NFκB in the pathogenesis of CRPS, independent whether NFκB is the final molecule in the pathway or not, new targets for drug therapy could be provided. Current therapy in CRPS is aimed at targeting separate mechanisms. However, targeting one mechanism in CRPS is not enough, as made apparent by the limited success rate for the majority of treatments. Based on the new hypothesis, targeting the NFκB pathway may provide a way to target all the underlying mechanisms at once. Thereby, progression of the disorder may be more effective, since it will be prevented at more than one level. Independent from the inciting event or principle disturbed mechanism, NFκB pathway inhibitors might remove the 'engine' that keeps the process running.

Targeting NFκB as a therapy in sepsis, inflammatory diseases and neuronal diseases is proposed by several authors.[46,49,96,97] It is suggested to be very promising for a wide variety of patients, but due to the involvement of NFκB in a wide variety of physiological processes, there are safety implications. New drugs that target one of the components of the NFκB pathway are currently under development. One of these drugs, bortezomib, was already launched in the United States in 2003 for the treatment of multiple myeloma. Other drugs are in various stages of development (table 3). The NFκB pathway inhibitors are designed for the use in treatment of a variety of disorders, including inflammatory diseases, autoimmune diseases, atopic disorders, arteriosclerosis and malignancies. None of the drugs currently under development is mentioned for treatment of CRPS. When these drugs have proven to be safe, the therapeutic effect of NFκB pathway inhibitors could be studied in CRPS patients.

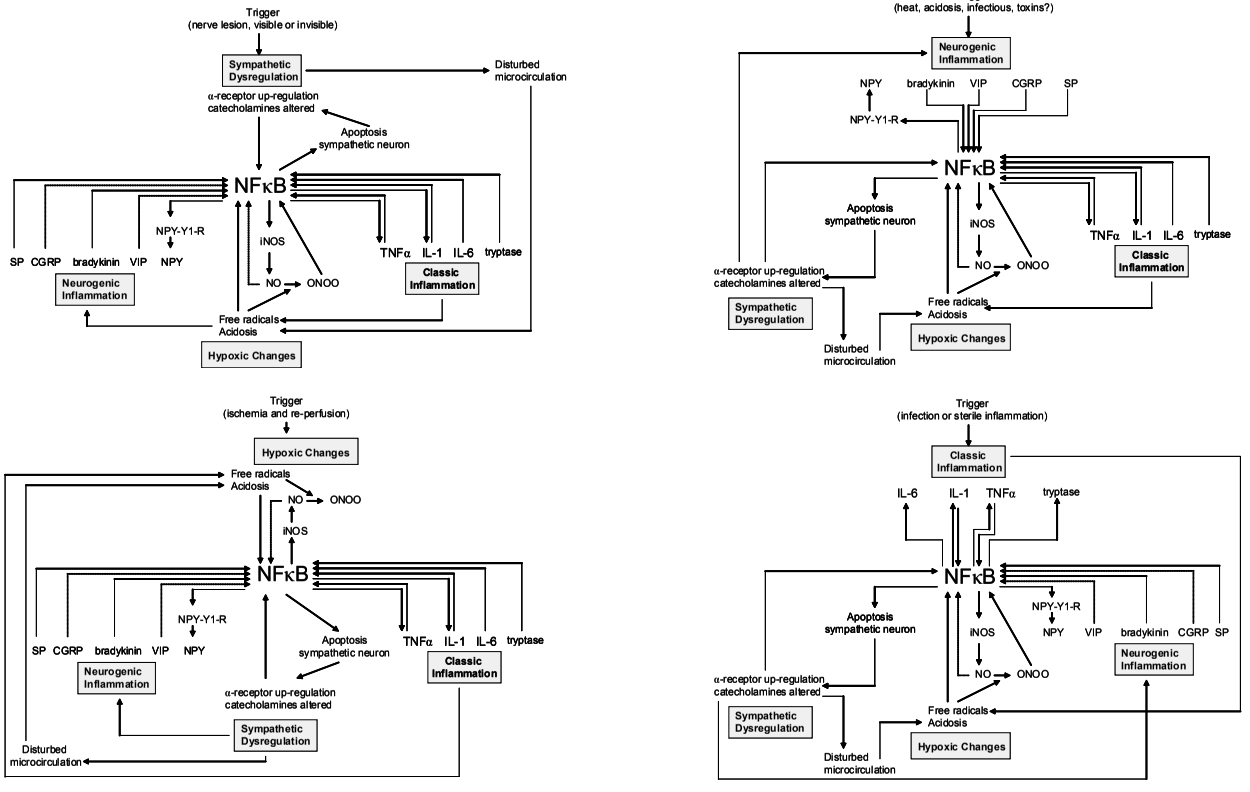

Figure 3A-D. The entire CRPS pathogenesis from several starting points. Mediated by NFκB, a trigger that induces either sympathetic dysregulation (A), classic inflammation (B), neurogenic inflammation (C), hypoxic changes (D) can induce the entire pathogenesis of CRPS. SP=Substance P, CGRP=Calcitonin Gene-related Protein, VIP=Vasoactive Intestinal Protein, NPY=Neuropeptide Y, NPY-Y1-R= Neuropeptide Y-Y1 Receptor, NEP=Neutral-Endopeptidase, ACE=Angiotensin Converting Enzyme, SMP=Sympathetically Maintained Pain, NO=Nitric Oxide, iNOS=inducible Nitric Oxidase, ONOO=peroxynitrite

Table 3. Current development status of NFκB pathway inhibitors. (Investigational Drugs DataBase, Nov 15 2005 (107), advanced search by activity field on Nuclear factor kappa B inhibitor).

Development status	No. of drugs
Discovery Research	22
Clinical (unspecified)	1
Phase I clinical trials	4
Phase II clinical trials	4
Phase III clinical trials	0
Pre-registered	0
Registered	0
Launched	1 (bortezomib)
Research tools	1
Suspended	0
Withdrawn	0
No development reported	4
Discontinued	4

Conclusion

Computer-assisted literature analysis to support the generation of novel and testable hypotheses has previously been proven useful.[4,5] This study builds upon this research, and extends it by bringing together researchers and clinicians with a wide variety of backgrounds. An experimental exercise was performed in which a text-mining/entity relationship tool has been applied to systematically synthesize the knowledge in existing literature about a complex disorder, exemplified by CRPS. Within the created literature network, a simple knowledge gap analysis was performed, using node connectivity. This approach was essential in formulating the hypothesis that NFκB might be a key player molecule in the pathogenesis of CRPS. This example of multidisciplinary research illustrates how the collaborative efforts of investigators from different fields of expertise can demonstrate new directions for future biological and epidemiological research on a complex disease.

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4.2 | Role of NFκB in an Animal Model of Complex Regional Pain Syndrome type I (CRPS-I)

Submitted

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Abstract

Introduction: NFκB is involved in several pathogenic mechanisms that are believed to underlie the complex regional pain syndrome (CRPS), including ischemia/reperfusion (IR) injury, inflammation and sensitization. Chronic post-ischemia pain (CPIP) has been developed as an animal model that mimics the symptoms of CRPS-I. The possible involvement of NFκB in CRPS-I was studied in CPIP rats.

Methods: Under sodium pentobarbital anesthesia a tourniquet was placed around the rat left ankle joint to produce 3 hrs ischemia, followed by rapid reperfusion. Tissue from muscle and spinal cord was obtained at 2 hrs, 48 hrs and 7 days after IR injury. NFκB was measured in nuclear extracts using ELISA. At 48 hrs post-reperfusion, rats were tested for mechanical (von Frey filaments) and cold allodynia (acetone drop test) before and after systemic (10, 30 or 100 mg/kg), intrathecal or intraplantar (both 250 μg per rat) administration of the NFκB inhibitor pyrrolidine dithiocarbamate (PDTC).

Results: At 2 and 48 hrs after IR injury, NFκB was elevated in muscle and spinal cord of CPIP rats compared to sham rats. At 7 days NFκB levels were normalized in muscle, but were still elevated in spinal cord tissue. Systemic PDTC treatment relieved mechanical and cold allodynia in a dose-dependent manner, lasting for at least three hrs. Also intrathecal, but not intraplantar, administration relieved mechanical allodynia.

Discussion: The results suggest that muscle and spinal NFκB plays a role in the pathogenesis of CRPS. Since current treatments for CRPS are often ineffective, NFκB might make an interesting new target for therapy in CRPS patients.

Introduction

Complex regional pain syndrome (CRPS) is considered to evolve from several pathological mechanisms, including oxidative stress[1,2], classic[3-6] and neurogenic[3,4] inflammation, and autonomic and sensory nerve system alterations.[7,8] A previous described automated analysis of literature has revealed that the transcription factor nuclear factor kappa B (NFκB) is involved in all these disease mechanisms.[9] For example, affected limbs of human CRPS patients show signs of chronic ischemia[1,10], which can induce NFκB activation, mediated by the formation of reactive oxygen species (ROS) and peroxinitrite.[11,12] Inflammatory mediators, including tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1) and IL-6 have been demonstrated in blister or spinal cord fluid of CRPS patients[5,13], and can activate or are activated themselves by NFκB.[14-16] Moreover, NFκB interacts with neuropeptides that have been found abnormally expressed during CRPS[4,17], such as calcitonin gene related protein (CGRP)[18] and substance P (SP).[19] Finally, animal studies have revealed that NFκB is involved in nociceptive sensitization through up-regulation of glutamate receptors in dorsal root ganglion cells[20], and through interactions with cytokines.[21]

NFκB resides in the cytosol of many different cell types, and can be activated by many triggers, including ultraviolet radiation, free radicals, cytokines and products of bacterial and viral infections.[22] Upon activation, inhibitory kappa B (IκB) protein is cleaved from the NFκB complex, which subsequently forms dimers that are capable of passing through the nuclear membrane. In the nucleus, NFκB promotes the transcription of a wide variety of genes. NFκB has been attracting considerable scientific attention over the past years as a key factor in inflammation, apoptosis, and neuronal-glial interactions.[23] Excessive NFκB activity has been attributed to the pathogenesis of several chronic inflammatory disorders and oncological diseases.[24] Since 2005, the NFκB pathway inhibitor bortezomib (Veldane®) has been applied successfully in the therapy of multiple myeloma and other malignancies.[25]

Pyrolidine dithiocarbamate (PDTC) is a chemical with metal chelating and antioxidant properties, which inhibits NFκB activity by blocking the phosphorylation of IκB.[26,27] Systemically administrated PDTC and other dithiocarbamates have been shown to be protective and therapeutic in animal models for ischemia and reperfusion (IR) injury[28], acute inflammation[29] and neuropathic pain.[30] The aim of the present study was to investigate the potential involvement of NFκB in the pathogenesis of CRPS. NFκB levels and the effect of NFκB inhibition by PDTC were studied in rats with chronic post-ischemia pain (CPIP). These rats develop CRPS-like symptoms, such as edema, hyperemia and allodynia, after IR injury of the hind paw, without evident nerve damage.[31]

Methods

Study design

NFκB levels were measured in muscle and spinal cord tissue of CPIP animals and compared to sham animals at 2 hrs (CPIP: N = 15, sham: N = 9), 48 hrs (CPIP: N = 15, sham: N = 9 - 10) and 7 days (CPIP: N = 6, Sham: N = 7) after IR injury.

The effect of NFκB inhibition by systemic PDTC administration on allodynia was studied in CPIP rats using four treatment groups (saline and 10, 30, and 100 mg/kg of PDTC; 10 rats per group) and in sham rats using two treatment groups (saline and 100 mg/kg of PDTC; 10 rats per group). PDTC/saline was administered intraperitoneally (i.p.) 48 hrs after IR injury. Animals were tested for mechanical and cold allodynia in the ipsilateral hind paw just before treatment, and at 30, 60, 90, 120 and 180 minutes after treatment.

Additionally, to investigate the site of PDTC effects, intrathecal or intraplantar administrations (250 μg per rat) were performed in two additional groups of animals (N = 10 per group) and compared to saline treatment using both administration routes (N = 10 per group). Intrathecal injections (20 μl volume) were performed by L6 lumbar puncture under brief anesthesia with isofluorane, whereby intrathecal delivery was confirmed by observing an injection induced tail-flick.[32] Intraplantar injections (50 μl volume) were performed in the ipsilateral foot of awake animals. Mechanical allodynia was measured in both the ipsi- and the contralateral hindpaw at 30 and 60 minutes after PDTC administration.

All treatment and testing procedures were performed in a blinded manner by a single experimenter per test. PDTC was obtained from Sigma Aldrich and was freshly dissolved daily in saline.

Animals

Male Long Evans rats (275-300 g, Charles River, Quebec) arrived at least 5 days before the start of the experiments. They were kept under a 12 hrs/12 hrs light-dark cycle (lights on at 7:00 h), with free access to food and water. All experiments were performed during the light cycle. Methods were approved by the Animal Care Committee at the McGill University, and conformed to the ethical guidelines of the Canadian Council on Animal Care.

Chronic post-ischemia pain (CPIP)

CPIP was induced by ischemia and reperfusion (IR) injury of the left hind paw as described by Coderre and colleagues.[31] Briefly, animals were anesthetized over a three hour period with a bolus (55 mg/kg, i.p.) and chronic i.p. infusion of sodium pentobarbital for 2 hrs (27.5 mg/kg/h). After induction of anesthesia, a Nitrile 70 Durometer O-ring (O-rings West, Seattle, WA) with 7/32" internal diameter was placed around the rat's left ankle joint. After three hrs the O-ring was cut, allowing reperfusion of the hind limb. Sham animals underwent anesthesia similar to the CPIP animals, but an O-ring was not placed around the ankle.

Tissue sampling and preparation

Animals were euthanized by decapitation under anesthesia with isofluorane. Immediately, muscle samples of the superficial plantar layer (one each from the Flexor Hallucis Brevis, Flexor Digiti Minimi Brevis and Flexor Digitorium Brevis, each weighting between 29 and 50 mg) and spinal cord samples at L5-L6 (each weighting 12 to 20 mg) were obtained and quickly frozen in isopentane, kept on dry ice, and stored at -80°C until processing. Samples were thawed at 4°C and homogenized either mechanically (muscle) or by sonification (spinal cord) in 12.0 µl/mg tissue of RIPA buffer containing 50 mM Tris-HCl, 150 mM NaCl, 1mM EDTA, 1% Igepal (Sigma, St.Louis, MO), 1% Sodium deoxycholate and 0.1% SDS (Ph 7.4), to which was added a 1% protease inhibitor cocktail (Sigma, St.Louis, MO). Tissue homogenates were centrifuged at 3,000 g for 10 min, and the supernatant was collected and processed for nuclear fraction extraction following the recommended procedure of a commercially produced extraction kit (Chemicon Nuclear Extraction Kit, Millipore Corp., Billerica, MA). Briefly, after spinning at 250 g for 5 min, samples were diluted 1/5 (vol/vol) in cytoplasmic lysis buffer and incubated at 4°C for 20 min. The homogenates were mechanically sheared by repeatedly drawing and ejecting each sample through a series of 25 ga, 26 ga and finally 27 ga needles and centrifuged at 8,000 g for 20 min at 4°C. Subsequently, the pellets were resuspended in nuclear extraction buffer, mechanically disrupted with a 27 ga needle, incubated for 60 min, and then centrifuged at 16,000 g for 6 min at 4°C, in order to obtain the supernatant that contained the nuclear fraction. Nuclear fractions were concentrated by centrifugal filtration at 14,000 g for 20 min using cellulose filters with a 30 kDa cut-off (Microcon YM-30, Millipore Corp., Billerica, MA). Nuclear fractions remaining after filtration were collected and diluted in buffer to a final volume of 100 µl for muscle and 50 µl for spinal samples. Total sample protein content was determined by the Bradford method.[33] Nuclear extracts were stored at -80°C until further analysis.

Measurement of NFκB by ELISA

NFκB measurements were performed using a commercially supplied NFκB transcription factor binding assay (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's suggested protocol. Briefly, duplicate 10 µl samples of nuclear extract were first incubated overnight at 4°C in wells pre-coated with a dsDNA sequence corresponding to the NFκB consensus motif. After five washes, the samples were incubated overnight at 4°C with a rabbit polyclonal antibody to the p50 subunit of NFκB at a final dilution of 1:100 (sc-7178, Santa Cruz Biotechnology, Santa Cruz, CA). Subsequently samples were incubated for 60 min with an HRP-conjugated goat anti-rabbit secondary antibody (Cayman Chemical, Ann Arbor, MI), followed by colorimetric detection (measured as absorbance at 450 nm; Versamax, Molecular Devices, Sunnyvale, CA). After background subtraction, absorbance measures were referred to a standard curve obtained from a series of duplicate wells containing measured amounts of human recombinant p50 (Cayman Chemical, AnnArbor, MI) and then converted to an estimate of the quantity of p50/well, which was normalized by dividing the p50 estimate by the total amount of protein measured in the sample.

Mechanical and cold allodynia

Mechanical allodynia was assessed by measuring the 50% withdrawal response to stimulation with von Frey filaments, according to a modified method as described by Chaplan and colleagues.[34] Briefly, rats were placed in Plexiglas® cages with a wire grid bottom. Filaments (Stoelting, Illinois, USA) were applied to the plantar surface of the hind paw for approximately five seconds in either ascending or descending strength, to determine the filament closest to the threshold of response. CPIP rats that had not developed mechanical allodynia at 48 hrs post IR injury ('non-responders', 50% threshold > 10 g) were excluded from the further measurements of mechanical allodynia after PDTC treatment.

Cold allodynia was assessed using a modification of the acetone drop method as described by Choi and colleagues.[35] A drop of acetone was placed on the plantar surface of the foot and the response was measured as the amount of seconds of nociceptive behavior observed during the first min after acetone application. Again, non-responders for cold allodynia at 48 hrs post IR injury (pain behavior for one second or less) were excluded from the further measurements of cold allodynia after PDTC treatment.

Mechanical and cold allodynia were tested in the same animals, whereby mechanical allodynia was always tested first. When both sides were tested, the contralateral side was tested before the ipsilateral side.

Statistics

All statistical analyses were performed using the statistical package for social sciences (SPSS) version 12.0. Significance was established at $p < 0.05$. Data were plotted as the mean +/- standard error of the mean (SEM).

NFκB in tissue from the ipsi and contralateral side of CPIP rats was compared to sham rats using a Mann-Whitney U test. Ipsi- versus contralateral differences within rats were compared using a Wilcoxon signed rank test.

Baseline mechanical and cold allodynia test results were compared with one-way ANOVA. Post-treatment differences between groups were analyzed using repeated measures ANOVA with a Greenhous-Geisser correction for sphericity. In post hoc analyses, each treatment group was compared to the saline control group applying a Bonferoni correction. In CPIP rats only, decreases in cold allodynia relative (percentage) to pre-treatment values were calculated and compared to the saline control group. For mechanical allodynia, a delta area under the curve (ΔAUC) relative to pre-treatment values was calculated over the period of observation and compared with the saline group using one-way ANOVA followed by a post hoc LSD test. Pre- and post-treatment values within one treatment group were compared using a Wilcoxon signed rank test.

Results

NFκB in muscle and spinal cord

The results of NFκB measurements in muscle and spinal cord are depicted in figure 1. Because in sham rats NFκB levels at both sides were similar ($p = 0.162$ for muscle and $p = 0.694$ for spinal cord), the right and left side measures of sham rats were combined in the comparison to CPIP rats. At both 2 and 48 hrs after IR injury, NFκB was increased

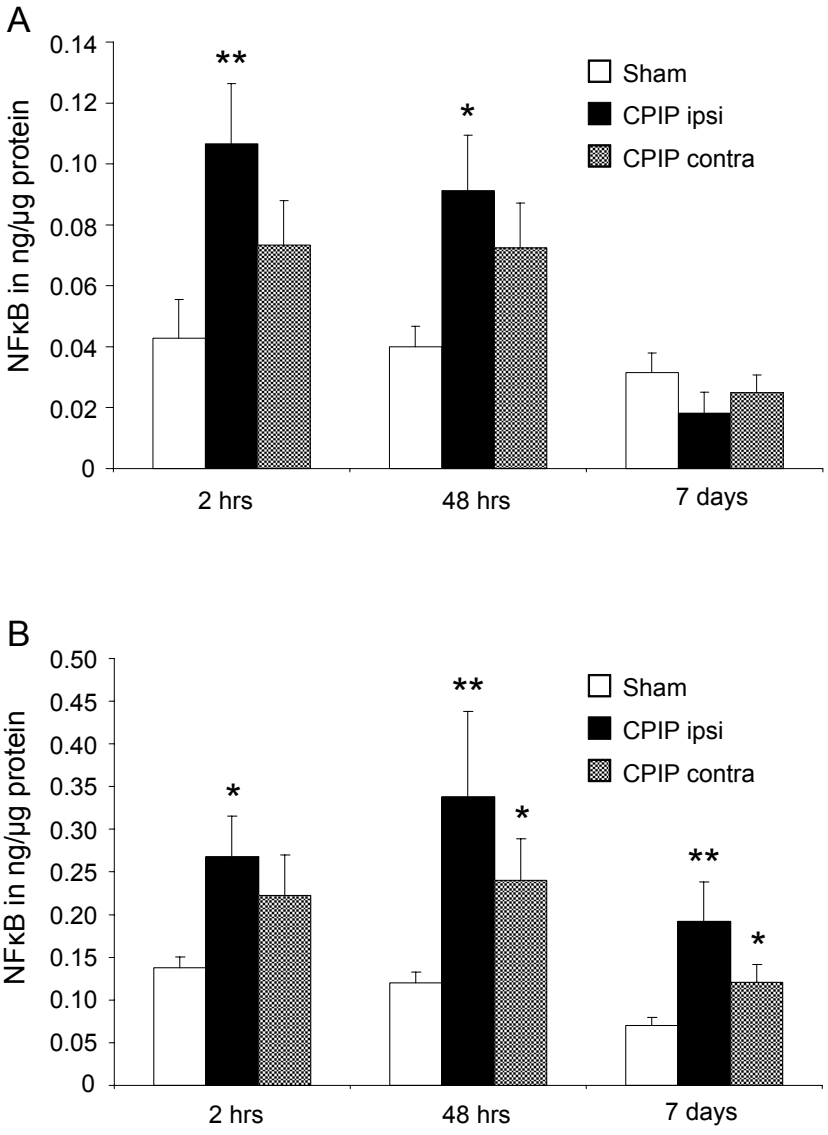

Figure 1. A. NFkB levels in muscle from the ipsilateral (ipsi) and contralateral (contra) hind paw of CPIP rats compared to shams at 2 hrs, 48 hrs and 7 days post-reperfusion, measured by ELISA. CPIP ipsilateral at 2 and 48 hrs: N = 15, at 7 days: N = 6. CPIP contralateral at 2 and 48 hrs: N = 15, at 7 days: N = 6. Sham at 2 and 48 hrs: N = 18, at 7 days N = 14. *p < 0.05, **p < 0.005, Mann-Whitney U test compared to shams. **B.** NFkB levels in spinal cord from the ipsilateral (ipsi) and contralateral (contra) side of CPIP rats compared to shams at 2 hrs, 48 hrs and 7 days post reperfusion, measured by ELISA. CPIP ipsilateral at 2 and 48 hrs: N = 15, at 7 days: N = 6. CPIP contralateral at 2 and 48 hrs: N =15, at 7 days: N = 6. Sham at 2 and 48 hrs: N = 18, at 7 days N=14. *p < 0.05, **p < 0.005, Mann-Whitney U test compared to shams.

compared to sham rats in muscle ($p = 0.004$ at 2 hrs, $p = 0.020$ at 48 hrs) as well as spinal cord ($p = 0.027$ at 2 hrs and $p = 0.001$ at 48 hrs) from the ipsilateral side of CPIP rats. At 7 days after IR injury, NFκB levels in muscle did not differ between CPIP and sham rats. However, in spinal cord the ipsilateral NFκB levels from CPIP rats were still elevated ($p = 0.001$). Remarkably, also on the contralateral side of CPIP rats NFκB was increased compared to shams in muscle and spinal cord tissue, although this was not statistically significant. Moreover, within CPIP rats there was no significant difference between ipsi- and contralateral sides.

Systemic PDTC treatment

Paw-withdrawal thresholds of the ipsilateral hind paw at baseline did not differ between CPIP and sham rats (13.2 +/- 3.6 g and 13.4 +/- 3.0 g respectively, $p = 0.823$). At 48 hrs after IR injury, CPIP rats developed a decrease in paw-withdrawal threshold (mean 50% von Frey threshold of 6.83 +/- 3.64 g) compared to shams (mean 50% von Frey threshold of 11.88 +/- 3.42 g) ($p < 0.0001$). Within the CPIP group, 32 rats (80%) displayed a 50% von Frey threshold < 10.0 and were regarded as responders for mechanical allodynia.

Acetone responses at baseline were similar between CPIP and sham rats (1.075 +/- 1.8 s and 1.10 +/- 1.6 s respectively, $p = 0.957$). Compared to shams (2.05 +/- 3.1 s), acetone responses were increased in CPIP rats at 48 hrs after IR injury (3.78 +/- 4.3 s), although the difference was not significant ($p = 0.113$). In the CPIP group, 26 rats (65%) displayed pain behavior for more than one second, and were considered as responders for cold allodynia.

The effect of systemic PDTC treatment at 48 hrs after IR injury on mechanical allodynia is displayed in figure 2A. A significant main effect of time was observed ($F(4,121) = 14.3$, $p < 0.001$), as well as a significant main effect of treatment ($F(3,28) = 8.1$, $p < 0.001$), but there was no significant time × treatment interaction ($F(13,121) = 1.29$, $p = 0.229$). In post hoc analyses, CPIP rats that had received the highest dosage of PDTC (100 mg/kg) showed a decrease in mechanical hypersensitivity compared to the saline group ($p = 0.02$) and the group that received the lowest dose of PDTC (10 mg/kg, $p = 0.02$). The ΔAUC showed an effect of treatment for the highest (100 mg/kg, $p = 0.002$) and middle (30 mg/kg, $p = 0.021$) doses of PDTC, compared to saline controls. In the sham rats, no significant effect of time was observed, but there was a significant main effect of treatment ($F(1,17) = 7.3$, $p = 0.015$) and a significant time × treatment interaction ($F(6,102) = 3.1$, $p = 0.008$). Also within sham rats, the ΔAUC differed between the PDTC and the saline treated group ($p = 0.028$).

Regarding the absolute values for cold allodynia, a significant main effect of time was observed ($F(5,104) = 5.7$, $p < 0.001$), but the main effect of treatment ($F(3,22) = 2.6$, $p = 0.079$) and the time × treatment interaction ($F(14,104) = 1.7$, $p = 0.057$) just failed to reach significance. In sham rats, there was no significant main effect of time ($F(3,50) = 1.8$, $p=0.164$) or treatment ($F(1,17) = 0.8$, $p=0.377$) nor was there a significant time × treatment interaction ($F(3,50) = 1.2$, $p = 0.318$). For CPIP rats only, the relative (percentage) decrease in cold allodynia after systemic PDTC treatment is depicted in figure 2B. A significant main effect of treatment was observed ($F(3,22) = 4.0$, $p = 0.020$), and post hoc analyses demonstrated a decrease in cold allodynia in rats that had received the highest dose of PDTC compared to saline treatment ($p = 0.035$) and the lowest PDTC dose ($p = 0.045$).

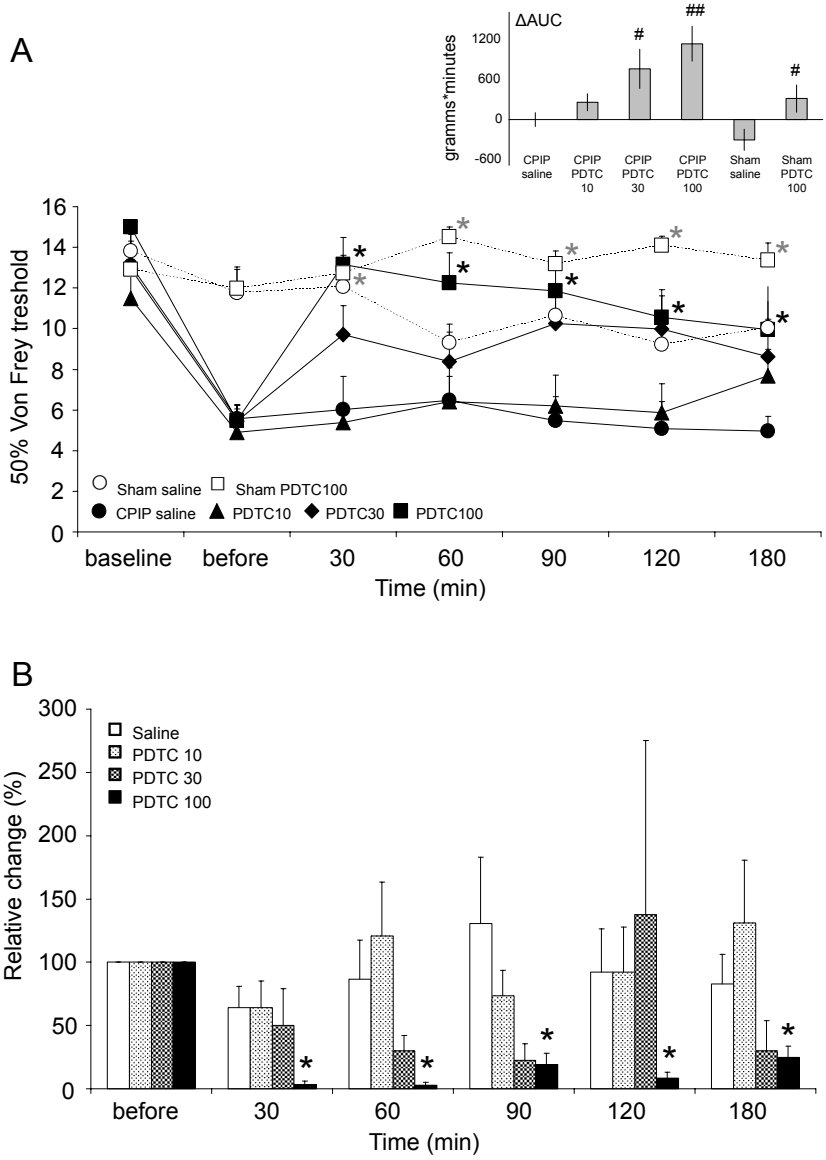

Figure 2.A. Mechanical paw-withdrawal threshold in CPIP and sham rats after systemic PDTC treatment at 48 hrs post-reperfusion, compared to saline treatment. CPIP saline: N = 7; CPIP PDTC 10 mg/kg: N = 9; CPIP PDTC 30 mg/kg: N = 9; CPIP PDTC 100 mg/kg: N = 7; Sham saline: N = 10; Sham CPIP: N = 10. * $p < 0.05$, repeated measurements ANOVA followed by a post-hoc Bonferoni test compared to the saline control group. # $p<0.05$ ## $p<0.005$, one-way ANOVA followed by a post-hoc LSD test compared to the saline control group. **B.** Relative changes in aceton responses in the ipsilateral hind paw of CPIP rats after systemic saline or PDTC treatment at 48 hrs post-reperfusion, compared to aceton responses before treatment. CPIP saline: N = 9; CPIP PDTC 10 mg/kg: N = 6; CPIP PDTC 30 mg/kg: N = 4; CPIP PDTC 100 mg/kg: N = 7. *$p < 0.05$, repeated measurements ANOVA followed by a post-hoc Bonferoni test compared to the saline control group.

Intrathecal and intraplantar PDTC treatment

At baseline, there was no difference in mechanical sensitivity between the right and left hind paw (13.5+/-2.15 g and 13.3+/-2.20 g, $p = 0.689$), while after IR injury, 26 out of 40 rats (65%) had developed mechanical allodynia.

The effects of intrathecal and intraplantar PDTC treatment are depicted in Fig 3. For intrathecal treatment, there was a significant main effect of time ($F(2,27) = 21.6$, $p < 0.001$), but there was no significant main effect of treatment ($F(1.14) = 2.6$, $p = 0.132$) or time × treatment interaction ($F(2,27) = 2.6$, $p = 0.094$). However, in the PDTC treatment group the mean 50% VF threshold was increased at both 30 min ($p = 0.017$) and 60 min ($p = 0.012$) post-treatment, compared to the pre-treatment threshold. No pre versus post differences were observed in the saline treatment group ($p = 0.687$). The ΔAUC was significantly larger for the PDTC group compared to the saline group ($p = 0.034$).

For intraplantar PDTC treatment, a significant main effect of time was observed ($F(2,14) = 45.9$, $p<0.001$), but there was no significant main effect of treatment ($F(1,8) = 0.0$, $p=0.980$) or time × treatment interaction ($F(2,14) = 1.9$, $p=0.195$). The mean 50% von Frey thresholds did not differ significantly between before and after treatment. Additionally, the ΔAUC was not significantly different.

Discussion

We investigated the involvement of NFκB in chronic post-ischemia pain (CPIP), an animal model of CRPS-I. NFκB was increased in muscle and spinal cord from CPIP rats compared to shams at both 2 and 48 hrs after IR injury. At 7 days after IR injury, NFκB levels were equal to shams in muscle tissue, but still elevated in the spinal cord. Systemic PDTC administration at 48 hrs after IR injury relieved mechanical and cold allodynia in a dose-dependent manner. Mechanical allodynia was also relieved upon intrathecal treatment, but not upon intraplantar treatment.

Considering previous studies, a role of NFκB in CPIP was a plausible expectation. First, several studies in different tissue types have demonstrated increased NFκB activity early after hypoxia, for example in myocardial tissue, brain, hepatic tissue, and skeletal muscle.[26,27,36,37] In all of these studies, the extent of the damage caused by IR injury could be attenuated by administration of an NFκB inhibitor. Second, CPIP rats display signs of inflammation.[31] IR injury is known to provoke a well documented cascade of inflammatory events[38], and NFκB is an important mediator in such inflammatory responses. Third, CPIP rats develop neuropathic pain-like symptoms, including mechanical and cold allodynia. Previously, increased NFκB activity has been demonstrated in animal neuropathic pain models, while these symptoms can be relieved by an NFκB inhibitor.[21,39,40]

The hypothetical involvement of NFκB in CPIP is confirmed by our present observations. NFκB elevation in muscle tissue is consistent with a previous report of muscular NFκB activation upon ischemia by arterial clamping.[26] On the contrary, increases in spinal NFκB levels following peripheral IR injury is to our knowledge a new finding, although spinal NFκB activation has been reported following peripheral nerve section[41] and nerve inflammation.[42,43] These peripheral triggers can induce an intra-spinal cytokine release, a process that may be mediated by NFκB.[21,40] However, neuropathic pain-like symptoms and spinal NFκB activation in CPIP rats are subsequent

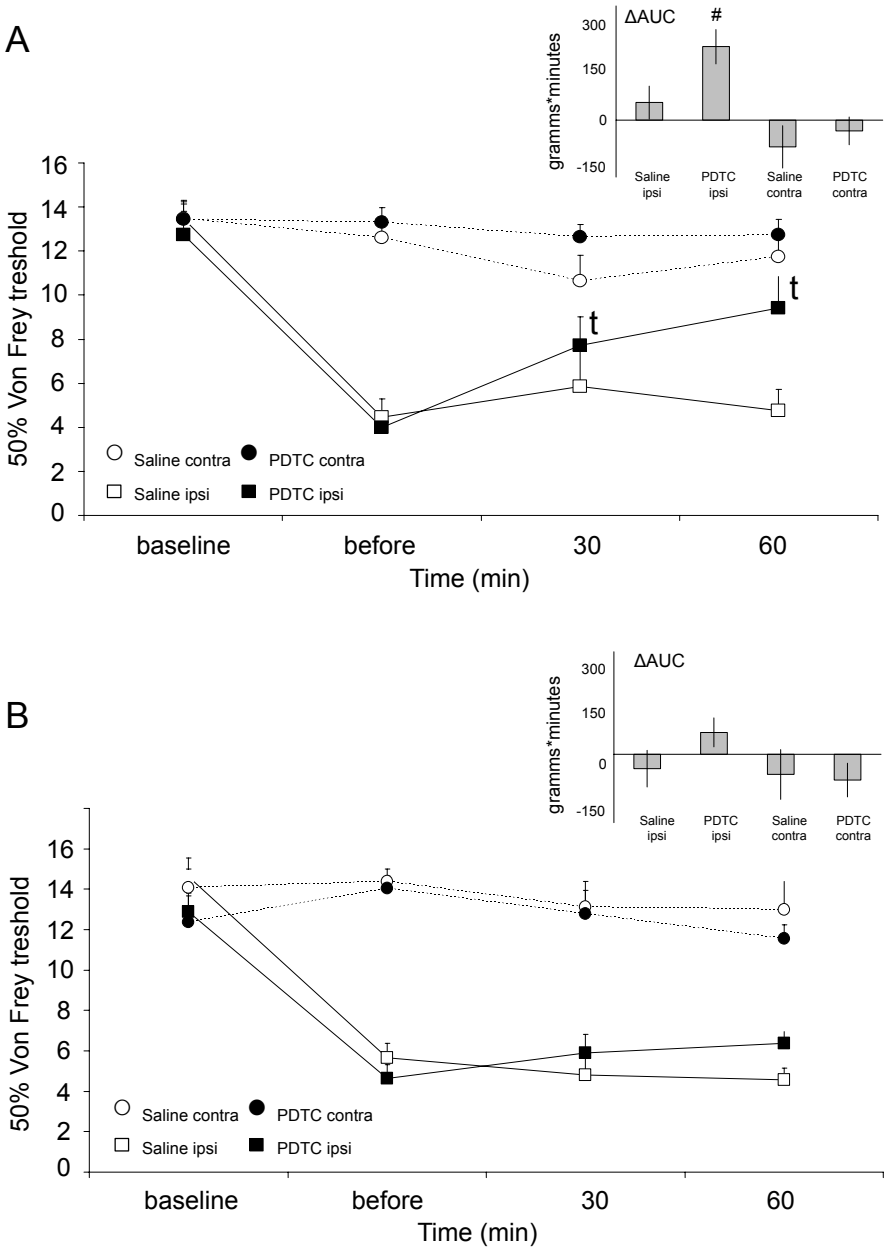

Figure 3. A. Mechanical paw-withdrawal thresholds in CPIP rats after intrathecal treatment with PDTC 48 hrs post-reperfusion. Saline ipsilateral (ipsi): N = 8; PDTC ipsilateral: N = 8; Saline contralateral (contra): N = 8; PDTC contralateral: N=8. t $p < 0.05$, Wilcoxon signed rank test comparing each post-treatment value with its pre-treatment value. # $p<0.05$, One-way ANOVA compared to the saline control group. **B.** Mechanical paw-withdrawal thresholds in CPIP rats after intraplantar treatment with PDTC 48 hrs post-reperfusion. Saline ipsilateral (ipsi): N = 5; PDTC ipsilateral: N = 5; Saline contralateral (contra): N = 5; PDTC contralateral: N = 5.

to IR injury instead of traumatic nerve injury or direct immunological stimulation.[31] Presumably, IR injury can induce pathological responses in the central nervous system (CNS) similar to those induced by mechanical or inflammatory nerve damage, either through direct activation of nociceptors by reactive oxygen species (ROS) or by ROS-induced inflammatory reactions. The observation in CPIP rats of prolonged spinal NFκB activity (until at least 7 days after IR injury), when peripheral levels were normalized, suggests that eventually the CNS pathology becomes independent of its initial peripheral trigger. This is consistent with previous observations showing that CPIP rats display ongoing mechanical allodynia at 7 days after IR injury, while plasma extravasation in the affected hind paw had been normalized within 24 hrs.[31]

NFκB activity is also increased in the contralateral muscle tissue of CPIP rats, although not as profound as on the ipsilateral side, and within CPIP rats a significant difference between the ipsi- and contralateral sides not observed. A possible explanation may be the spread of free radicals and subsequently activated inflammatory mediators from the side of IR injury to the opposite side by blood circulation. In support of this, contralateral allodynia has been found in the CPIP rats in the past[31], although not consistently in all studies.[44] It may be that contralateral allodynia depends on spinal sensitization which may be mediated by NFκB that is increased in the contralateral spinal cord dorsal horn.

The anti-allodynic effect of systemic PDTC administration was clear and dose-dependent. Results from intrathecal administration were less pronounced, and no effect was obtained by intraplantar treatment. Presumably, at 48 hrs after IR injury mechanical allodynia is mainly caused by the enhanced central, and not the peripheral NFkB activity. PDTC passes the blood brain barrier and systemic doses will likely produce CNS effects, whereas low concentration intraplantar injections should not. However, it is also possible that we were unable to determine the effective dose for local treatment.

Few animal models exist that mimic the clinical pattern of CRPS-I. The CPIP model resembles human CRPS-I in several aspects. CPIP rats display features that represent both inflammatory and neuropathic pain-like symptoms of human CRPS. Moreover, CPIP rats express sympathetically maintained pain[45], a phenomenon that in the past has been considered almost pathognomic for CRPS, although currently it is acknowledged to be present in only a subset of CRPS patients. Similar to CRPS-I patients, CPIP rats receive poor pain relief from classical anti-inflammatory and anti-neuropathic pain treatments[44]. Inhibitors of NFκB have been suggested as interesting new therapeutics for (chronic) inflammatory disorders, such as asthma[46], rheumatoid diseases[47] and inflammatory bowel disorders.[48] CRPS may very well be another candidate disorder for such treatment, since NFkB is likely involved in both peripheral (ischemia, inflammation) and central (sensitization) underlying disease mechanisms. However, because of serious immunological consequences there may be safety issues associated with targeting NFkB in human patients[24], and the current therapeutic indications are restricted to oncology. On the other hand, some non-specific modulators of NFkB activity have already been demonstrated beneficial in attenuating CRPS in animal models, as well as in human trials, including N-acetylcysteine[31,49] and corticosteroids.[44,50,51] Currently, these drugs are not applied generally for CRPS treatment, but these new mechanistic insights warrant further clinical studies to establish their clinical benefits.

In conclusion, we have demonstrated increased NFkB activity in both muscle and spinal cord, as well as a therapeutic effect of NFkB inhibition on allodynia, in CPIP rats. We believe that these results encourage further exploration of the role of NFkB in the pathogenesis of inflammatory pain disorders, like CRPS-I. Safety issues warranted, these studies might provide new treatment opportunities for CRPS-I.

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Chapter 5

General Discussion

Introduction

The complex regional pain syndrome (CRPS) was first described near the end of the 19th century and has carried a long lasting image of obscurity. An inflammatory origin had already been suggested by Sudeck[1], but it was only recently that (neuro-) inflammatory mediators in CRPS were actually demonstrated.[2-4] Mechanisms of neuroplasticity are assumed to underlie the sensory and functional impairments, but they remain largely to be elucidated.[5,6] Sympathetic hyperactivity has been considered pathognomic for CRPS in the past, but nowadays its contribution to the pathogenesis is subject of debate[7,8], as is the contribution of stress and psychological factors.[9-11] The aim of the present thesis was to increase the insight into the incidence, general characteristics, determinants and etiology of CRPS.

The introductory chapter provided a review of literature reflecting the current knowledge on CRPS. Subsequently, the second chapter presented three descriptive studies regarding the incidence, disease outcome, and treatment and referral patterns of CRPS in the Netherlands. The third chapter included three studies on determinants associated with CRPS, namely medical history, antihypertensive drug use, and estrogen exposure. All research described in the second and third chapter was performed within the Trauma Related Neuronal Dysfunction (TREND) consortium, a Dutch knowledge consortium that integrates research on CRPS.[12] The fourth chapter described a search for new mechanistic hypotheses. The initial step was performed within the Infobiomed consortium, a European Union funded network that aims at enforcing biomedical informatics as integrative discipline.[13] Basically, this study was set up to see whether benefit could emerge from an advanced information retrieval approach towards a clinical problem. A new mechanistic hypothesis was generated, involving the role of NFκB in CRPS, and further explored in a CRPS animal model during a collaborative project with the McGill University in Montréal (Canada). The main findings, methodological considerations and suggestions for future studies will be discussed in this final chapter.

Main findings

CRPS Incidence in the Netherlands

After Sandroni et al.[14] we have performed in IPCI only the second published population based CRPS incidence study and have reported an incidence rate (IR) of 26.2 per 100,000 person years (PY)[15] for the study period from 1996 until 2005. According to our findings, CRPS is more common than for example multiple sclerosis (5.5 per 100,000 PY in United Kingdom)[16] or systemic lupus erythematodes (3.0 per 100,000 PY in Quebec, Canada).[17] It implies that in the Netherlands there are approximately 4,000 new CRPS patients each year.

In the IPCI database we identified incident CRPS cases by using a search algorithm on the narratives of the electronic patient records. Subsequently we asked the GPs to confirm or reject the diagnosis in a short questionnaire. Before us, in Olmsted County

(USA) Sandroni et al. used the computerized diagnostic indexes (1989-1999) of the electronic medical file system of that area and identified initially 389 new CRPS cases over 1,336,088 person years. An IR calculation based on these numbers would have yielded an IR of 29.1 per 100,000 PY in Olmsted County, which would have been in line with the IR of 26 that we found in the IPCI database. However, in their article Sandroni et al. presented an IR that was based on only the cases that, by review of the medical records, fulfilled the diagnostic criteria from International Association for the Study of Pain (IASP). Because of non fullfillment, 81% of the initially identified cases was exluded and the final IR reported by Sandroni et al. was only 5.5 per 100,000 PY. The difference in case validation (IASP criteria in the Sandroni study versus GP confirmation in our study) apparently may, at least partially, explain why we found a much higher IR of 26 per 100,000 PY. However, the conveniency of medical record review for application of the IASP criteria has been questioned and the suggestion that 81% of the physician established CRPS diagnoses was incorrect seems irrealistically high[18]. The concise and unstandardized registration of clinical features in medical records might have leaded to an (overly) strict exlusion of cases in the Sandroni study, and thereby to an underestimation of the IR.

Initially we based our IR calculation on the GP confirmed CRPS diagnoses, without taking the fulfillment of diagnostic criteria into account. We realised the risk of overdiagnosis, but we restrained from futher validation by clinical featurs, because we considered the electronic medical journal not reliable for this. However, in a later stadium of our studies we visited (a subset) of the CRPS patients and retrospectively we collected information on their past and present clinical features in a standardized manner. Applying the IASP criteria on this information, combined with the medical records and requested specialist letters, we rejected 24% of the original indentified cases as false positives (compared to 81% in the Sandroni study). With the new numbers we performed a second IR calculation and found 20 new CRPS cases per 100,000 PY (instead of 26), which is still much more than previously reported. While our original IR may have been affected by overestimation, this overestimation has been minimalised in our revised IR, due to the extensive case validation procedure. Underestimation will now be more of relevance, since during the case identification procedure we used a free text search that was broad, but presumably not all including and not all CRPS cases might have been acknowlegded as such by the GP.

Descriptions of disease outcome, referrals and treatment

The disease outcome, prognostic factors, referral patterns and applied treatment strategies for CRPS have been scarcely described. As a consequence, prognostic information and uniform treatment can hardly be provided to patients. Generally, CRPS is regarded as a long lasting disorder that causes severe discomfort and impairments, perhaps even for life. Participants of reported studies are often affected for many years.[19-22] However, this somewhat pessimistic scenario has been contradicted by others, who suggest that the patients in these studies represent only a subgroup of severe cases, that are recuited in specialized tertiary care centers.[14,23]

Outcome measures for disease recovery in CRPS are not well defined and this complicates research concerning the prognosis. When is a CRPS patient recovered? As

CRPS is mainly defined by its clinical presentation one could argue that resolution of its symptoms and signs should be the standard. Oerlemans et al. therefore have developed a multicomponent impairment level sumscore (ISS) in which clinical features are not only assessed (as in the diagnostic criteria) but also quantified and combined into one score.[24] A low ISS suggests good recovery. However, these impairments do not necessarily reflect the activity of the underlying disease mechanisms, since it has been demonstrated that patients who improved according to the ISS still had increased inflammatory mediators in their blister fluids.[25] Presumably, a distinction could be made between the clinical features that indicate active ongoing CRPS (perhaps the inflammatory signs) and clinical features that should be regarded as remaining impairments while the actual CRPS has resolved (perhaps the motor disturbances). Such a distinction remains yet to be defined.

We have found in our GP based CRPS patient cohort that persistent complaints are present in more than 80% of the patients at an average of 5.7 years (range: 2-11) since CRPS onset (defined as the date of the precipitating injury). In over 60% one or more disturbances were also objectified during physical examination. According to self reports only 30% considered themselves completely recovered, while most patients (54%) reported the CRPS to have been stabilised. In a minority (16%) the CRPS was still progressive. The impact on work was high: more than half of the patients had permanently changed (28%) or even aborted (31%) their former working activities.

A medical specialist consultation (usually an anesthesiologist or rehabilitation physician) was needed in most cases (80%), and the majority had received physical therapy (89%) or pharmacotherapy (93%). Only 18% received nerve blocks, with variable success according to the patients.

All the above mentioned findings contradict the previous suggestions that CRPS is generally mild and spontaneously resolving.[14] Despite the absence of direct evidence it is generally assumed that early intervention favours the disease course and outcome. According to the Dutch treatment guideline[26] free radical scavengers, such as dimethylsulfoxide crème and N-acetylcysteine, form the recommended first pharmacologic treatment step for CRPS, combined with physiotherapy. However, only half of the patients in our study had received this treatment within the first three months after the onset of CRPS (precipitating injury). Perhaps the treatment delay can be limited by encouraging GPs, to whom 64% of the CRPS cases presents first, to initiate basic treatment in an early stadium, while waiting for the results of futher consulation of a CRPS specialist.

Risk Factors and Leads to Etiology

Over the past decade the disease mechanisms underlying CRPS have become increasingly clarified, although we are still far from complete understanding. The results from fundamental and preclinical studies, as well as the heterogeneous clinical presentation of CRPS all point at a multifactorial disease origin. The five main contributing disease mechanisms (as reviewd in the introductory chapter) include disturbances of the autonomic (sympathetic) and somatic nervous system, (neurogenic) inflammation, ischemia, and psychological aspects. Until today, only limited epidemiological studies have contributed to the better understanding of CRPS. However, apart from revealing clinically relevant risk factors, the study of determinants of CRPS could aid in confirming the mechanistic

theories that were derived from experimental and (pre-) clinical studies. The other way around, determinants may provide new leads for further mechanistic studies.

Since we had a special interest in the neuroimmune aspects of CRPS we focused in our case-control studies on potential determinants of CRPS that are related to a neuroinflammatory pathogenesis. We performed two studies that both strengthened the idea that neurogenic inflammation is of importance in CRPS onset. In the first study we found that CRPS patients have more often than controls a medical history of asthma and migraine, both disorders that involve mast cells and neuroinflammatory peptides, such as substance P (SP) and calcitonin gene related protein (CGRP). In the second study, we found a strong association between CRPS onset and the use of drugs that inhibit the activity of the angiotensin converting enzyme (ACE). ACE is a known metabolizer of SP and bradykinin (involved in inflammation and periphere nociceptor sensitization) and thereby a potential modulator of neuroinflammatory responses. SP, CGRP, bradykinin and mast cells have all been demonstrated to be abnormally expressed or activated in CRPS patients[27-30] and many clinical features of CRPS are consistent with the effects of neuropeptides.[31] Moreover, symptom relieve in CRPS patients can be achieved by compounds that interfere with the release or activity of SP[32,33] and CRPS patients respond stronger in experimentally induced neurogenic inflammation.[34,35] Our results affirm the previous findings from experimental and (pre-) clinical studies by showing that in an epidemiological perspective there is indeed a connection between the risk of CRPS and the presence of exaggerated neuroinflammatory processes.

Estrogens are also known to affect inflammatory responses. The high incidence of CRPS in women compared to men and the increasing incidence during the post-menopausal years of life have both raised the interest toward a potential role of estrogens in the immunologic mechanisms that underlie CRPS. However, in our case-control study we demonstrated no association between cumulative endogenous estrogen exposure and the risk of CRPS. Moreover, CRPS onset in pre-menopausal women was not affected by the use of oral contraceptive drugs. Oral contraceptives influence the actual endogenous estrogen levels only very moderately, and may therefore hardly affect eventual inflammatory responses. On the contrary, hormonal replacement therapy (HRT) in post-menopausal women affects endogenous estrogen levels to a much larger extent. We observed a probable protective effect of HRT use on the risk of CRPS, but the results were not significant due to low power in study. Additionally, a protective effect of preganancy (high estrogen levels) was suggested by our results, as well as an increased risk of CRPS shortly after delivery (drop of estrogen levels), although again this was based on very small numbers. Unfortunately the power issues withholds us from drawing sound conclusions, but the actual observations do warrant the further exploration of the role of estrogens in the pathogenesis of CRPS.

The role of NFκB

In the past two decades the nuclear transcription factor κB (NFκB) has gained major scientific attention as a mediator in inflammatory responses and in cell survival mechanisms. NFκB resides inactive in the cytoplasm of many cell types and becomes activated when its inhibitory protein (IκB) is cleaved off by phosphorylation through the enzyme IKK. A wide variety of stimuli can induce NFκB activity, including

bacterial and viral products (via Toll like receptor activation), cytokines, growth factors, neurotransmitters, neuropeptides, reactive oxygen species (ROS), physical stress, and various chemical stimuli.[36] Upon activation, NFκB forms dimers that translocate to the cell nucleus, where it regulates the expression of over 150 target genes.[36] NFκB has been attributed a role in the disease mechanisms underlying acute and chronic inflammatory responses, cancer and neurodegenerative disorders.[37]

In order to find previously unacknowledged potential mediators in CRPS we used an automated information retrieval tool (PathwayAssist™) to overview all abstracts in Medline. We discovered a high connectivity between NFκB and the assumed disease mechanisms of CRPS, including inflammation, ischemia/reperfusion injury and sensitization. This leaded to the idea that NFκB may be a common mediator in the multifactor origin of CRPS. We explored this hypothesis further by using an animal model for CRPS type I, known as the chronic post ischemia pain (CPIP) model. Involvement of NFκB in this model was demonstrated in two ways. First, NFκB levels were increased in muscle and spinal cord at the affected side of CPIP rats compared to controls. Second, systemic administration of an NFκB inhibitor (pyrrolidine dithiocarbamate) relieved mechanical and cold allodynia in a dose-dependent way.

The potential unifying role of NFκB in the pathogenesis of CRPS may provide, once further explored, new pharmacotherapeutic opportunities. Beneficial results have already been observed from treatment with non-specific NFκB inhibitors such as N-acetylcysteine and vitamine C. Targeting NFκB specifically has been suggested as a promising strategy for autoimmune diseases, neurodegenerative disorders and cancer. Perhaps CRPS would be another candidate for such treatment. However, targeting the NFκB pathway may also prove a complex and dangerous effort.[37] NFκB mediated responses are regulated in a complex network of positive and negative feedback mechanisms. Interference might disturb the physiological balance between cell stimulation (inflammation) and cell death (apoptosis), which can lead to immunosupression as a dangerous adverse effect.

Methodological considerations

Setting

All the epidemiologic population based studies that are described in the second and third chapter of this thesis have been performed within the Integrated Primary Care Information (IPCI) project, a Dutch general practice database that is maintained by the Medical Informatics department of the Erasmus Medical Center. The database is representative of the general population of The Netherlands regarding age and gender distribution.[38] Since in the Dutch health care system the GP acts as a gatekeeper and information receiver for secondary care[39], the GP journals can be considered to store the necessary medical information of the patients. To maximize completeness of the data GPs participating in the IPCI project do not keep additional paper records, except for specialist letters, that are available for the investigators on request. The data have proven valid for (pharmaco)-epidemiological studies.[40]

The large source population in the database provided the opportunity to identify a relative large subset of CRPS patients, as well as controls with detailed matching on injury. A unique feature of the IPCI project is that, with mediation of the GP, patients

can be contacted and visited, which allowed for diagnostic validation and additional data collection. The population based setting makes the results representative for CRPS patients in general.

Study population: cases and controls

All CRPS patients and controls were derived from the source population in the IPCI database. The final study population varied per study as is depicted in figure 1.

In any study on CRPS, validation of the cases is an issue of major concern. The absence of a reasonably sensitive and specific biomarker leaves the clinical presentation as a single basis for diagnosis. The interpretation of the findings during anamnesis and physical examination[41,42], the choice for a specific set of criteria[43], and the time of clinical evaluation all affect the final case definition.[44]

For patients identified in the IPCI database diagnostic validation was of particular concern since it had to be done in retrospect. However, by using multiple sources for the gathering of clinical information, including the electronic GP journals, requested specialist letters, and patient reports when a visit was possible, we were able to apply the IASP diagnostic criteria for CRPS relatively accurate. Moreover, at the time of CRPS onset, the diagnosis was confirmed by a medical specialist in almost 80% of the cases. We excluded all cases for whom the history of symptoms appeared questionable during the visit, especially when a potential alternative diagnosis was not sufficiently investigated.

Controls were matched to cases on age (year of birth) and gender. Moreover, controls were required to have experienced an identical injury as their matching case within a two year band of calendar time. This meant that each case with a fracture was compared to controls with a fracture; each case with a soft tissue injury to controls with a soft tissue injury, etc. Since CRPS generally occurs after an injury, matching on this risk factor is important in order to properly study other determinants of interest. A detailed injury matching was possible due to the large number of patients in the GP database. We believe that matching on injury has been a unique and strong feature of our studies.

Observational studies: selection, bias and confounding

Using a population based GP source population we intended to generate knowledge that is representative of all CRPS patients. However, by visiting only a subset of cases confirmed by the GP for additional data collection, some selection has been introduced. This was inevitable since 17% could not be traced for invitation and another 32% refused participation. Despite our clear statements in the invitation letter that participation was relevant regardless CRPS severity we suspect that well recovered patients were less eager to cooperate than patients with ongoing complaints. In this sense there may be some selection of severe cases present in our study population, although presumably this is much less than in a hospital based setting. We believe this selection is however confined, as participants and non participants were not significantly different on characteristics as age, gender and medical history.

In association studies bias can be introduced by misclassification of the determinants or the outcome (or both). In our studies information on the determinants of interest (prior to CRPS history, medication use) was retrieved from the electronic GP journals. As the data registration by GPs originally serves a care and not a scientific purpose

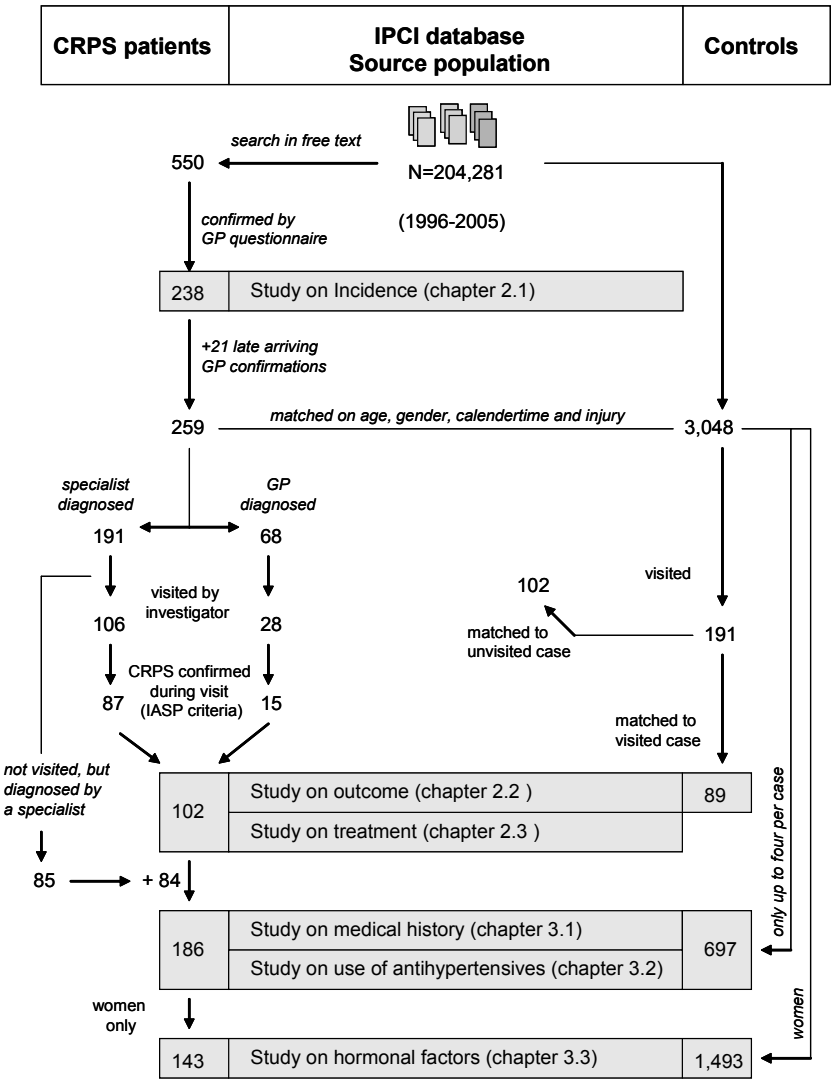

Figure 1. Flow chart for the inclusion of the study populations for different studies described in chapters 2 and 3 of this thesis.

some misclassification will be of relevance. However, since the registration occurred in a prospective manner by the GP before the onset of CRPS (both patient and GP were unaware of the patients' susceptibility to CRPS) this misclassification is probably random and should therefore lead only to an underestimation of the true associations. Misclassification of antihypertensive drug use according to the GP prescription records was investigated by comparison with pharmacy dispensing lists that were available for 19% of the patients in the study population. This revealed a sensitivity of 81% and a specificity of 99%, indifferent between cases and controls. Misclassification of the outcome (CRPS diagnosis) was limited by the extensive case validation procedure. Moreover sensitivity analyses were always conducted, wherein only cases confirmed during a visit were included (most valid diagnosis).

Confounding occurs when a variable is associated with the determinant of interest as well as the outcome, while not being part of the causal pathway. It leads to an incorrect estimate of the association under study. The most well known risk factors for CRPS are age, gender and physical trauma, all of which we dealt with by matching. Relevance of other potential confounders, such as prespecified diseases and concomitant use drug use, was tested by including them in the multivariate conditional logistic regression model and observing the change in the odds ratio's. Effect modification was studied by performing subgroup analyses on gender and on warm and cold type CRPS, and by including interaction terms in the regression model.

Automated information retrieval

The large and rapidly expanding amount of biological information stored in scientific databases demands for advanced methodologies of analysis and interpretation. The extensive data quantity that is generated in the genomics and proteomics area needs to be integrated with clinical and epidemiological knowledge. Bioinformatics tools are designed to aid in these complex efforts and are increasingly applied, for example in autoimmunity research (immunoinformatics)[45] and drug development (toxicogenomics).[46] PathwayAssist™ is such a (commercially available) bioinformatics tool, developed for navigation and analysis of biological pathways, gene regulation networks, and protein interaction maps.[47] It has been applied in many different manners with the purpose to increase the insight in the underlying molecular mechanisms of normal physiological responses and several diseases.[48]

We used PathwayAssist™ and its incorporated text mining module MedScan to retrieve information on the interactions between prespecified CRPS concepts (mediators, receptors, drugs), from all abstracts available in Medline. The emerging visualized network enabled us to see connections that previously had been overlooked due to information overload. Having better overview, we recognized the central position of NFκB, a transcription factor that had never been connected with CRPS before. Our methodology, using text mining and automated information retrieval to obtain new hypotheses on a clinical problem, was experimental. As we found a new direction for further research, we might conclude that in our particular case the approach was successful. However, we did not demonstrate generalizability of this methodology in the sense that automated information retrieval will always aid in the development of new ideas for clinical or biomedical research. Repeated efforts performed with other poorly understood diseases are recommended to demonstrate the general benefit.

CPIP model

Several animal models have been applied to study the disease mechanisms of CRPS. Some of these are general models for neuropathic pain, such as the sciatic nerve section[32] model and the chronic constriction injury model.[49] CRPS symptoms in these models follow upon actual nerve injury and therefore they are more representative for CRPS type II, as in human this is defined by the presence of a nerve lesion. Animal models whereby symptoms were induced without direct nerve injury, similar to human CRPS type 1, included straight forward tibia fracture and casting[50], local infusion with a free radical donor[51], intra-arterial infusion of SP[52], and local ischemia/reperfusion (IR) injury.[53]

The IR injury model, known as the chronic post ischaemia pain (CPIP) model, had several advantages above the others, which made it preferable for our study on NFκB involvement in CRPS: 1. since there is no nerve injury it reflects CRPS type I; 2. both inflammatory and sensory aspects of human CRPS are reflected in the model; 3. pathology is induced by physical injury (instead of chemical infusion), resembling a physical trauma in human CRPS; 4. significant allodynia is present in approximately 70% of the animals as early as 48 hours after IR injury. This high response rate limits the number of animals needed to be included, while the rapid onset of allodynia confines the duration of experiments. 5. CPIP animals respond poorly to treatment with standard analgesics, similar to human CRPS.[54]

However, being an animal model, extrapolation of the results to human should occur with appropriate reserve.

Conclusions from the thesis

We provided population based descriptions of the incidence, disease outcome and treatment of CRPS in the Netherlands. Our results suggest that CRPS occurs more frequently than previously thought and that its disease outcome is less favorable than assumed, since the far majority still has complaints after two or more years of disease duration. This somewhat unfortunate reality emphasizes the relevance of further efforts to improve the understandings of CRPS. Hopefully this will result in better prevention and treatment opportunities that offer eventually a more optimistic perspective.

Our case-control comparisons revealed substantial new indications that neurogenic inflammation is of relevance in CRPS, while recent experimental studies and clinical observations point in the same direction.[31] Mediators of neurogenic inflammation may be interesting targets for pharmacotherapy, since it is a plausible idea that early confinement of the neuroinflammatory responses prevents further damage. However the exact interaction between peripheral (neuro-) inflammatory mechanisms and central nervous system alterations remains unclarified. Advanced information retrieval methods led to the speculation that NFκB might play a role in this, being a mediator in both (neuro-) inflammatory and neuropathological disease mechanisms. The results from an animal (CPIP) study confirmed the actual involvement of NFκB in the pathogenesis of CRPS.

Proposed further research

An issue of major concern in all human CRPS research remains the ongoing vagueness in CRPS definition and the differentiation of potential subtypes. The distinction between type I and type II has become debatable, since sub-clinical nerve injury may commonly be present after fractures or other injuries. Also discrimination between the warm and cold type may not always accurately reflect the underlying disease mechanisms as inflammation and autonomic dysregulation. However, as long as the diagnosis and classification of patients is suboptimal, study populations will remain heterogeneous, which makes it difficult to find determinants, treatment effects or biomarkers. Therefore, a strong need exists for prospective studies wherein symptoms and signs are assessed in a large cohort of post-trauma patients. Cluster analysis techniques could aid in pattern recognition, which would eventually derive CRPS subtypes and also discriminate between CRPS and physiological trauma responses. Ideally, the clinical spectrum of CRPS could be linked to a (composition of) biomarker(s).

Because of retrospective symptom/sign assessments the study design of the present thesis was inconvenient for improving clinical definition and phenotype classification. However, this would be possible if a prospective setup could be embedded within the IPCI project. IPCI is unique above most other GP databases, as it allows patient contact mediated by the GP. If the GP could alert the investigator each time CRPS is diagnosed or suspected, patients could be visited for clinical assessments early after disease onset (preferably within three months). Repeated symptom/sign evaluations from that moment onwards would provide accurate disease course descriptions, which are lacking until today. Technical improvements are expected to allow expanding of the IPCI database with new participating GPs and the perspective is to have one million patients in active follow-up by 2009. Based on our estimated incidence rate this would imply 260 new cases in IPCI each year. Considering a participation rate of 60% (as in the present thesis), theoretically a four year follow-up period would yield over 500 early assessed cases from a population based setting. However, the complex logistic issues bound to such a setup are not to be underestimated and require careful consideration in advance.

Once CRPS is better defined and classified, the further study of risk factors and determinants should be resumed. Eventually, this may improve anticipation of CRPS onset in high risk patients, as well as the understanding of underlying mechanisms of disease. A specific genetic profile could be such a determinant, for example one that predisposes for neurogenic inflammation or rapid sensitization. Also trauma related factors (trauma type, immobilization) and specific patients' characteristics (age, gender, medical history, drug use, life style, etc) may be relevant. A GP database as IPCI is highly convenient for such studies because much information on determinants of interest is usually recorded by the GP. Patient visits make additional data collection possible, for example through questionnaires or by obtaining body materials (blood, sputum, urine, skin, etc.). Also from patients who were visited in regard of the present thesis venous blood has been obtained and stored. Further analysis of these samples could include genome-wide association assays, determination of infection history, and assessment of autoimmunity markers.

Although the contribution of (neurogenic) inflammation to CRPS remains to be clarified further, results until this far warrant clinical attempts to attenuate exaggeration of this disease mechanism. For example, corticosteroids have occasionally been shown effective in the treatment of CRPS[55] and well performed randomized blinded clinical trials could prove its true benefit. Corticosteroids are notorious for their adverse effects, but this has not restrained physicians from applying them in other inflammatory disorders. Another promising pharmacotherapy option in this view is provided by free radical scavengers, as ROS are common triggers as well as products of neurogenic inflammation. Apart from confirming the positive results from previous trials, the relevance of timing (i.e. administration early in the disease course) could be studied, as well as the costs and benefits of standardized high dose vitamin C prescription after fractures. Finally, drugs that prevent sensitization are of interest, such as inhibitors of SP (may also be effective against a neurogenic inflammation) and NK-1 or NMDA receptor antagonists.

On a more fundamental level, the exact interaction between the several disease mechanisms involved in CRPS demands further exploration. NFκB might be a linking factor, but although its involvement is supported by observations in the CPIP model, the actual connection between NFκB activity and the expression of other mediators in CRPS has not yet been demonstrated. As NFκB potentially promotes the transcription of over 150 genes, it would be useful to understand which of these are particularly promoted in CRPS, and at which point in the disease process. Additionally, mechanistic questions can be raised regarding the interference with the NFκB pathway for therapeutic purposes. This can be done on different levels and the optimal target should be determined: it may be NFκB itself, but also one of its effector products such as substance P or TNFα. Moreover, the safety of such treatment is an issue of concern.

More than a century has past since Sudeck published the first article on “die akute untzündliche Knochenartrophie”. Since then many physicians and scientists have attempted to unravel the complex etiology and slowly we progress to a better understanding of CRPS, but we need to keep on searching.

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Summary

The complex regional pain syndrome (CRPS) is a painful and potentially disabling complication of a physical injury, for example a fracture or contussion. It usually occurs in the distal end of the affected extremity and is marked by a variety of symptoms, including pain and sensory abnormalities, vasomotor and sudomotor disturbances, and motor/trophic impairments. Although the general insights in CRPS have increased over the past decade, there is still limited understanding of the actual etiology, pathogenesis, risk factors, and prognosis.

The aim of this thesis is briefly introduced in **chapter 1.1** as to gather more insight in CRPS as a clinical entity and into its pathogenesis and etiology. In **chapter 1.2** we provide a review of the current knowledge regarding the underlying disease mechanisms, including disturbances in the autonomic (sympathetic) and the somatic nervous system, neurogenic inflammation, hypoxia, and psychological factors. Mediators involved in all these separate mechanisms are likely to interact and thereby to contribute all together to the complex, multifactor pathogenesis of CRPS. Additionally, we summarize currently known risk factors and emphasize the need for further epidemiologic studies to reveal determinants of CRPS.

Chapter 2 comprises three descriptive studies on CRPS in the Netherlands. All studies were conducted in a cohort of CRPS patients who were identified in a general practice database, the Integrated Primary Care Information (IPCI) project. In **chapter 2.1** we describe a population based incidence rate (IR) calculation for CRPS, revealing an IR of 26 per 100.000 person years. The mean age at CRPS onset was 52 years and the peak incidence occurred between the fifth and seventh decade. Women were affected 3.4 times more often than men. The most common precipitating event was a facture, (44%), followed by a sprain (18%) and surgery (12%). In a minority (10%) of patients CRPS had developed spontaneously.

In **chapter 2.2** we studied the outcome of CRPS. We assessed symptoms and signs in 102 patients at on average 5.8 years (range: 2.1-10.8) after CRPS onset. More than 80% of the patients still reported CRPS symptoms (subjective) that could be objectified (signs) in over 60%. This was significantly more than in reference patients with a similar precipitating injury but no CRPS. Symptom and sign prevalences were not associated with time since onset. In addition to clinical features, we studied other parameters of CRPS outcome: 64% of the patients still fulfilled the IASP diagnostic for CRPS; 27% received ongoing treatment. Based on self reports, 30% of the patients was recovered, in 54% CRPS was stabilized, and 16% suffered from progressive severe disease. Of the patients that had a job at the moment of CRPS onset, 31% had become completely incapable to work, while 28% worked with adjustments. Finally, with the purpose to distinct patients further by disease outcome, we performed a K-means cluster analyses on sign and symptom prevalences. Of the three emerging clusters, one clearly represented the poorest outcome subgroup. Poor outcome was associated with an affected upper extremity, an atypical precipitating injury (no fracture), and cold CRPS.

Chapter 2.3 addresses the referral and treatment patterns for CRPS in the Netherlands from 1996 until 2005. CRPS patients received a large diversity of treatments from many

different physicians. Scavengers and common analgesics were applied early in the disease course, while vasodilative drugs and anti-neuropatics were prescribed later on. More that 90% of the patients received additional non-pharmacological treatments, such as physiotherapy. A minority (18%) received invasive pain blocks. Most (63%) patients consulted the GP before referral to a medical specialist. Dutch guidelines for CRPS treatment were released in 2006 (after the study period) and recommend free radical scavenger prescription as general first treatment step for all patients, combined with analgesics and physiotherapy. This therapy is harmless and since an early start may favor the prognosis of CRPS, we would encourage GPs and other physicians to commence the therapy in an early stadium, while waiting further evaluation by a CRPS specialist.

Chapter 3 includes three case-control studies, which were also performed in IPCI, regarding potential determinants of CRPS. In these studies the controls were matched to the cases on type of injury, additional to age and gender. First, in **chapter 3.1**, we evaluated the medical history of CRPS patients by reviewig the complete electronic GP records. CRPS patients more often had a prior to CRPS history of migraine (OR: 2.43, 95%CI: 1.18-5.02) and asthma (OR: 3.0, 95%CI: 1.3-6.9). Neuro-inflammatory mediators, for example substance P (SP), are involved in both disorders, which may connect them to CRPS. Additionally, a history of osteoporosis (OR: 2.44, 95%CI: 1.17-5.14), menstrual cycle related disorders (OR: 2.60, 95%CI: 1.16-5.83) and neuropathies (OR: 5.7, 95%CI: 1.8-18.7) was more common in CRPS patients, suggesting that these are risk factors. In contrast to previous suggestions, psychological factors were not associated with CRPS.

Since some antihypertensive drugs interact with mediators or disease mechanisms in CRPS (neuropeptides, adrenergic receptors, vasoconstriction), we studied their association with CRPS onset in **chapter 3.2**. Current use of ACE inhibitors was associated with CRPS onset (OR: 2.7, 95%CI: 1.1-6.8) and the risk increased even further with a longer duration of use and with a higher dose. Calcium channel blockers, angiotensin II receptor antagonists, β-blockers, and diuretics did not affect the risk of CRPS onset. Since ACE is involved in the metabolism of SP and bradykinin we hypothesize that ACE inhibitor induced accumulation of these peptides facilitates the neurogenic inflammation underlying CRPS.

The pronounced female predominance of CRPS, as well as the increasing incidence in women at post-menopausal age, leaded us to investigate the relation between CRPS and estrogen exposure in **chapter 3.3**. Cumulative estrogen exposure was assessed as the ages of menarche and menopause, menstrual life, parity, and cumulative time of pregnancy and breast feeding. None of these measures were significantly associated with CRPS. Actual estrogen exposure, defined by the current use of oral contraceptive drugs or hormonal replacement therapy (HRT), did not significantly affect the risk for CRPS either, although HRT use showed a non significant protective effect (OR: 0.5, 95%CI: 0.1-1.6) During the first six months post partum (fast decreasing estrogen levels) CRPS onset was increased (OR:5.6, 95%CI: 1.0-32.4). However, power limitations demand cautious interpretation of the results. The overall results are in line with those for other painful inflammatory disorders with a similar gender distribution pattern, such as rheumatoid arthritis and multiple sclerosis.

Chapter 4 describes the development and testing of a new hypothesis on the molecular pathogenesis of CRPS. First, elaborated on in **chapter 4.1**, we used the text mining based information retrieval tool PathwayAssist™ to visualize a Medline based network of interactions between known concepts of relevance in CRPS (proteins, small molecules, drug, etc). This network provided the overview that was needed to recognize the central position of the nuclear transcription factor κB (NFκB) amongst acknowledged other mediators in CRPS. NFκB has been attributed an important role in inflammatory disorders, for example in rheumatologic diseases and asthma. Although it has never been connected to CRPS before, its involvement in the disorder is theoretically very plausible.

This hypothesis was tested in an animal model for CRPS, which is decribed in **chapter 4.2**. In the chronic post ischemia pain (CPIP) model CRPS-like symptoms are induced in rats by ischemia and reperfusion (IR) injury of the left hind paw. At 2 and 48 hours post IR injury, we observed increased NFκB activity (using ELISA) in the muscle and spinal cord of the affected sides of CPIP rats compared to shams. At day 7 post IR injury NFκB activity in muscle was equal between CPIP rats and shams, while in the spinal cord of CPIP rats the NFκB levels sustained increased. Additionally, the mechanical and cold allodynia that developes following IR injury could be reversed in a dose dependent manner by the systemic administration of an NFκB inhibitor (pyrrolidine dithiocarbamate). The results suggest a contribution of NFκB to allodynia in CPIP rats, and perhaps to human CRPS.

Finally, in **chapter 5** the main results and methodological aspects are discussed. Suggestions for further research are provided.

Samenvatting (in Dutch)

Het complex regionaal pijn syndroom (CRPS) is een pijnlijke en soms invaliderende complicatie van een lichamelijk letsel, bijvoorbeeld een fractuur of contussie. Het komt meestal voor distaal in de aangedane extremiteit en kan leiden tot veel verschillende symptomen, waaronder pijn en sensorische afwijkingen, maar ook vasomotore, sudomotore en motor/trofische stoornissen. Hoewel er in het afgelopen decenium steeds meer over CRPS bekend is geworden, bestaat nog steeds veel onduidelijkheid omtrent de etiologie, pathogenese, risicofactoren en de prognose.

Het doel van dit proefschrift wordt kort geïntroduceerd in **hoofstuk 1.1**, waar het wordt omschreven als het verkrijgen van meer inzicht in het algemene voorkomen van CRPS en in de onderliggende pathogenese en etiologie. In **hoofdstuk 1.2** wordt een overzicht gegeven van wat momenteel bekend is over de onderliggende ziektemechanismen. Hierbij komen pathologie van het autonome en somatische zenuwstelsel, neurogene inflammatie, hypoxie en de rol van psychologische factoren aan de orde. Veelvuldige interactie tussen de verschillende betrokken mediatoren is waarschijnlijk en dit leidt tot een complexe en multifactoriële etiologie. Ook de tot nu toe bekende risicofactoren voor CRPS worden besproken en het belang van toekomstig epidemiologisch onderzoek wordt benadrukt.

Hoofdstuk 2 omvat drie beschrijvende studies over CRPS in Nederland. Deze studies werden verricht in een cohort van CRPS patiënten die waren geïdentificeerd in een huisartsendatabase, het 'Integrated Primary Care Information' (IPCI) project. In **hoofdstuk 2.1** beschrijven we een incidentie berekening voor CRPS, die uitkomt op een schatting van 26 nieuwe patiënten per 100.000 persoonsjaren. De gemiddelde leeftijd waarop patiënten CRPS kregen was 52 jaar en de incidentie was het hoogt in de leeftijd van 50 tot 70 jaar. Vrouwen kregen 3.4 keer vaker CRPS dan mannen. Een fractuur was het meest voorkomende voorafgaande letsel (44%), gevolgd door een contussie (18%) en een operatie (12%). In een minderheid (10%) had CRPS zich spontaan ontwikkeld.

Hoofdstuk 2.2 behandelt het herstel van CRPS. De subjectieve en objectieve symptomen werden onderzocht in 102 patiënten met een gemiddelde tijdsduur van 5.8 jaar (spreiding 2.1-10.8) sinds het ontstaan van CRPS. Meer dan 80% rapporteerde nog steeds CRPS klachten, die in meer dan 60% ook konden worden geobjectiveerd. Dit was significant meer dan in controle patiënten die hetzelfde letsel hadden gehad, maar geen CRPS. Binnen de groep van CRPS patiënten waren de klachten niet afhankelijk van de ziekteduur. Naast het klinisch beeld werden ook een aantal andere operationalisaties van herstel bekeken: 64% van patiënten voldeed nog steeds aan de IASP criteria voor CRPS; 27% kreeg nog steeds enige vorm van therapie. Zelf raporteerde 30% van de patiënten een goed herstel, 54% beschouwde de aandoening als gestabiliseerd en 16% ondervond nog steeds achteruitgang. Van degenen die voorafgaand aan de CRPS een baan hadden, had 31% deze blijvend moeten opgeven vanwege CRPS, terwijl 28% alleen aangepast werk kon verrichten. Om verder onderscheid te maken ten aanzien van herstel werd een cluster analyse verricht op basis van de aanwezigheid van subjectieve en objectieve symptomen. Van de drie verkregen clusters omvatte één duidelijk slechter herstelde patiënten ten opzichte van de andere twee. Slecht herstel was geassocieerd met CRPS

aan de bovenste extremiteit, een atypisch voorafgaand letsel (geen fractuur) en de koude vorm van CRPS.

Hoofdstuk 2.3 gaat over de verwijs- en behandelpatronen van CRPS in Nederland tussen 1996 en 2005. CRPS patiënten in Nederland kregen veel verschillende behandelingen die werden voorgeschreven door een variëteit aan behandelaars. Scavengers, of wel 'vrije radicaal wegvangers' en algemene pijnstillers werden vooral voorgeschreven vroeg in het ziektebeloop, terwijl vaatverwijdende medicatie en co-analgetica pas later werden gestart. Meer dan 90% van de patiënten onderging tevens niet farmacologische behandelingsvormen, zoals fysiotherapie. Een minderheid (18%) onderging pijn blokkades. De meeste patiënten (63%) bezochten eerst de huisarts voordat ze voor de CRPS een medisch specialist bezochten. In 2006 (na de studie periode) is in Nederland een behandelingsrichtlijn voor CRPS verschenen, waarin een algemene eerste stap wordt geadviseerd voor alle patiënten. Deze stap omvat, naast fysiotherapie en pijnstilling, het voorschrijven van een scavenger met als doel de beschadiging, die wordt veroorzaakt door vrije radicalen, te ondervangen. Omdat deze vorm van therapie relatief onschuldig is en een vroege start wellicht het ziektebeloop ten goede komt, zouden huisartsen, maar ook specialisten, deze behandeling in een vroeg stadium moeten beginnen, terwijl het oordeel van een geconsulteerd CRPS specialist verder wordt afgewacht.

In hoofdstuk 3 worden drie case-control studies besproken waarin werd gekeken naar mogelijke determinanten van CRPS, allen verricht in IPCI. In deze studies werden de controles gematched met de cases voor de aard van het voorafgaande letsel, alsook voor de leeftijd en het geslacht. In **hoofdstuk 3.1** werd gekeken naar de medische voorgeschiedenis, waarbij gebruik werd gemaakt van het complete electronische dossier van de huisarts. CRPS patiënten hadden vaker dan controle personen migraine (OR: 2.43, 95%CI: 1.18-5.02) en astma (OR: 3.0, 95%CI: 1.3-6.9) in de voorgeschiedenis. Neuro-inflammatoire eiwitten, bijvoorbeeld substance P (SP), spelen een rol in beide aandoeningen, wat hen mogelijk in verband brengt met CRPS. Ook osteoporose (OR: 2.44, 95%CI: 1.17-5.14), menstruele cyclus gerelateerde klachten (OR: 2.60, 95%CI: 1.16-5.83) en neuropathieën (OR: 5.7, 95%CI: 1.8-18.7) kwamen frequenter voor bij CRPS patiënten en zouden daarom als risicofactoren kunnen worden beschouwd. In tegenstelling tot wat in het verleden is gesuggereerd, bleek het risico op CRPS niet geassocieerd met psychologische factoren in de voorgeschiedenis.

Sommige antihypertensiva hebben een effect op mediatoren en mechanismen die een rol kunnen spelen in CRPS (neuropeptiden, adrenoreceptoren, vasoconstrictie). Daarom werd in **hoofdstuk 3.2** de associatie tussen het gebruik van antihypertensiva en CRPS onderzocht. Het gebruik van ACE-remmers was geassocieerd met een verhoogd risico op CRPS (OR: 2.7, 95%CI: 1.1-6.8). Dit effect nam toe bij langdurig gebruik en bij hoge dosering. Andere antihypertensiva, waaronder calcium antagonisten, β-blockers, angiotensine-II receptor antagonisten en diuretica, waren niet geassocieerd met CRPS. ACE is van belang voor het metabolisme van de neuroinflammatoire eiwitten SP en bradykinine. Via accumulatie van deze eiwitten kunnen ACE-remmers mogelijk de neuroinflammatore processen in CRPS patiënten faciliteren.

Omdat CRPS een duidelijk vrouwelijke predominantie kent, alsmede een verhoogde incidentie op post-menopausale leeftijd, werd in **hoofdstuk 3.3** de relatie tussen CRPS en oestrogenen onderzocht. De cumulatieve expositie aan endogene oestrogenen werd

benaderd op basis van de leeftijd van menarche en menopauze, de menstruele levensduur, de pariteit en het totaal aantal maanden zwangerschap en borstvoeding. Geen van deze maten bleek echter geassocieerd met het risico op CRPS. Expositie aan oestrogenen ten tijde van de start van CRPS werd onderzocht door te kijken naar het gebruik van orale anticonceptiva (in pre-menopausale vrouwen) of van hormonale suppletie therapie (in post-menopausale vrouwen). Ook hierbij werden geen significante associaties met CRPS gevonden, hoewel een niet-significant beschermend effect werd gezien tijdens het gebruik van hormonale suppletie therapie (OR:0.5, 95% betrouwbaarheids interval 0.1-1.6). In de eerste zes maanden post partum (snelle daling oestrogeen spiegels) was het risico op CRPS verhoogd (OR:5.6, 95%CI: 1.0-32.4). Door de lage aantallen moeten resultaten van deze studie terughoudend worden geïnterpreteerd. Echter, de bevindingen zijn grotendeels in overeenstemming met die voor andere pijnlijke inflammatoire aandoeningen die eveneens een vrouwelijke predominantie kennen, zoals rheumatoïde artritis en multipele sclerose.

Hoofdstuk 4 beschrijft de ontwikkeling en het verdere onderzoek van nieuwe ideeën over de moleculaire achtergrond van CRPS. In **hoofdstuk 4.1** bespreken we hoe een 'text mining' applicatie werd toegepast (PathwayAssist™) om de interacties tussen CRPS concepten (eiwitten, kleinere moleculen, medicijnen) te visualeren in een netwerk, waarbij Medline als onderliggende informatie bron werd gebruikt. Het overzicht in het netwerk maakte het mogelijk de centrale positie van de nucleaire transcriptie factor κB (NFκB) te herkennen tussen de al bekende bij CRPS betrokken mediatoren. NFκB wordt al langer verondersteld een belangrijke rol te spelen in inflammatoire ziekten, bijvoorbeeld rheumatische aandoeningen en astma. Een verband met CRPS was nog niet eerder gesuggereerd, maar is theoretisch aannemelijk, gezien de veelvuldige interactie met andere mediatoren.

Deze hypothese werd onderzocht in een dier model voor CRPS, zoals beschreven in **hoofdstuk 4.2**. In het 'chronic post ischemia pain' (CPIP) model ontwikkelen ratten CRPS-achtige verschijnselen na een periode van ischemie en reperfusie (IR) in de linker achterpoot. Zowel 2 uur als 48 uur na de IR periode werd een verhoogde NFκB activiteit (ELISA) gevonden in spier en ruggemerg weefsel aan de aangedane zijde van de CPIP ratten, in vergelijking tot controle ratten. Op dag 7 na de IR periode was de NFκB activiteit het spierweefsel gelijk voor CPIP en controle ratten, maar nog steeds verhoogd in het ruggemerg van CPIP ratten. De door de IR periode veroorzaakte mechanishe en koude allodynie in CPIP ratten verbeterde dosisafhankelijk na toediening van een NFκB remmer (pyrrolidine dithiocarbamaat). De resultaten wijzen op een rol van NFκB in het CPIP model en daarmee mogelijk ook in CRPS bij mensen.

Tenslotte worden in **hoofdstuk 5** de hoofdresultaten en methodologische aspecten besproken. Tevens worden enige suggesties gedaan voor toekomstig onderzoek.

Abbreviations

n.a.	not applicable
ACE	Angiotensin Converting Enzyme
AMPA	Apha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of Variances
ATC code	Anatomical Therapeutical Chemical code
AT2	Angiotensin-II
CGRP	Calcitonin Gene Related Peptide
CI	Confidence Interval
CNS	Central Nervous System
CPIP	Chronic Post Ischemia Pain
CRPS	Complex Regional Pain Syndrome
DDD	Defined Daily Dose
DM	Diabetes Mellitus
DMSO	Dimethylsulfoxide
ELISA	Enzyme Linked Immuno Sorbent Assay
ET-1	Endotheline-1
GP	General Practitioner
HLA	Human Leukocyte Antigen
HRT	Hormonal Replacement Therapy
IASP	International Association for the Study of Pain
IL	Interleukin
IPCI	Integrated Primary Care Information
IR	Incidence Rate
IR injury	Ischemia and Reperfusion injury
MS	Multiple Sclerosis
NEP	Neutral Endopeptidase
NFκB	Nuclear Factor κB
NK-1	Neurokinin 1
NMDA	N-Methyl-D-Aspartate
NO	Nitric Oxide
NPY	Neuropeptide Y
NSAID	Non Steroidal Anti-inflammatory Drug
OC	Oral Contraceptive drugs
OR	Odds Ratio
PDTC	Pyrrolidine Dithiocarbamate
PNS	Peripheral Nervous System
PY	Person Years
RA	Rheumatoid Arthritis
ROS	Reactive Oxygens Species
SMP	Sympathetically Maintained Pain
SMR	Standardazid Morbidity Ratio
SP	Substance P
SSRI	Selective Seretonin Reuptake Inhibitor
TCA	Tricyclic Antidepressant
TENS	Transcutaneous Electronic Neurostimulation
TNF	Tumor Necrosis Factor
TP53	Tumor Protein 53
VIP	Vasoactive Intestinal Protein

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Curriculum Vitae

The author was born on the 3th of June 1978 in Voorburg, the Netherlands. After obtaining secondary education at the Gymnasium Haganum in The Hague, she started to study Biomedical Sciences in 1996 and Medicine in 1999 at the University of Leiden, for both of which she graduated in 2002. During her studies she worked as a nursing assistant in a housing facility for psycho-geriatric patients. As part of her education she participated in scientific projects at the departments of Molecular Cell Biology, Infectious Diseases and General Practitionering at the University of Leiden. Additionally she obtained the first year diploma for Psychology (University of Leiden) and fulfilled a medical elective course at the Kanti Children's Hospital in Katmandu (Nepal). The Medical Doctor degree was received *cum laude* in August 2004, after finishing the clinical internships. Subsequently, she worked for one year as a resident of Internal Medicine in 't Lange Land Ziekenhuis in Zoetermeer and kept working there occasional shifts afterwards until 2008. In August 2005 she started to work as a PhD student at the department of Medical Informatics and the Pain Treatment Center of the Erasmus Medical Center in Rotterdam, where she performed the research that is described in the present thesis, under the supervision of Prof.dr. Miriam Sturkenboom, Prof.dr. Bruno Stricker, and Dr. Frank Huygen. Most of the studies were performed within the Trauma Related Neuronal Dysfunction (TREND) consortium. One chapter was performed in collaboration with the European Infobiomed consortium and one with the Anesthesia research departement of the McGill University in Montréal (Canada). During her research period she obtained a Master of Science degree in clinical epidemiology at the National Institute of Health Sciences (Nihes). As of October 2008 she has started her medical specialist training in Anesthesiology at the Erasmus Medical Center. Marissa is married to Niels Vrolijk. They live in The Hague (The Netherlands).

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M. de Mos, F.J.P.M. Huygen, J.P. Dieleman, J.S.H.A. Koopman, B.H.Ch. Stricker, M.C.J.M. Sturkenboom. Medical history and the onset of Complex Regional Pain Syndrome. *Pain 2008 (in press)*

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M. de Mos, M.C.J.M. Sturkenboom, F.J.P.M. Huygen. Current understandings on complex regional pain syndrome. *Submitted*

M. de Mos, F.J.P.M. Huygen, B.H.Ch. Stricker, J.P. Dieleman, M.C.J.M. Sturkenboom. The association between ACE inhibitors and the complex regional pain syndrome: suggestions for a neuroinflammatory pathogenesis of CRPS. *Submitted*

J.S.H.A. Koopman, J.P. Dieleman, F.J.P.M. Huygen**, M. de Mos**, C.G.M. Martin, M.C.J.M. Sturkenboom. Incidence of facial pain in the general population *Submitted*

A. Laferrière, M. Millecamps, D.N. Xanthos, W.H. Xiao, C. Siau, **M. de Mos**, C. Sachot, F.J.P.M. Huygen, G.J. Bennet, T.J. Coderre. Deep tissue microvascular dysfunction as a mechanism of persistent cutaneous tactile allodynia. *Submitted*

Post-traumatic dystrophy (PD), morbus Sudeck, algodystrophy, sympathetic reflex dystrophy (SRD), these are just a few of the more than 70 names that over the past have been used to address the poorly understood disorder that sometimes occurs after an injury and that, above a variety of other symptoms, is marked by a persistent, disabling pain, usually described as burning. Today we call it the complex regional pain syndrome (CRPS), a descriptive name that was established in 1995 by the International Association for the Study of Pain with the aim to expel from its nomenclature all speculations about the disease etiology. The name complex regional pain syndrome should be temporary; a new one may be assigned once the nature of the disease will be better understood.

Although we are still far from complete understanding, the insights in the potential underlying disease mechanisms of CRPS have increased significantly over the past decade. The central nervous system was classically assumed to be involved, but nowadays the roles of inflammation and ischemia also have become acknowledged. The influence of stress and other psychological factors is still a subject of interest. Mediators of these mentioned disease mechanisms are likely to interact in a complex network. To unravel this and discover the crucial pathways, it is needed that various types of scientists and physicians collaborate in a multidisciplinary approach.

The present thesis describes the search towards a better understanding of CRPS mainly (but not only) from an epidemiological perspective. Using a population based cohort of CRPS patients that was derived from the Integrated Primary Care (IPCI) project, we described the clinical entity of CRPS (incidence, outcome, treatment) and sought for leads to its etiology through the investigation of determinants and risk factors. Additionally we attempted to develop new mechanistic hypotheses by automated information retrieval and tested a new hypothesis, that involves the role of the transcription factor NFκB, in an animal model for CRPS type 1.