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Distinguishing phenotypes of Complex Regional Pain Syndrome

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Dedicated to my dear partner, my family and all study participants.

Without their support this work would have been impossible.

1. Introduction

1.1. Definition of pain

Pain is a complex and multidimensional sensory phenomenon. Each human being has its own very personal experiences with pain and what it feels like. Some will perhaps think of pain related to severe illnesses such as cancer or complex bone fractures following an accident. Others will mention the pain they felt when seeing a close relative or friend suffering from cancer. However, pain can also be associated with positive memories like the birth of a child or aching muscles after winning a triathlon. These examples illustrate how variable the experience of pain can be. All this makes the development of an exhaustive pain definition a delicate task. The International Association for the Study of Pain (IASP) accepted this challenge and published the first definition of pain in 1978. Since then, the IASP has worked on many revisions as advances in science opened up new perspectives on this special sensory phenomenon. The last update was published in 2020 and defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." [1] This definition highlights that pain is more than just a bare sensation informing our mind of potentially harmful processes but also includes an affective component.

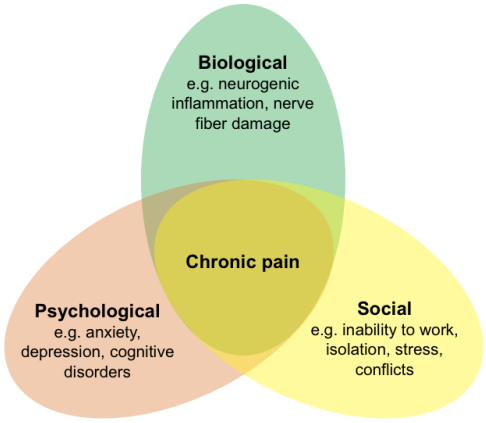


Figure 1. Biopsychosocial model for chronic pain
Biological, psychological and social factors are involved in the pathogenesis of chronic pain

1.2. Differentiation of acute and chronic pain

In general, the ability to perceive pain was an evolutionary advantage and helped our ancestors surviving. This is especially true for acute pain making the differentiation of pain into acute and chronic pivotal. Acute pain often has a protective function by warning

the individual and inducing protective behavior [2]. In acute pain, there is a good understanding of the interplay of nociceptors, ion channels and (supra-) spinal structures [2]. However, pain can also develop into an independent phenomenon leading to chronic pain. Chronic pain disease can be classified as either primary or secondary disease [3]. In primary chronic pain disease, pain is perceived in absence of a causative tissue damage [4]. In secondary chronic pain disease, pain is related to tissue damage such as cancer, surgery or a trauma to just name a few examples. This is also introduced in the current version of the International Classification of Diseases (ICD-11) [3]. From the clinical perspective chronic pain disease is variable not only in the way it presents itself to physicians but also in pathological mechanisms or epidemiological observations. It includes lower back pain or joint pain as frequent causes. Whereas fibromyalgia and also Complex regional pain syndrome (CRPS) are examples for pain diseases that occur less often and presumably have distinct pathologies. General considerations on the chronification of pain are based on the biopsychosocial model (**Figure 1**) [4], which encompasses biological, social and psychological alterations. From a mechanistical, neurobiological perspective three phases of chronification have been proposed [5]. In the first phase, a nociceptive stimulus triggers the perception of pain similar to acute pain perception. The second phase is characterized by peripheral and central sensitization facilitating pain perception and eventually causing hyperalgesia. In phase three, biological modifications – e.g. in gene expression or neuronal damage as seen in neuropathic pain – cause abnormal reactions such as allodynia and persistent pain in absence of a painful stimulus [5]. Social aspects of pain chronification include deprivation and isolation [6] or can be work-related [7]. The psychological aspects of pain chronification are manifold. Involved are behavioral and cognitive mechanisms but also psychological disorders i.e. depression or anxiety [6]. Beside the importance of discovering mechanisms of pain chronification treating chronic pain disease is one of the major challenges in medicine [8]. Modern chronic pain treatment acknowledges the biopsychosocial model by designing multimodal treatments including drug therapy, psychological support, physiotherapy and training as well as occupational therapy. This emphasizes that an improved mechanistic understanding also promotes new treatment strategies.

1.3. Complex regional pain syndrome - CRPS

1.3.1. CRPS as primary chronic pain disease

Complex regional pain syndrome (CRPS) is a primary chronic pain disease [9]. CRPS usually develops after limb injury or fracture. In some cases, the onset of CRPS is preceded by a bagatelle trauma only. Beside chronic pain, CRPS is accompanied by a variety of additional signs and symptoms forming the characteristic clinical picture. Typical symptoms are swelling of the affected extremity, skin color changes, sensory disturbances and motor changes (e.g. dystonia or tremor) [9]. Besides, loss of bone substance is a typical feature of CRPS, which was already described on X-ray images by the German surgeon Paul Sudeck in 1900. He emphasized that despite clinically obvious signs there were more features to be discovered [10]. Few years later, the term sympathetic reflex dystrophy was shaped and first described the theory that vegetative dysfunction was a major pathological cause of this disease. Many descriptive terms have been used before the disease was named CRPS in 1995 [10]. Reliable diagnostic criteria and a uniform terminology helped CRPS research and are the basis for adequate diagnosis and treatment.

CRPS phenomenology is characterized by abnormalities of the somatosensory function. Lowered thresholds for the perception of painful stimuli are described as hyperalgesia whereas elevated sensory thresholds are generally referred to as hypoesthesia. In CRPS, hyperalgesia towards pressure pain and dynamic mechanical allodynia are typical findings and often accompanied by hypoesthesia towards non-painful stimuli. This combination of a gain and loss of function phenotype partially resembles sensory findings in patients with peripheral nerve lesion [11, 12]. However, in these patients, strong hypoesthesia is more common whereas pressure pain hyperalgesia is more pronounced in CRPS patients [11]. A comparison of CRPS patients and patients with arthritis emphasized that sensory disturbances cannot be explained by pain alone, but an additional pathology is needed as explanation for the observed disturbances [13].

Until today, CRPS pathophysiology is the subject of a scientific discussion. However, important aspects of pathophysiology and risk factors have been discovered: Complicated fractures, high pain intensity after the injury and preexisting rheumatological diseases are known risk factors [9]. An interplay of numerous pathological alterations – including

inflammation, central nervous system (CNS) pathology, autoimmunity and vegetative dysfunction – is likely responsible for the development of CRPS. Especially in acute CRPS an inflammatory process including neurogenic inflammation seems to be involved [9, 14, 15]. Clinically, this becomes visible through edema, increased skin temperature and changed skin color. Central nervous aspects of CRPS pathology explain functional impairment in later disease stages [16-20]. In detail, neglect-like symptoms [17] or mirror-like spread of symptoms [21] are most likely of central nervous origin. Neglect-like symptoms in CRPS include involuntary movements of the affected limb or the perception of the limb as foreign or alien. Some symptoms e.g. increased sweating (hyperhidrosis) or edema that are attributed to inflammation in early stages remain even if inflammation is likely to resolve. It is hypothesized that altered sympathetic innervation and an imbalanced vegetative response trigger these symptoms. This is supported by the finding of auto-antibodies targeting adrenergic and muscarinergic receptors [22, 23]. Furthermore, antibody dependent CRPS-like symptoms in animal models suggest the involvement of autoimmune processes [24].

CRPS treatment is often designed as multimodal therapy and follows recommendations of the German Neurological Society (Deutsche Gesellschaft für Neurologie, [25]). Drug therapy typically comprises of conventional analgesics and adjuvant analgesics (antidepressants, anticonvulsants). Steroids are used in early, inflammatory disease stages. Bisphosphonates known from the treatment of osteoporosis or bone metastasis are used because of anti-inflammatory properties and their influence on spinal microglia. Psychotherapy for coping with pain and cognitive behavioral therapy is provided if patients suffer from depression or other psychiatric comorbidities. In occupational therapy it is e.g. mirror therapy that is successfully applied in CRPS patients [26]. Therapeutic blockades of the spinal sympathetic trunk can also lead to symptom amelioration. More invasive treatment options such as spinal cord or dorsal root ganglion stimulation should only be considered in severe or refractory cases. Even though effective treatment is available, symptom control can be challenging and evidence from randomized controlled clinical trials is sparse [9].

1.3.2. Clinical heterogeneity

The large heterogeneity of CRPS pathology requires clinical phenotyping or 'subgrouping' of CRPS patients to streamline treatment. Commonly three subgroups of CRPS are distinguished [27] based on sex, the existence of nerve lesions (absent: CRPS type I, present: CRPS type II) and temperature phenotype. Within subgroups remains considerable variation. Therefore, we formed two additional subgroups. One based on the identity of the affected extremity (e.g. hand or foot) and a second based on the existence of hyperalgesia on the contralateral, non-CRPS extremity to grasp inter-individual differences in sensation. Focusing on subgroups and improving the understanding of the variable clinical picture can be an important step in the direction of personalized treatment. It can also help in designing new studies e.g. addressing treatment response and disease outcome which might differ in subgroups.

Sex differences are of importance in chronic pain disease. One key factor raising interest in sex differences is the higher prevalence of chronic pain conditions among women [28]. As summarized by Mills et al., the evidence from literature suggests that pain perception, pain processing including cognitive aspects and also coping strategies differ between women and men [28]. CRPS as specific chronic pain disease also affects more female patients [9, 29]. In fact, sex differences have been described in CRPS [30, 31]. Male patients use more extreme words to describe pain, choose more passive coping strategies and exhibit higher levels of depressiveness and kinesiophobia [30]. There is also evidence for sex differences in sensory function. Regardless of chronic pain disease, women generally tend to have lower perception thresholds. This phenomenon has to be accounted for when comparing female and male patients with respect to sensory thresholds. However, after correcting for this general trend, sex-depending hypersensitivity towards pressure pain remained in female CRPS patients indicating disease-specific sex differences [31].

If nerve lesions are associated with CRPS it is classified as CRPS type II, otherwise as CRPS type I. Nerve injury can arise from trauma or surgical procedures e.g. surgery for carpal tunnel syndrome. Patients suffering from type II CRPS respond less sensitive than type I CRPS patients to non-painful mechanic stimulation (hypoesthesia). Apart from this, both types are similar in their sensory function [11]. Therefore, the clinical relevance of dividing CRPS patients in type I and type II is under debate [32].

CRPS patients can be classified in “warm” and “cold” CRPS based on their temperature phenotype [33, 34]. This differentiation is based on the perception of the affected extremity either as warm or cold. The warm phenotype is often accompanied by increased skin temperature, skin reddening and edema. It is therefore interpreted as inflammatory disease stage and more common in early CRPS. In contrast, the cold phenotype is typically seen in later disease stage and associated with decreased skin temperature, blueish skin [33] and dystonia [34]. Warm CRPS might also be associated with a more favorable disease course [33, 35]. Furthermore, sensory loss in quantitative sensory testing (QST) [34] as well as signs of central sensitization [35] were more often seen in cold CRPS whereas dynamic mechanical allodynia was more common in warm CRPS [34].

Upper and lower extremities can be affected by CRPS but potential differences in sensory profiles between extremities have rarely been addressed. This knowledge gap becomes even more obvious when noting that patients with lower extremity CRPS are sometimes excluded from studies [20]. However, before pooling patients with upper and lower extremity CRPS it has to be assessed if disease characteristics actually differ. From a mechanistic perspective differences are likely. Hand and foot are used in a completely different manner which is also visible in cortical representation where areas responsible for fine-tuned motor skills of the hand are larger than the corresponding areas of the foot. In addition, sparing a foot during fracture healing is difficult potentially resulting in more pain.

The impact of CRPS on the contralateral extremity has been of scientific interest for a while. It was noticed that pain spreading to areas distant from the original injury site is a common phenomenon [36, 37]. Further, results indicate that hyperalgesia and painful symptoms can occur on the presumably unaffected, contralateral extremity [36, 38-41]. This is accompanied by sensory disturbances as abnormalities in thermal QST which were seen in both extremities [40]. It was shown that hypersensitivity towards capsaicin exposure in CRPS was not only limited to the ipsilateral CRPS-affected extremity but also seen on the contralateral side. This hypersensitivity was not accompanied by changes in local skin reddening as expected if peripheral sensitization was causing the hypersensitivity suggesting the involvement of the CNS [38]. In a longitudinal study it has also been shown that contralateral hyperalgesia can develop within 16–53 months

[39]. These truly interesting observations highlight the importance of the CNS in CRPS including central sensitization as one aspect of CRPS pathology [42].
In my thesis, I evaluate the five presented subgroups with regard to sensory profiles, and patient and disease characteristics in a large cohort. With this, I contribute robust results that substantially add to the debate on CRPS heterogeneity and clinical subgrouping. Furthermore, hyperalgesia on the contralateral extremity is investigated as novel candidate subgroup.

1.3.3. CRPS and fractures

Delayed diagnosis of CRPS is a relevant clinical problem [43, 44]. The duration of the normal healing process and the sensation on the injured extremity during this process vary, which makes it difficult to differentiate between delayed normal healing and acute CRPS.
Sensory disturbances similar to CRPS and neuropathic pain disease can also occur after fractures. Pressure pain hyperalgesia [45] as well as cold pain hypersensitivity were seen in fracture patients [45] and patients after experimental forearm immobilization [46]. In addition to sensory abnormalities, difficulties in conducting imagined movements of the fracture limb were noted. This indicates sensorimotor dysfunction in fracture patients [45]. Besides, further clinical features of CRPS such as edema/limb swelling were documented in patients after limb immobilization due to hand surgery [45, 47]. Fracture healing and immobilization partially imitate features of CRPS hence complicating the differentiation of fracture and CRPS patients. A better understanding of the normal healing process in fracture patients – i.e. through comparing sensory profiles with those of CRPS patients – contributes to a better understanding of early CRPS pathology and could help to achieve earlier diagnosis and tailored treatment. This study sets out to a direct comparison of sensory profiles and disease characteristics between fracture and acute CRPS patients by capturing a broad spectrum of normal fracture healing and marking differences to CRPS.

1.3.4. Disease course

The longitudinal outcome of CRPS is of eminent interest for patients when being confronted with the diagnosis of CRPS. However, only few longitudinal studies on long-term outcome are available and data on the development of chronic cases are missing. Even though symptoms improve over time, many patients suffer from chronic CRPS. There is evidence suggesting that symptom amelioration is most likely to occur within the first six to twelve months after disease onset [48]. Data from a prospective, observational trial showed that many clinical signs and symptoms improve especially within the first six months. However, after a one-year follow-up only 5.4% were free of symptoms and more than 25% still met the diagnostic criteria for CRPS. Interestingly, spreading of pain to areas outside the actual injury site became more frequent during follow-up [48]. This highlights once more the relevance of the sensory dysfunction as clinical feature of CRPS. Follow-up investigations revealed that hypersensitivity towards thermal and mechanical pain increases while pressure pain hyperalgesia decreases. The described increase also involved the contralateral extremity [39]. In a six-months follow-up, treatment responders were characterized by improved pressure pain hyperalgesia. However, sensory profiles could not predict therapeutic success and were rather similar at baseline and follow-up [20].

Psychological parameters are also of interest in long-term observations. Lower levels of anxiety and disability were associated with lower pain scores during follow up. Similarly, patients with less pain and lower levels of pain related fear showed less disability after follow-up [49].

Beside clinical outcome parameters, it is essential to include the patient's perspective on recovery into treatment decision and definition of treatment response. In a survey, patients described improvement in pain, range of motion, stiffness and independence from medication as most relevant for their recovery [50].

In my study, sensory profiles and disease characteristics including psychological parameters are followed up in acute as well as in chronic CRPS patients to get further insight in the plasticity of sensory signs, clinical disease characteristics and psychological parameters.

1.4. Research questions

This study on CRPS sets out to improve the differentiation of subgroups through adding new clinically relevant subgroups and questioning others in a multifaceted approach. Results are based on clinical symptoms (e.g pain intensity), psychological questionnaires and QST. Further, this work elucidates the long-term progression of sensory alterations in CRPS. In particular, this thesis addresses the following questions:

I. Do CRPS patients show sensory disturbances and is the sensory function linked to clinical disease characteristics?

II. Do clinical and sensory data support the clinical differentiation of the following CRPS phenotypes based on
   a. Female or male sex?
   b. Absence or presence of a nerve lesion (CRPS type I or type II)?
   c. Warm or cold temperature phenotype?
   d. The affection of the upper or lower extremity?
   e. The affection of the contralateral extremity?

III. How similar are fracture and CRPS patients? Can sensory function and clinical disease characteristics help in differentiating fracture and CRPS patients?

IV. Do clinical disease characteristics and sensory disturbances improve over the time course of CRPS and are potential improvements linked?

2. Methods

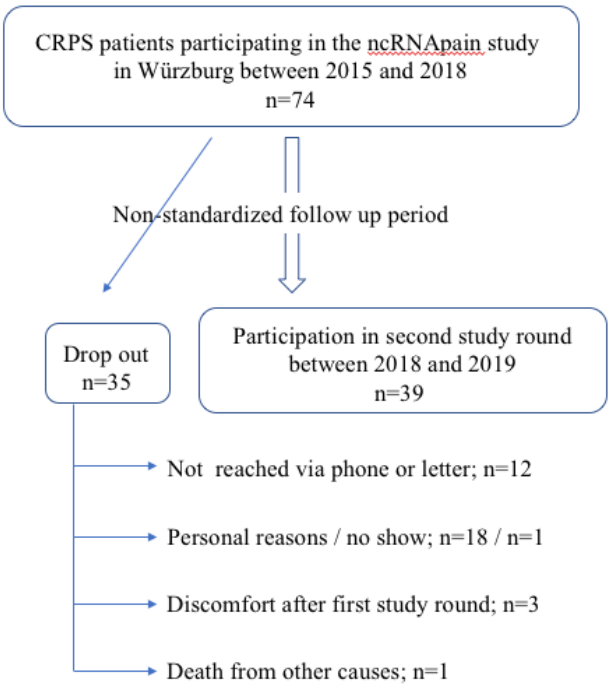
2.1. Patient recruitment

2.1.1. Baseline patient recruitment and general exclusion criteria

Patient recruitment began after ethical approval was obtained from the responsible ethic committees (approval numbers: AZ – 52/14, Würzburg; 9142-F, Mainz) and followed the ncRNApain study protocol registered at the German study register (DRKS00008964). CRPS patients were recruited in out- and inpatient pain clinics in the participating study centers at the Würzburg and Mainz University hospital. Fracture patients were recruited in the outpatient clinic of the surgical department at Würzburg University Hospital. Fracture patients were defined as patients recovering from an upper extremity bone fracture. All patients underwent nerve conduction velocity measurement at initial study inclusion to rule out neuropathy as cause of pain. Further exclusion criteria were acute infection, any surgery in the last four weeks and severe psychiatric comorbidities. All patients were overnight fasted and were asked to avoid heavy exercise, alcohol consumption and large meals the day before study participation. This was due to a blood sample taken for other study purposes not included in this work.
In the analysis of neuropathic pain characteristics in fracture and CRPS patients the data of 158 patients with diabetic polyneuropathy recruited at the Brno ncRNApain study center (Dept. of Neurology, University Hospital of Brno, Brno, Czech Republic) served as disease control [51].

2.1.2. Patient recruitment for a follow-up investigation

For follow-up investigations, CRPS patients seen at the Würzburg pain clinic between 2015 and 2018 were contacted again and asked for their volunteer participation in a second study round. A total of 74 patients was recruited in the mentioned interval and therefore suitable for follow-up study participation. Out of these, 39 patients could be recruited. Reasons for denial were documented and distributed as follows (**Figure 2**): Twelve patients were not reached. One patient did not show up for the study participation and one patient had died from other causes. From the remaining patients three explained that the first study participation led to discomfort and a second participation was not



wished. 18 patients named personal reasons such as a busy schedule for their denial of a second participation in the study. The mean follow-up interval was 2.5 years (range 0.8 – 4.3 years).

Figure 2. Patient recruitment for follow-up participation
Patients were recruited within the ncRNApain study in Würzburg. The follow up sample was based on participants in the Würzburg study center recruited between 2015 and 2018. Shown are reasons for drop out.

2.2. Clinical examination

Clinical examination took place at ncRNApain study centers and followed a structured protocol. Medical history taking included the trauma preceding CRPS onset and a brief neurological examination. The neurological examination focused on CRPS clinical signs and symptoms as well as pain intensity and characteristics.

2.2.1. Disease characteristics and Budapest criteria

The affected as well as the unaffected extremity were examined carefully and changes in skin color, temperature, texture, skin appendage or the presence of edema were documented. The range of motion of affected joints was measured. Patients were asked to rate minimum, maximum and last week´s mean pain intensity on a 0-10 numeric rating scale (0-10 NRS). The CRPS diagnosis was evaluated using the Budapest criteria for clinical diagnosis as shown in Table 1 [52]. Pain inappropriate to the preceding trauma is a prerequisite to the diagnosis. Further, the Budapest criteria combine results from patient reports and clinical examination and investigate four symptom categories: sensory, vasomotor, sudomotor/edema, motor/trophic. To fulfill the clinical diagnostic criteria the patient must report symptoms out of at least three categories. Additionally, the clinician

has to observe symptoms out of two categories. As the Valencia consensus criteria on the diagnosis of CRPS were published after the presented study was conducted they were not part of the diagnostic criteria used here [32].

Table 1. Budapest criteria for clinical diagnosis of CRPS [52]

Symptom categories	**Typical symptoms**
Sensory	Allodynia, hyperpathia
Vasomotor	Skin color and skin temperature changes
Sudomotor/Edema	Asymmetric swelling/sweating
Motor/Trophic	Tremor, dystonia, cramping, affected skin/hair/nails

2.2.2. Subgroup classification

Patients were assigned to five different subgroups depending on disease or patient characteristics. *Sex:* One subgroup divided female and male patients. *Temperature phenotype:* Patients were asked whether the affected extremity felt cold or warm at the onset of CRPS. Accordingly, they were grouped into warm or cold CRPS. *CRPS type:* CRPS cases in which a nerve lesion is evident represent type II. Cases without known nerve lesion have type I CRPS. *Extremity:* Based on the affected extremity patients were grouped in upper and lower extremity cases. *Contralateral hyperalgesia:* An affection of the contralateral extremity, referred to as contralateral hyperalgesia, was defined as allodynia, pressure or mechanical pain hypersensitivity on the contralateral limb. In detail, results from QST were used for group assignment. Contralateral hyperalgesia was assumed if pressure or mechanical pain thresholds of the contralateral extremity were outside the 95%-confidence interval ($z>1.96$) or dynamic mechanical allodynia was seen on the contralateral extremity.

2.2.3. CRPS severity score

The CRPS severity score [53] is an evaluated tool to measure the disease severity on a 0-17 scale. More pronounced CRPS symptoms lead to higher CRPS severity scores. Similar to the Budapest criteria this score combines results from patient report and clinical

examination. Table 2 summarizes the different symptom categories investigated in order to calculate the CRPS severity score.

Table 2. CRPS severity score [53]

Patient report	**Clinical examination**
Allodynia	Hyperpathia
Temperature asymmetry	Allodynia
Skin color asymmetry	Temperature asymmetry
Sweating asymmetry	Skin color asymmetry
Edema	Sweating asymmetry
Trophic changes	Edema
Motor changes	Trophic changes
Decreased range of motion	Motor symptoms
	Decreased range of motion
Σ 8	Σ 9
Maximum CRPS severity score = 17	

2.3. Quantitative Sensory Testing - QST

QST is a psychophysical testing method [54]. It was developed in order to establish a tool for measuring the function of the human somatosensory system in a reproduceable and objective way hence enabling its use in research. It combines eleven single tests that cover different modalities of the somatosensory nervous system. All tests are applied to the skin always defining a testing and control area. Here, the CRPS-affected extremity was used as testing side and the contralateral extremity as control side. By doing so, an internal control is guaranteed. Testing of the upper extremity took place in the thenar or hypothenar area, always choosing the more painful area. Accordingly, the plantar or dorsal foot were being tested in lower extremity cases. The QST procedure follows a standardized protocol and was carried out by trained investigators in certified laboratories only.

2.3.1. Principles behind detection and pain thresholds

Stimulation always begins with non-painful stimuli above the detection threshold. Detection thresholds are then found by decreasing the intensity of the stimulus until no sensation is reported anymore. The pain threshold describes the stimulus intensity where a painful sensation is reported for the first time. This threshold must not be confused with the point where pain intensity is getting unbearable. To capture the pain threshold a stimulus is increased until the sensation is starting to be overlaid by burning, pricking or tingling sensations.

2.3.2. Temperature testing

Temperature testing was performed with an MSA-II thermal stimulator (Somedic AB, Hørby, Sweden). There are four different tests measuring the temperature sensation. In each test the default temperature is set to 32°C and changes with 1°C/s until the subject reports a temperature sensation according to the tested sensory function. In that manner warm and cold detection thresholds (WDT, CDT) as well as heat and cold pain thresholds (HPT, CPT) are measured. In addition, the subject´s ability to detect temperature changes itself is recorded (TSL). To prevent skin damage, minimum and maximum temperature are set to 10°C and 50°C, respectively.

2.3.3. Mechanical and vibration detection

Mechanical detection is tested with von-Frey filaments (Marstock Nerve Test, Schriesheim, Germany) ranging from 0.25mN to 512mN. Von-Frey filaments in a descending order are applied to the skin until no sensation is reported anymore defining the mechanical detection threshold (MDT). A 64-Hz tuning fork is used to asses vibration detection (VDT). The vibration stimulus is repeated three times on each side.

2.3.4. Mechanical and pressure pain

Painful mechanical sensation is tested with metal pins (PinPricks®, MRC-Systems GmbH, Heidelberg, Germany). Minimal and maximal force applied are 8mN and 512mN, respectively.

The mechanical pain threshold (MPT) is calculated by increasing the intensity until a painful sensation is reported. In a separate test, a brush, a Q-tip and a cotton wool ball as naturally nonpainful stimulators are added. Each PinPrick® and all painless stimulators are applied five times in mixed order. Subjects have to rate the pain sensation after each stimulation on a 0-100 numeric rating scale. The mechanical pain sensitivity (MPS) is calculated from all ratings. Painful sensation after brush, Q-tip or cotton wool testing is recorded as dynamic mechanical allodynia. To receive information on temporal summation of pain stimuli a wind-up ratio (WUR) is calculated by dividing the pain intensity (0-100 NRS) after ten PinPrick®-repeats by the pain intensity of a single PinPrick® stimulus. Blunt pressure pain is induced with a pressure algometer (FPN 200; Wagner Instruments, Greenwich, CT, USA) to calculate pressure pain thresholds (PPT). Again, the stimuli are repeated three times on each side and the stimulus stops when the patient reports a painful sensation.

2.3.5. QST parameter selection, data processing and z-score interpretation

As proposed by Dimova et al., a selection of the QST parameters explained above was analyzed [55]. This approach excludes the mechanical pain sensitivity, the temperature threshold limen, the wind-up ratio and the vibration detection threshold. This focused approach reduces the alpha error inflation due to multiple testing.

Individual QST results are z-transformed and normalized using a reference data set published by the DFNS [56]. The provided DFNS data set normalizes with regard to sex, age group and tested body area. The following equation is used:

$$z = \frac{(individual\ value) - (mean\ of\ the\ published\ reference)}{SD\ of\ the\ published\ reference}$$

Z-values outside the 95% confidence interval (-1.96 > z > 1.96) indicate *gain of function* and *loss of function* in the tested sensory modality, respectively. *Gain of function* can thereby also be seen as hypersensitivity towards the tested stimulus and indicates lowered detection or pain thresholds. *Loss of function* means hyposensitivity or hypoesthesia towards the stimulus due to elevated thresholds.

2.4. Self-administered questionnaires

The German versions of self-administered questionnaires were used to assess functional impairment, pain characteristics as well as psychological comorbidities such as depression or anxiety.

2.4.1. Graded Chronic Pain Scale

The *Graded Chronic Pain Scale* (GCPS) [57] is a questionnaire grouping the perceived pain into four grades of chronic pain. It thereby includes items on pain persistence, pain intensity and pain-related disability. Here, the scores of pain intensity (range 0-100) and pain-related disability (range 0-100) were extracted and used for the analysis. Higher pain intensity and disability are represented by higher scores.

2.4.2. Neuropathic Symptom Inventory

The *Neuropathic Pain Symptom Inventory* [58] (NPSI, range 0-1) is based on ten questions addressing pain characteristics and temporal aspects of the perceived pain. Higher values indicate a stronger expression of neuropathic pain characteristics. Importantly, the NPSI has no cut-off values defining neuropathic and non-neuropathic pain. In detail, the NPSI includes five separate scores which asses the intensity of burning pain, pressure pain, pain attacks, evoked pain and paresthesia. The total score is equally based on these scores.

2.4.3. Beck Depression Inventory II

The *Beck Depression Inventory II* [59] (range 0-63) is a psychological questionnaire assessing depression. Depressive symptoms lead to higher scores. Values between 0 and 13 are considered minimal, between 14 and 19 mild, between 20 and 28 moderate and higher than 28 severe depression.

2.4.4. State-Trait Anxiety Inventory

Anxiety as trait and state characteristic was measured with the *State-Trait Anxiety Inventory* [60] (STAI-T, STAI-S, range 20-80). The questionnaire consists of 40 questions and uses a Likert scale. In the following chapters it is referred to anxiety as trait characteristic as visualized in the STAI-T. Values below 40 are considered normal, higher values indicate anxiety.

2.4.5. Disabilities of the Shoulder, Arm and Hand

In patients with upper extremity CRPS the disability of the shoulder, arm and hand was measured through the *Disability of the shoulder, arm and hand score* (DASH). This questionnaire comprises of 30 items addressing the functionality of the upper extremity in the week before study participation. The score ranges from 0-100 while higher score imply worse disability [61].

2.5. Statistical Analysis

2.5.1. Demographic, clinical and sensory data analysis

All data were tested for normal distribution. If data was not normally distributed nonparametric tests were applied in hypothesis testing. Throughout this work, mean and standard deviation are used to describe the data if not stated otherwise. Statistical significance was accepted when $p < 0.05$, always using two-tailed hypothesis testing. Categorical data was compared with a Pearson χ^2-test. Paired data was analyzed using a paired t-test or a Wilcoxon signed rank test and McNemar-test if data was categorical. Unpaired data was compared with a t-test or Mann-Whitney-U-test. The follow-up on

sensory function used a repeated measurement analysis of variance (rm-ANOVA) for each QST parameter. “Baseline vs. follow up” and “affected vs. unaffected extremity” served as within subject factors. In order to adjust to the alpha error inflation due to multiple testing in the comparison of single QST items the significance level was lowered depending on the number of comparisons (p = 0.05/number of hypothesis tested). The statistical analysis was conducted with IBM® SPSS® Statistics version 26. Figures were designed with GraphPad Prism 8 (Graph Pad Software LLC).

2.5.2. Principal Component Analysis - PCA

Principal component analysis (PCA) was used as method of dimension reduction. The dimension reduction can visualize underlying data structures. This is especially useful in data sets where many variables contribute to a larger construct, e.g. “pain” or “sensory function”. PCA can then condense these variables into principal components (PC) that ideally represent the larger construct standing behind the variables. In detail, the factor extraction was preceded by the calculation of the Kaiser-Maier-Olkin-Measure (KMO) and the Bartlett test for sphericity to test that the data structure was suitable for PCA. In the literature, KMO values between 0.7-0.8 are considered good [62]. Factors were extracted if eigenvalues were > 1 (Kaiser´s criterion). After extraction, a varimax rotation was carried out and factors finally saved as PC. PCA was conducted with IBM® SPSS® Statistics version 26.

3. Results

3.1. Disease characteristics and sensory profiles

3.1.1. Patient and disease characteristics

199 CRPS patients recruited from the ncRNApain study were included in this analysis (**Table 3**). The majority of patients suffered from short lasting (<12 months), upper extremity CRPS type I. The sample was dominated by female patients (n=153 vs. n=46). In 38% of the patients, mild depressive symptoms were seen (Beck depression inventory II > 13). Regarding anxious symptoms, half of the patients had state-trait anxiety inventory scores above the threshold of 40 indicating anxiety. Disease severity as expressed by the CRPS severity score was moderate.

Table 3. Demographics and disease characteristics of the whole CRPS cohort (n=199)[63]

Variables	**Complex regional pain syndrome full cohort (n = 199)**
Age, years (range)	51.3 (18- 91)
Sex (n (%) male/n (%) female)	46 (23.1)/153 (76.9)
Type (n (%) I/n (%) II)	175 (87.9)/24 (12.1)
Disease duration (n (%) < 12 months/n (%) >12 months)	161 (80.9)/38 (19.1)
CRPS severity score[1]	11 ± 3
Mean pain last week (numeric rating scale 0-10)	5.1 ± 2.0
Neuropathic pain symptom inventory[2]	0.38 ± 0.22
Beck depression inventory II [3] (n=192)	13 ± 11
State-Trait Anxiety Inventory, trait version[4] (n=194)	43 ± 13
Graded Chronic Pain Scale, disability score [5]	59 ± 24
Graded Chronic Pain Scale, pain intensity score[5]	60 ± 20

[1] CRPS severity score, range 0-17 [53]; [2] Neuropathic pain symptom inventory, range 0-1 [58]; [3] Beck depression inventory II, range 0-63 [59]; [4] State-Trait anxiety inventory, range 20-80 [60]; [5] Graded chronic pain scale – disability and pain intensity scores, range 0-100 [57]

3.1.2. QST and sensory profiles

QST as accepted tool in pain research was used to draw sensory profiles of this large CRPS cohort. Sensory disturbances as important feature of CRPS phenomenology can be visualized and in this special case were also used as baseline for future follow up investigations. Complete QST data sets were available from 192 patients (**Figure 3A**).

The affected extremity exhibited signs of hypoesthesia upon non-painful stimulation. Accordingly, cold, warm and mechanical detection thresholds were elevated (z-scores unaffected vs. affected limb for cold detection: -0.53 vs. -1.22; $Z=-5.42$, $p<0.001$; warm detection: -0.32 vs. -1.08; $Z=-6.63$, $p<0.001$; mechanical detection: 1.16 vs. 0.38; $Z=-4.76$, $p<0.001$). There was a weak but significant correlation between warm/mechanical detection thresholds and mean pain intensity indicating that higher pain intensities are associated with a loss of function in non-painful detection (warm detection: Pearson correlation coefficient =-0.20, $p=0.005$; mechanical detection: Pearson correlation coefficient =-0.20, $p=0.006$).

The opposite was observed in painful stimulation. Thresholds for cold and pressure pain were lower on the affected extremity indicating hypersensitivity (z-scores unaffected vs. affected limb cold pain: -0.07 vs. 0.56; $Z=-5.89$, $p<0.001$; pressure pain: -1.24 vs. 1.82; $Z=-10.64$, $p<0.001$). Paradoxical heat sensations were more frequently reported on the affected extremity (**Figure 3B**, McNemar test: $p=0.023$). Dynamic mechanical allodynia occurred more often on the affected limb (**Figure 3C**, McNemar test: $p<0.001$) and was detected in 19.6% (n=39) of the patients. In a smaller number of patients (n=7; 3.5%) allodynia was also seen on the contralateral extremity. This is accounted for in the subgroup based on hyperalgesia on the contralateral extremity.

For better visualization of the distribution of z-scores (**Figure 3D**) results were grouped into gain of function ($z>1.96$), loss of function ($z<-1.96$) and normal stimulus response ($-1.96<z<1.96$). The distribution was significantly different in all three tests displayed (McNemar test: all $p<0.001$). It is visible that the proportion of gain of function in cold and pressure pain is larger on the affected side. With regard to mechanical detection threshold loss of function is more frequent on the affected side even though a large proportion also exhibits signs of gain of function. However, it is also visible that the majority of z-scores was within the normal range.

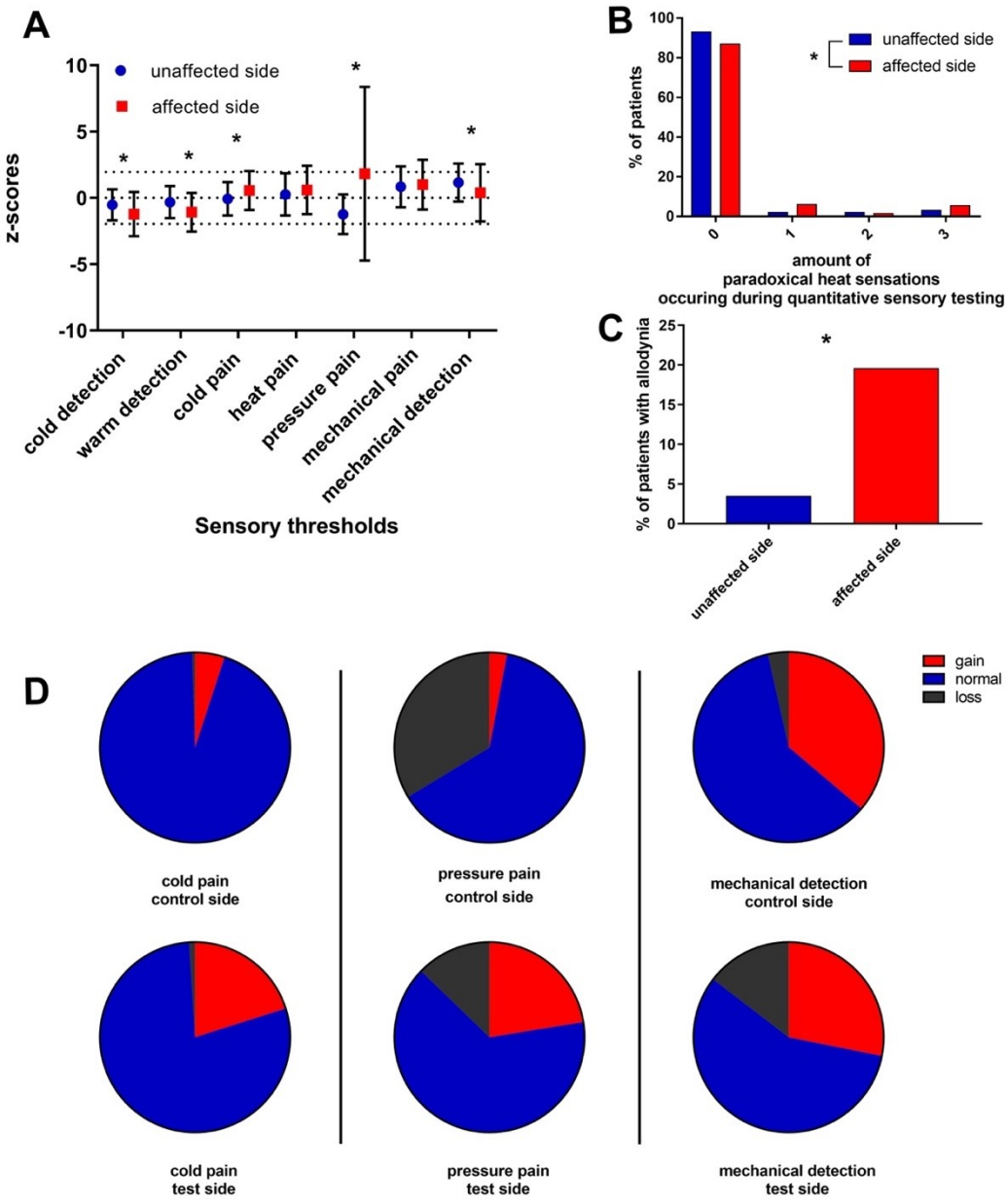


Figure 3. QST sensory profiles of CRPS patients (n=199) show signs of hyperalgesia as well as hypoesthesia [63]

(**A**) Detection and pain thresholds of the CRPS affected extremity (red squares) compared to the unaffected limb (blue dots). Shown are the mean z-values (± SD) for seven QST tests including cold and warm detection threshold, cold and heat pain threshold, pressure pain threshold, mechanical pain threshold and mechanical detection threshold. Doted lines at -1.96 and 1.96 mark the 95%-confidence interval referring to the reference mean. (**B**) Amount of paradoxical heat sensations occurring during QST. The number of patients reporting mechanical allodynia during QST is shown in (**C**). Based on the z-values QST results were divided into three groups: $z > 1.96$, gain (red): hypersensitivity towards the stimulus; $z < -1.96$, loss (grey): hyposensitivity towards the stimulus; $-1.96 < z < 1.96$, normal (blue): regular response to the stimulus. (**D**) shows the distribution into the three groups. * $p < 0.05$; Wilcoxon signed rank test (A) and McNemar test (B, C).

3.2. Clinical subgroups of CRPS patients

3.2.1. Sex differences

Beside a difference in age, no major sex differences were observed. The sample was dominated by female patients (76.8%) that on average were eight years older than male patients (**Table 4**).

Table 4. Patient and disease characteristics of female and male CRPS patients

Variables	Male subjects (n = 46)	Female subjects (n = 153)	p value
Age (range)	**45.3 (18; 85)**	**53.2 (21; 91)**	**< 0.001**
Sex (n (%) male/n (%) female)	37 (80.4); 9 (19.6)	138 (90.2); 15 (9.8)	0.075
Disease duration (n (%) <12 months; n (%) >12 months)	39 (84.8); 7 (15.2)	122 (79.7); 31 (20.3)	0.445
CRPS severity score[1]	11 ± 3	11 ± 3	0.729
Mean pain last week (numeric rating scale 0-10)	4.8 ± 1.9	5.2 ± 2.0	0.200
Max pain last week (numeric rating scale 0-10)	7.4 ± 1.6	7.15 ± 2.1	0.741
Neuropathic pain symptom inventory[2]	0.38 ± 0.21	0.38 ± 0.23	0.972
Beck depression inventory II[3]	14 ± 13 (n=42)	13 ± 11 (n=150)	0.620
State-Trait Anxiety Inventory[4], Trait version	41 ± 14 (n=45)	43 ± 12 (n=149)	0.375
Graded chronic pain scale[5], disability score	57 ± 26	60 ± 23	0.452
Graded chronic pain scale[5], pain intensity score	56 ± 22	61 ± 19	0.182

[1] CRPS severity score, range 0-17 [53]; [2] Neuropathic pain symptom inventory, range 0-1 [58]; [3] Beck depression inventory II, range 0-63 [59]; [4] State-Trait anxiety inventory, range 20-80 [60]; [5] Graded chronic pain scale – disability and pain intensity scores, range 0-100 [57]; Mann-Whitney-U test, Pearson χ^2 test for categorical data, p-values <0.05 are printed boldly.

In QST, female patients were more sensitive to pressure pain (z-score pressure pain threshold, female vs. male: 2.41 vs. -0.90; Z=-3.63, p<0.001) and had lower thresholds for non-painful mechanical detection (z-score mechanical detection threshold, female vs.

male: 0.65 vs. -0.50; Z=-3.82, p<0.001) than male patients (**Figure 4A**). Gain of function in pressure pain (**Figure 4B**) was seen in 25% of female patients and 13% of male patients (χ^2 =8.26, p=0.016). Hypersensitivity to non-painful mechanical stimulation also occurred more frequently in female patients (36% vs. 2%; χ^2 =25.73, p<0.001) while hypoesthesia was more common in male patients (30% vs. 10%) (**Figure 4B**). No difference was seen in the occurrence of allodynia (**Figure 4C**). These observations were made after correcting for general sex and age differences during z-transformation.

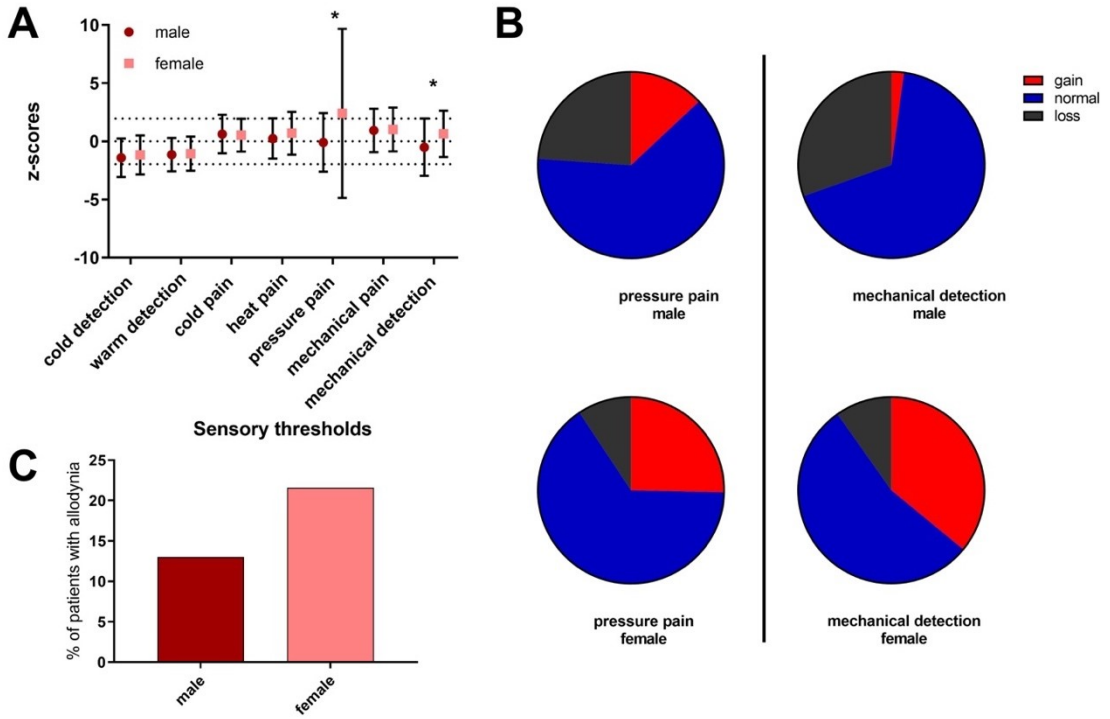


Figure 4. Sensory profiles of female patients (n=153) are characterized by pressure pain hyperalgesia and hypersensitivity to non-painful mechanical stimulation
(**A**) QST z-scores of affected extremities of male (dark red; n=46) and female (light red; n=153) CRPS patients. Shown are mean z-scores (±SD) for seven included QST tests. Dotted lines at -1.96 and 1.96 mark the 95%-confidence interval referring to the reference mean. (**B**) Based on the z-values QST results were divided into three groups: z > 1.96, gain (red): hypersensitivity towards the stimulus; z < -1.96, loss (grey): hyposensitivity towards the stimulus; -1.96 < z < 1.96, normal (blue): regular response to the stimulus. The pie charts illustrate the distribution into the three groups for pressure pain thresholds and mechanical detection thresholds. (**C**) shows the percentage of patients with allodynia on the affected extremity. *p<0.05, Mann-Whitney-U test (A), Pearson χ^2 test (B, C).

3.2.2. CRPS type

Only a minor difference was found depending on the presence of a nerve lesion. 87.9% of patients had type I CRPS without evident nerve lesion (**Table 5**). In CRPS type II patients the neuropathic pain character as measured by the NPSI was more pronounced. Pain intensity as well as other characteristics were similar. Sensory profiles were the same in type I and type II patients (**Figure 5**). The large standard deviation regarding pressure pain in type II patients is the result of some patients with extreme pressure pain sensitivity. It cannot be told if this is because of the nerve lesion or explained by another circumstance.

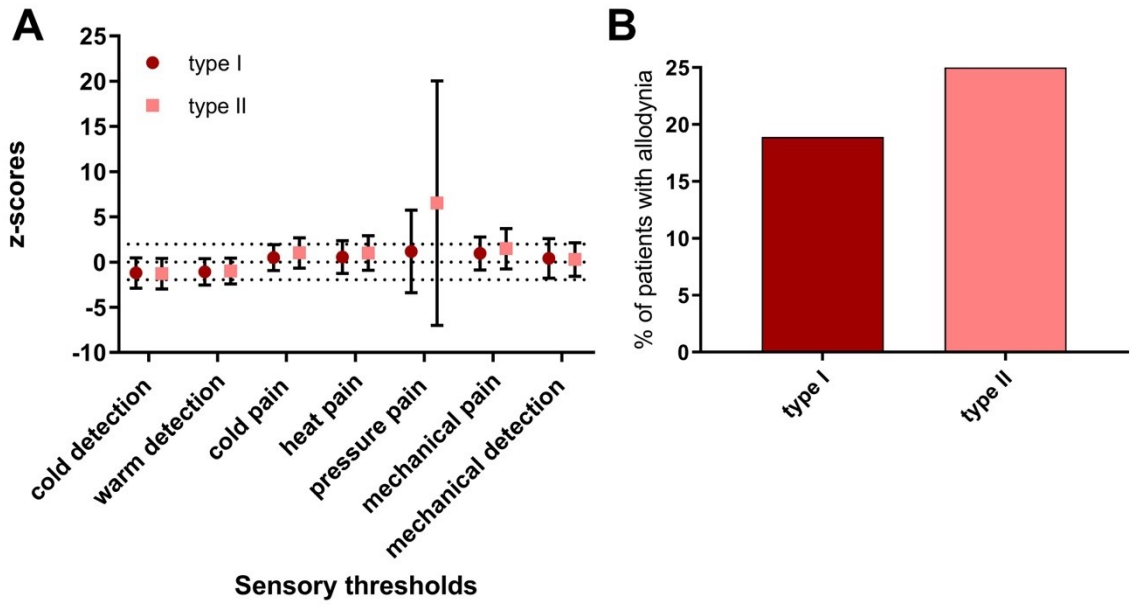


Figure 5. Sensory profiles of type I (n=175) and type II (n=24) CRPS patients are similar
(**A**) Detection and pain thresholds on the affected extremities of type I (dark red) and type II (light red) CRPS patients. Shown are mean z-scores (±SD) for seven included QST tests. Dotted lines at -1.96 and 1.96 mark the 95%-confidence interval referring to the reference mean. (**B**) The percentage of patients with allodynia on the affected extremity. *p<0.05, Mann-Whitney-U test (A), Pearson χ^2 test (B).

Table 5. Patient and disease characteristics of patients with type I and type II CRPS

Variables	CRPS I (n=175)	CRPS II (n=24)	p value
Age (range)	51.2 (18; 91)	52.3 (23; 72)	0.668
Sex (n (%) male/n (%) female)	37 (21.1)/138 (78.9)	9 (37.5)/ 15 (62.5)	0.075
Disease duration (n (%) <12 months/ n (%) >12 months)	144 (82.3)/31 (17.7)	17 (70.8)/ 7 (29.2)	0.181
CRPS severity score[1]	11 ± 3	11 ± 3	0.762
Mean pain last week (numeric rating scale 0-10)	5.1 ± 2.1	4.9 ± 1.6	0.581
Max pain last week (numeric rating scale 0-10)	7.2 ± 2.1	7.5 ± 1.3	0.742
Neuropathic pain symptom inventory[2]	**0.37 ± 0.22**	**0.48 ± 0.17**	**0.008**
Beck depression inventory II[3]	14 ± 12 (n=168)	12 ± 6	0.964
State-Trait anxiety inventory[4], trait version	43 ± 13 (n=170)	40 ± 11	0.356
Graded chronic pain scale[5], disability score	60 ± 24	57 ± 21	0.477
Graded chronic pain scale[5], pain intensity score	60 ± 20	60 ± 18	0.807

[1] CRPS severity score, range 0-17 [53]; [2] Neuropathic pain symptom inventory, range 0-1 [58]; [3] Beck depression inventory II, range 0-63 [59]; [4] State-Trait anxiety inventory, range 20-80 [60]; [5] Graded chronic pain scale – disability and pain intensity scores, range 0-100 [57]; Mann-Whitney-U test, Pearson χ^2 test for categorical data, p-values <0.05 are printed boldly.

3.2.3. CRPS temperature phenotype

Based on the temperature phenotype slight differences in sensory function were noted. The warm CRPS temperature phenotype was more common (75.3%) and occurred more often in acute cases with disease durations shorter than 12 months (**Table 6**). The overall disease severity was only 1 point higher in warm CRPS cases, but perceived disability through pain was significantly higher. Patients with the cold temperature phenotype were slightly less sensitive to mechanical detection (**Figure 6A**) with no differences in the frequency of allodynia (**Figure 6B**) (Z-score mechanical detection threshold, cold vs. warm: -0.28 vs. 0.60; Z=-2.79, p=0.005). Accordingly, hypersensitivity to mechanical detection was more often seen in warm CRPS patients (**Figure 6C**) (35% vs. 8%, χ^2 = 13.13, p=0.001)

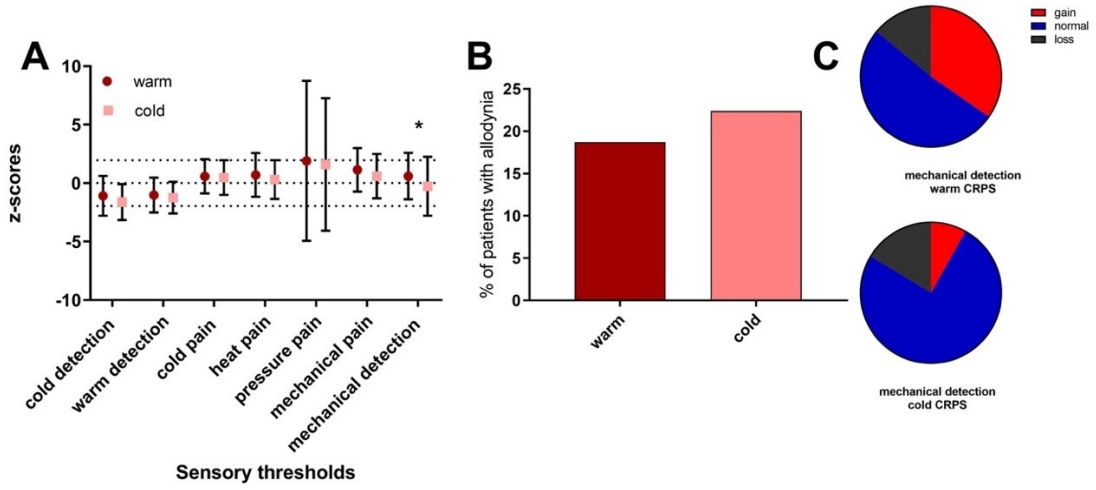


Figure 6. Patients with warm CRPS (n=150) show a higher sensitivity towards non-painful mechanical stimulation than patients with cold CRPS (n=49)
(**A**) Sensory profiles of the affected extremities of patients with warm (dark red) and cold (light red) CRPS. Shown are mean z-scores (±SD) for seven included QST tests. Dotted lines at -1.96 and 1.96 mark the 95%-confidence interval referring to the reference mean. (**B**) shows the percentage of patients with allodynia on the affected extremity. (**C**) Based on the z-values QST results were divided into three groups: z > 1.96, gain (red): hypersensitivity towards the stimulus; z < -1.96, loss (grey): hyposensitivity towards the stimulus; -1.96 < z < 1.96, normal (blue): regular response to the stimulus. The pie chart illustrates the distribution into the three groups for mechanical detection thresholds. *p<0.05, Mann-Whitney-U test (A), Pearson χ^2 test (B).

Table 6. Patient and disease characteristics of patients with warm and cold CRPS temperature phenotype

Variables	Warm CRPS (n = 150)	Cold CRPS (n = 49)	p value
Age (range)	**53.3 (18; 91)**	**45.5 (20; 70)**	**0.002**
Sex (n (%) male/n (%) female)	31 (20.7)/119 (79.3)	15 (30.6)/34 (69.4)	0.152
Type (n (%) I/n (%) II)	132 (88.0)/18 (12.0)	43 (87.8)/6 (12.2)	0.964
Disease duration (n (%) < 12 months/n (%) > 12 months)	**133 (88.7)/17 (11.3)**	**28 (57.1)/21 (42.9)**	**< 0.001**
CRPS severity score[1]	**11 ± 3**	**10 ± 3**	**0.003**
Mean pain last week (numeric rating scale 0-10)	5.2 ± 2.0	4.8 ± 2.2	0.260
Max pain last week (numeric rating scale 0-10)	7.3 ± 1.9	6.9 ± 2.4	0.565
Neuropathic pain symptom inventory[2]	0.38 ± 0.23	0.39 ± 0.21	0.530
Beck depression inventory II[3]	13 ± 11 (n=143)	14 ± 10	0.624
State-Trait anxiety inventory[4], trait version	42 ± 13 (n=146)	43 ± 12 (n=48)	0.511
Graded chronic pain scale[5], disability score	**61 ± 24**	**53 ± 23**	**0.037**
Graded chronic pain scale[5], pain intensity score	61 ± 20	57 ± 19	0.145

[1] CRPS severity score, range 0-17 [53]; [2] Neuropathic pain symptom inventory, range 0-1 [58]; [3] Beck depression inventory II, range 0-63 [59]; [4] State-Trait anxiety inventory, range 20-80 [60]; [5] Graded chronic pain scale – disability and pain intensity scores, range 0-100 [57]; Mann-Whitney-U test, Pearson χ^2 test for categorical data, p-values <0.05 are printed boldly.

3.2.4. Upper and lower extremity CRPS

Generally, CRPS of the upper and the lower extremity is similar. In the majority of patients, the upper extremity was affected by CRPS (**Table 7**). Lower extremity patients were on average 4.8 years younger. The z-scores on which the sensory profiles are based are corrected for general differences depending on the extremity tested. Consequently, observed differences would be rather disease-specific than caused by general sensory differences between hand and foot. However, sensory profiles were comparable between both groups with the exception of paradoxical heat sensations that occurred more frequently on the lower extremity (**Figure 7**).

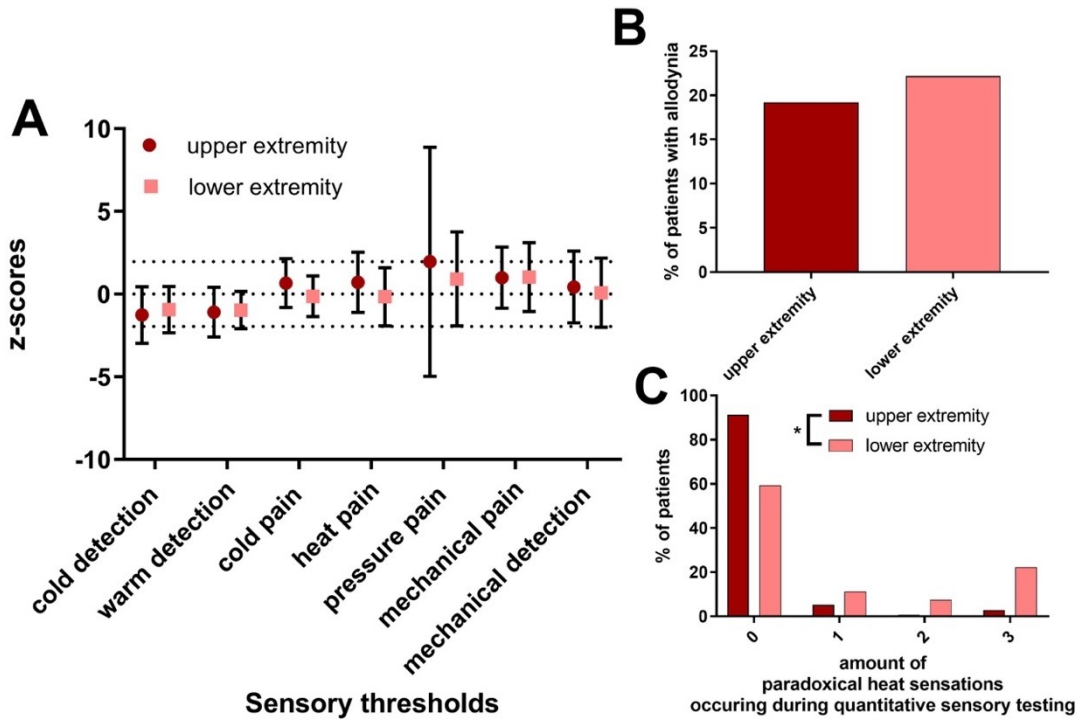


Figure 7. Sensory profiles of patients with upper (n=172) and lower extremity CRPS (n=27) are similar
(**A**) QST z-scores of the affected extremities of patients with upper (dark red) and lower extremity (light red) CRPS. Shown are mean z-scores (±SD) for seven included QST tests. Dotted lines at -1.96 and 1.96 mark the 95%-confidence interval referring to the reference mean. (**B**) Occurrence of allodynia on the affected extremity. (**C**) shows the amount of paradoxical heat sensations reported during QST. *p<0.05, Mann-Whitney-U test (A), Pearson χ^2 test (B, C).

Table 7. Patient and disease characteristics of patients with upper and lower extremity CRPS

Variables	CRPS upper extremity (n=172)	CRPS lower extremity (n=27)	p value
Age (range)	**52.0 (18; 91)**	**47.2 (23; 62)**	**0.019**
Sex (n (%) male/n (%) female)	41 (23.8)/131 (76.2)	5 (18.5)/22 (81.5)	0.542
Type (n (%) I/n (%) II)	151 (87.8)/21 (12.2)	24 (88.9)/3 (11.1)	0.871
Disease duration (n (%)<12 months/n (%)>12 months)	139 (80.8)/31 (19.2)	22 (81.5)/5 (18.5)	0.935
CRPS severity score[1]	11 ± 3	10 ± 3	0.269
Mean pain last week (numeric rating scale 0-10)	5.2 ± 2.0	4.8 ± 2.2	0.430
Max pain last week (numeric rating scale 0-10)	7.3 ± 2.0	6.8 ± 1.9	0.175
Neuropathic pain symptom inventory[2]	0.38 ± 0.22	0.38 ± 0.22	0.874
Beck depression inventory II[3]	14 ± 11 (n=165)	12 ± 8	0.969
State-Trait anxiety inventory[4], trait version	43 ± 13 (n=168)	41 ± 11 (n=26)	0.663
Graded chronic pain scale[5], disability score	59 ± 24	61 ± 20	0.861
Graded chronic pain scale[5], pain intensity score	60 ± 20	58 ± 16	0.253

[1] CRPS severity score, range 0-17 [53]; [2] Neuropathic pain symptom inventory, range 0-1 [58]; [3] Beck depression inventory II, range 0-63 [59]; [4] State-Trait anxiety inventory, range 20-80 [60]; [5] Graded chronic pain scale – disability and pain intensity scores, range 0-100 [57]; Mann-Whitney-U test, Pearson χ^2 test for categorical data, p-values <0.05 are printed boldly.

3.2.5. Contralateral hyperalgesia

Hyperalgesia on the contralateral extremity was common and seen in 31.2% of patients. In most patients, contralateral hyperalgesia was seen because of hypersensitivity to mechanical pain on the contralateral extremity (n=53). Only a minority exhibited signs of pressure pain (n=6) or mechanical allodynia (n=7) on the contralateral extremity.

Patients with contralateral hyperalgesia had higher pain intensities and the neuropathic pain character was more pronounced. Patients with upper extremity CRPS showed higher disability scores in the DASH questionnaire when contralateral hyperalgesia was present (**Table 8**). Furthermore, a trend towards a generally higher disease severity could be observed. Sensory profiles of the affected ipsilateral extremity were different in mechanical pain thresholds (mechanical pain threshold z-score; without vs. with contralateral hyperalgesia: 0.24 vs. 1.77; $Z=-5.8$, $p<0.001$; **Figure 8A**). Significant differences in ipsilateral pressure pain sensitivity were only seen before correcting for multiple comparisons (pressure pain threshold z-score; without vs. with contralateral hyperalgesia: 0.71 vs. 4.34; $Z=-2.52$, $p=0.012$). In summary, patients with hyperalgesia on the contralateral extremity were also more sensitive to mechanical pain on the CRPS extremity. Accordingly, gain of function in mechanical pain detection and pressure pain sensitivity was the predominant phenotype on the affected extremity ($\chi^2=32.46$, $p<0.001$ for mechanical pain; $\chi^2=10.05$, $p=0.007$ for pressure pain) (**Figure 8B**).

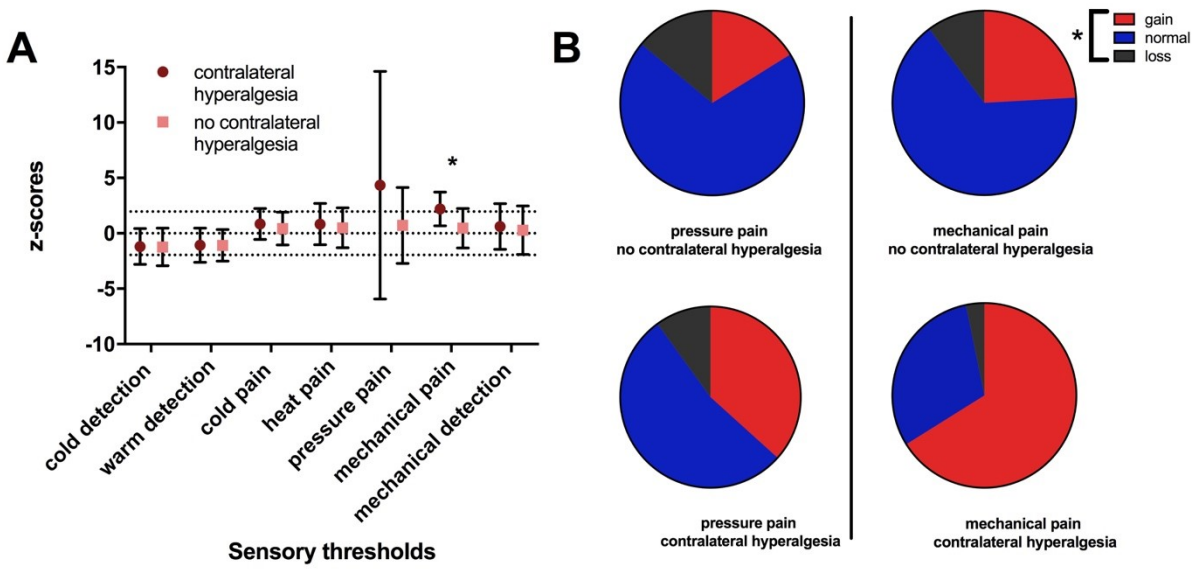


Figure 8. Sensory profiles of patients with contralateral hyperalgesia are characterized by ipsilateral mechanical hyperalgesia [63]

(**A**) Sensory function of affected extremities of patients with (dark red; n=99) and without (light red; n=100) contralateral hyperalgesia. Shown are mean z-scores (±SD) for seven included QST tests. Dotted lines at -1.96 and 1.96 mark the range of ± 2 SD referring to the reference mean. (**B**) Based on the z-values QST results were divided into three groups: $z > 1.96$, gain (red): hypersensitivity towards the stimulus; $z < -1.96$, loss (grey): hyposensitivity towards the stimulus; $-1.96 < z < 1.96$, normal (blue): regular response to the stimulus. The pie chart illustrates the distribution into the three groups for mechanical and pressure pain thresholds. *p<0.05, Mann-Whitney-U test (A), Pearson χ^2 test (B).

Table 8. Patient and disease characteristics of CRPS patients with and without contralateral hyperalgesia [63]

Variables	Contralateral hyperalgesia (n = 62)	No contralateral hyperalgesia (n = 137)	p value
Age (range)	51.6 (23; 72)	51.2 (18; 91)	0.830
Sex (n (%) male; n (%) female)	**6 (10%); 56 (90%)**	**40 (29%); 97 (71%)**	**0.002**
Type (n (%) I; n (%) II)	54 (87%); 8 (13%)	121 (88%); 16 (12%)	0.806
Affected extremity (n (%) upper extremity; n (%) lower extremity)	54 (87%); 8 (13%)	118 (86%); 19 (14%)	0.854
Disease duration (n (%) <12 months; n (%) >12 months)	50 (81%); 12 (19%)	111 (81%); 26 (19%)	0.950
CRPS severity score[1] (mean ± SD)	12 ± 2	11 ± 3	0.052
Mean pain last week (numeric rating scale 0-10)	5.5 ± 2.1	4.9 ± 2.0	0.056
Max pain last week (numeric rating scale 0-10)	**7.7 ± 2.0**	**7.0 ± 2.0**	**0.007**
Current pain (numeric rating scale 0-10)	**5.2 ± 2.3**	**3.8 ± 2.4**	**<0.001**
Neuropathic pain symptom inventory[2]	**0.43 ± 0.22**	**0.36 ± 22**	**0.044**
Beck depression inventory II[3]	14 ± 12 (n=59)	13 ± 10 (n=133)	0.719
State-Trait anxiety inventory[4], trait version	43 ± 13 (n=60)	43 ± 12 (n=134)	0.633
Graded chronic pain scale[5], disability score	64 ± 21	57 ± 25	0.071
Graded chronic pain scale[5], pain intensity score	**66 ± 18**	**58 ± 20**	**0.009**
Disability of the arm, shoulder and hand[6]	**60 ± 19**	**51 ± 23**	**0.024**

[1] CRPS severity score, range 0-17 [53]; [2] Neuropathic pain symptom inventory, range 0-1 [58]; [3] Beck depression inventory II, range 0-63 [59]; [4] State-Trait anxiety inventory, range 20-80 [60]; [5] Graded chronic pain scale – disability and pain intensity scores, range 0-100 [57]; [6]Disability of arm, shoulder and hand, range 0-100 [61]; Mann-Whitney-U test, Pearson χ^2 test for categorical data, p-values <0.05 are printed boldly.

3.3. CRPS patients compared to Fracture Controls

Injuries of the extremities including fractures are the most common cause of CRPS [9]. The following chapter investigates pain characteristics as well as sensory profiles in CRPS patients and patients recovering from fractures, so called fracture controls.

3.3.1. Patient and disease characteristics

For this analysis a total of 105 CRPS patients with short lasting (max. 12 months) CRPS type I of the upper extremity was selected and compared to 34 fracture controls with upper extremity fractures [51]. Both groups were of similar age, but the proportion of male patients was larger in fracture controls (**Table 9**). Importantly, QST z-values were normalized for sex differences. Pain intensity was 3-4 points higher in CRPS patients (**Figure 9A**). This was true for the current, mean and maximum pain intensity in the week before study participation (**Table 9**).

Table 9. Patient and disease characteristics in patients with CRPS type I of the upper extremity and fracture controls [51]

Variables	CRPS type I upper extremity (n=105)	Fracture Controls (n=34)	p value
Age ± SD (range)	52.8 ± 12.6 (20;91)	47.8 ± 14.1 (20;78)	0.1
Sex (n (%) male/n (%) female)	**19 (18.1)/ 86 (81.9)**	**17 (50.0)/17 (50.0)**	**< 0.001**
CRPS severity score[1]	**11 ± 3 (5;17)**	**1 ± 1 (0;5)**	**< 0.001**
Mean pain last week (NRS 0-10)	**5 ± 2 (0;10)**	**2 ± 2 (0;6)**	**< 0.001**
Current pain (NRS 0-10)	**5 ± 2 (0;10)**	**1 ± 2.0 (0;10)**	**< 0.001**
Maximum pain last week	**7 ± 2 (1;10)**	**3 ± 3 (0;10)**	**< 0.001**
Graded chronic pain scale[2], disability	**61 ± 25 (0;100)**	**22 ± 26 (0;100)**	**< 0.001**
Graded chronic pain scale, pain intensity	**60 ± 21 (3;100)**	**21± 23 (0;100)**	**< 0.001**

[1] CRPS symptom severity score, range 0-17 [53]; [2] Graded chronic pain scale, range 0-100 [57]; Mann-Whitney-U test, Pearson χ^2 test for categorical data, p-values <0.05 are printed boldly.

Fracture controls were not free of pain with five patients reporting pain intensities higher than one on the 0-10 NRS at the day of study participation. Careful chart review of these cases highlighted that despite rather high pain reports the CRPS severity score was still low and the Budapest criteria were not fulfilled (**Table 10**). The high pain ratings are most likely explained by preexisting painful conditions or prolonged healing after fracture.

Table 10. Fracture controls with current pain intensity > 1 [51]

No	sex	age	time since diagnosis (years)	Budapest criteria fulfilled	current pain (NRS)	mean pain (NRS)	max pain (NRS)	CSS	Presumable etiology of the pain
1	m	35	0.30	no	2	3	6	0	Chronic shoulder instability and pain
2	f	31	0.70	no	4	5	7	0	Elbow fracture, prolonged healing
3	f	63	0.25	no	10	5	10	4	Traumatic shoulder luxation, osteoarthritis
4	f	26	0.30	no	2	6	10	1	Elbow fracture, prolonged healing
5	f	61	0.15	no	2	3	5	0	Proximal humerus fracture, prolonged healing

Shown are patient and disease characteristics of patients with current pain intensity ratings > 1 on a 0-10 NRS. CRPS was ruled out and the presumable etiology of the high pain intensity is documented in the last column.

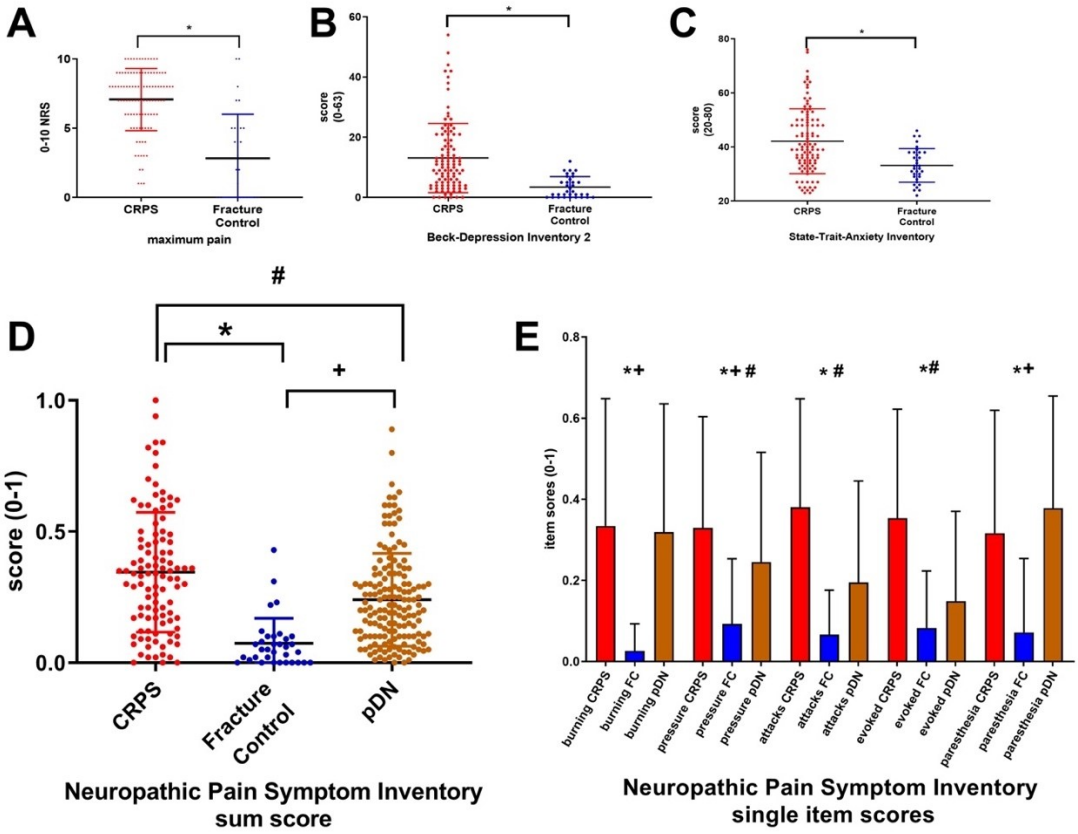


Figure 9. Pain intensity and characteristics as well as psychological parameters differ in CRPS (n=105) and fracture controls (n=34) [51]
(A) Maximum pain intensity on a 0-10 numeric rating scale is higher in CRPS (red) than in fracture controls (blue); Depressiveness **(B)** and anxiety **(C)** are more pronounced in CRPS patients (red); A neuropathic pain character is typical in CRPS. Shown are the results of the Neuropathic pain symptom inventory comparing CRPS patients (red), fracture controls (FC, blue) and patients with diabetic polyneuropathy (pDN, n=158, brown). **(D)** Neuropathic pain symptom inventory sum score, **(E)** single item scores. *, +, # p <0.05; * CRPS vs. fracture controls; # CRPS vs. pDN; + fracture controls vs. pDN; Mann-Whitney-U test
Figure printed with permission from Wolters Kluwer Health, Inc., license number 5239471173940: Dietz C. et al. What is normal trauma healing and what is complex regional pain syndrome I? An analysis of clinical and experimental biomarkers. Pain. 2019 Oct; 160(10):2278-2289, https://journals.lww.com/pain/pages/default.aspx

Beside differences in pain intensity, the NPSI depicted a less pronounced neuropathic pain character in fracture controls (0.07 vs. 0.35; Z=-6.6, p<0.001) (**Figure 9D**). Surprisingly high values in CRPS patients indicating neuropathic pain burden - in a pain disease without definite neuropathic damage - prompted further evaluation. Therefore, NPSI values obtained from patients with diabetic polyneuropathy (n=158; Brno, ncRNApain study center) were added as disease control (**Figure 9D**) and compared to fracture controls as well as CRPS patients. Interestingly, the values of CRPS patients

were even higher than those of patients with diabetic polyneuropathy (0.35 vs. 0.24, $p<0.001$). The NPSI values of fracture controls were significantly lower than those of patients with diabetic polyneuropathy. In summary, this suggests pronounced neuropathic pain symptoms in CRPS patients as typical pain characteristic. Next, a comparison of each NPSI subscore was conducted (**Figure 9E**). CRPS patients reached especially high scores regarding pressure pain, pain attacks and evoked pain. In general, fracture controls reached low scores in all categories particularly with regard to burning and pressure pain as well as paresthesia. Burning pain, pressure pain and paresthesia can therefore be considered discriminating pain features that help separating CRPS patients and fracture patients.

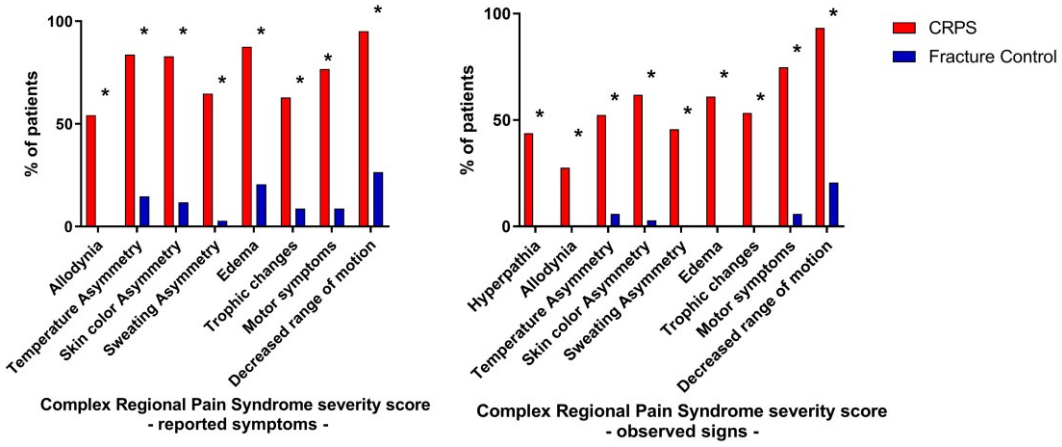


Figure 10. Higher frequency of symptom report and observation in CRPS patients than in fracture controls (adopted from [51])
Shown is the frequency of symptom report and observation of clinical signs in the different categories of the CRPS severity score. CRPS patients (red) compared to fracture controls (blue). * $p<0.05$, Pearson χ^2
Figure adapted with permission from Wolters Kluwer Health, Inc., license number 5239471173940: Dietz C. et al. What is normal trauma healing and what is complex regional pain syndrome I? An analysis of clinical and experimental biomarkers. Pain. 2019 Oct; 160(10):2278-2289, https://journals.lww.com/pain/pages/default.aspx

The CRPS severity score as validated tool for overall CRPS severity and disease burden was higher in CRPS (10.7 ± 2.8; range 3-16). In fracture controls the mean CRPS severity score was 1.56 ± 1.5 (0;5) and only few items were positive (**Table 9**). Beside the comparison of mean values, single items were compared (**Figure 10**). The frequency of reported symptoms or observed signs was low in fracture controls. In contrast, the frequency of reported symptoms in CRPS patients was ranging between 54.3% for

allodynia and 95.2% for motor dysfunction. Accordingly, the frequency of observed signs was also higher in CRPS patients with observed allodynia in 27.6% and motor dysfunction in 93.3%. Interestingly, a discrepancy between reported and observed signs can be noted with a tendency to a higher frequency of symptom reports.

3.3.2. QST and sensory profiles

Sensory profiles of fracture controls showed side differences and were not easily distinguishable from sensory profiles of CRPS patients. The selected subgroup of CRPS patients had sensory profiles (**Figure 11A**) comparable to the profile of the complete CRPS cohort. The analysis of the fractured versus healthy extremity in fracture controls (**Figure 11B**) showed a relative hypersensitivity of the affected extremity towards cold pain emphasizing sensory disturbances of the fractured limb (cold pain threshold fractured vs. unaffected extremity: 0.42 vs. 0.14; Z=-3.0, p=0.003). The visual comparison of sensory profiles obtained from the affected extremities in CRPS and fracture controls suggested that sensory profiles are similar between the two groups. However, the z-scores of pressure and mechanical pain threshold are significantly different (**Figure 11C**) with higher values in CRPS patients indicating a higher sensitivity towards painful pressure and mechanical stimulation (pressure pain threshold CRPS vs. fracture control: 0.87 vs. -0.61; Z=-2.89, p=0.004; mechanical pain threshold: 1.15 vs. 0.30; Z=-2.92, p=0.004). Further, allodynia occurred more often in CRPS patients (n=19 vs. n=1). The sensory profiles of the unaffected extremities were similar in both groups. As the range of variation in QST results is rather large the z-scores of the significant variables (pressure pain and mechanical pain) were grouped into three groups depending on their value: -1.96 to 1.96 = normal; <-1.96= loss of function; >1.96 = gain of function (**Figure 11D**). An analysis of the distribution into the categories *normal, loss of function* and *gain of function* showed a higher frequency of mechanical pain hypersensitivity in CRPS patients ($\chi^2(2) = 12.1$; p=0.002).

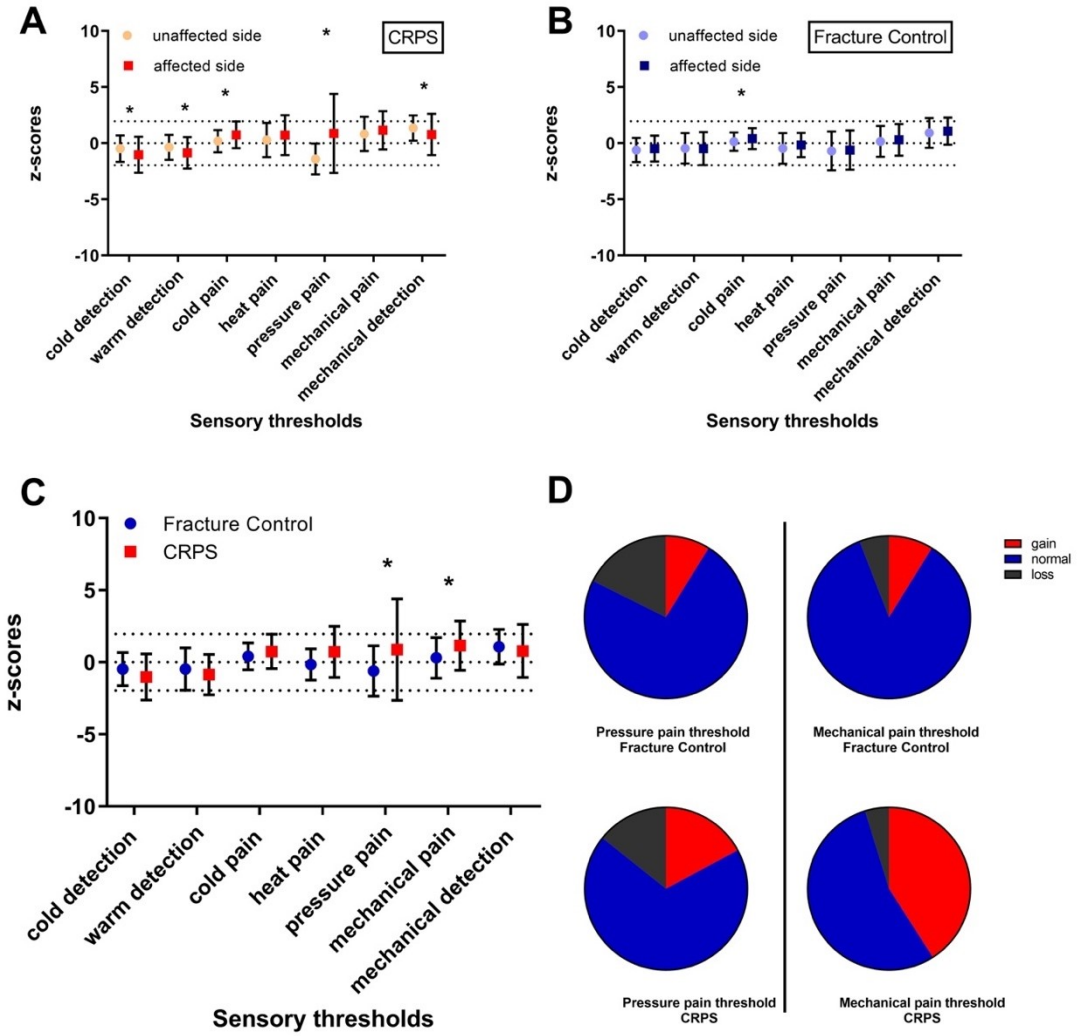


Figure 11. QST sensory profiles comparing CRPS patients (n=105) and fracture controls (n=34) depict a higher sensitivity towards pressure and mechanical pain in CRPS [51]
(**A**) QST profiles of the selected CRPS cohort comparing the affected (red) and the unaffected (orange) extremity. (**B**) QST profiles of fracture controls comparing the fractured limb (dark blue) to the unaffected limb (light blue). (**C**) Sensory profiles comparing the affected extremities of CRPS patients (red) and fracture controls (blue). Shown are mean z-scores (±SD) of seven included QST tests. Dotted lines at -1.96 and 1.96 mark the ± 2 SD range referring to the reference mean. (D) Based on the z-values QST results were divided into three groups: $z > 1.96$, gain (red): hypersensitivity towards the stimulus; $z < -1.96$, loss (grey): hyposensitivity towards the stimulus; $-1.96 < z < 1.96$, normal (blue): regular response to the stimulus. (**D**) shows the distribution into the three groups regarding pressure pain thresholds and mechanical pain thresholds; * $p < 0.05$, Wilcoxon signed rank test (A, B); Mann-Whitney-U test (C).

3.3.3. Patient and disease characteristics

Psychological comorbidities frequently occur in pain diseases. Here, self-administered questionnaires were used to describe patient reported outcomes (**Figure 9B + C**). The analysis depicted differences with a higher psychological disease burden in CRPS patients. Depressive symptoms as measured through the Beck Depression Inventory 2 (**Figure 9B**) exclusively occurred in CRPS patients (CRPS vs. fracture controls: 13.1 vs. 3.4; $Z=-5.5$, $p<0.001$). 35.2% of CRPS patients scored higher than the cut-off for mild depression whereas no fracture control reached the cut-off. Anxiety was measured through the State-trait anxiety inventory (**Figure 9C**) and seen in 50 CRPS but only four fracture control patients (CRPS vs. fracture controls mean STAI- values: 42.1 vs. 33.2; $Z=-4.1$, $p<0.001$).

Disability through pain as expressed by the Graded chronic pain scale was more pronounced in CRPS also emphasizing the disease impact on perceived disability (CRPS vs. fracture controls: 60.7 vs. 21.7; $Z=-6.1$, $p<0.001$).

3.3.4. Principal Components

A principal component analysis (PCA) was used to visualize the underlying data structure and condense many different variables into few principal components. Given the relatively large interindividual variances of the above described variables PCA can be of help by clearing the view on the relevant data constructs with reduced dimensions. Ideally, this facilitates the discrimination of fracture controls and CRPS patients. I conducted a PCA with orthogonal rotation (varimax) and included the State-trait anxiety inventory, the Beck depression inventory II, the CRPS severity score, the NPSI, the Graded chronic pain scale, mean pain and detection threshold QST variables (cold, warm, mechanical detection threshold) in the

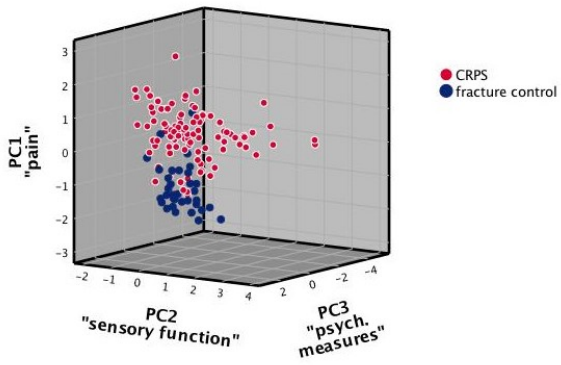


Figure 12. Principal Component 1 separates CRPS patients and fracture controls
3D-Scatterplot with principal components (PC) on the different axes. Shown are individual data points of CRPS patients (red) and fracture controls (blue).

analysis (**Figure 12**). Other QST variables could not be added due to insufficient cross-correlation. Cross-correlation is an assumption that needs to be met in order to allow correct PCA. The Kaiser-Meyer-Olkin measure for sampling adequacy was 0.817 and above the acceptable limit of 0.5 [62]. The Bartlett test for sphericity gave a $\chi^2(45) = 774.38$, $p<0.001$. This indicates sufficient correlation between the variables included in the PCA. Three principal components were extracted (**Table 11)**. Based on the impact the different variables have on each component they were named principal component 1 *pain*, principal component 2 *sensory function* and principal component 3 *psychological measurements*. In total, the three factors account for 74.1% of the overall variance. A Mann-Whitney-U test showed that principal component 1 *pain* is different between fracture controls and CRPS patients. The clustering of individual cases in a coordinate system with the three PC as axes (**Figure 12**) visualized how especially PC1 discriminates fracture controls and CRPS patients.

The comparison of CRPS patients and fracture controls revealed specific differences helping in distinguishing the two groups. CRPS patients were characterized by higher pain intensities and neuropathic pain symptoms. Sensory differences regard pressure and mechanical pain. Anxiety, depression and also perceived disability through pain were more pronounced in CRPS patients which points towards higher disease burden.

Table 11. Rotated factor loadings generated from Principal Component Analysis

Variables	Principal component 1 pain	Principal component 2 psychological measurements	Principal component 3 sensory function
Graded chronic pain scale – pain intensity	**0.91**	0.17	-0.12
Graded chronic pain scale - disability	**0.86**	0.18	-0.43
Mean pain	**0.86**	0.19	-0.10
CRPS severity score	**0.77**	0.14	0.23
Neuropathic pain symptom inventory	**0.74**	0.34	-0.11
Beck depression inventory 2	0.31	**0.90**	-0.06
State-Trait anxiety inventory	0.29	**0.91**	-0.11
Mechanical detection threshold	-0.061	-0.20	**0.80**
Cold detection threshold	-0.11	0.06	**0.79**
Warm detection threshold	-0.02	-0.05	**0.74**

Shown are the factor loadings of each variable included in PCA after varimax rotation. For better visualization of the impact on the three principal components values above 0.6 are printed boldly.

3.4. A follow-up of CRPS patients

3.4.1. Patient and disease characteristics

Baseline characteristics of the follow up cohort were compared to the characteristics of the complete CRPS study cohort (**Table 13**). The disease duration at baseline was significantly longer than in the large CRPS sample (0.9 years). To account for this difference, the differential development of chronic and acute CRPS cases is also described within the following chapter. Patient and disease characteristics were otherwise similar with comparable disease severity and pain intensities. Between the two study rounds patients received individual therapy following the German guidelines for CRPS therapy [25]. All patients had physiotherapy or occupational therapy, in 59 % as part of an interdisciplinary multimodal pain program. Most frequently used drugs were antidepressants, anticonvulsants and non-steroidal anti-inflammatory drugs (**Figure 13**).

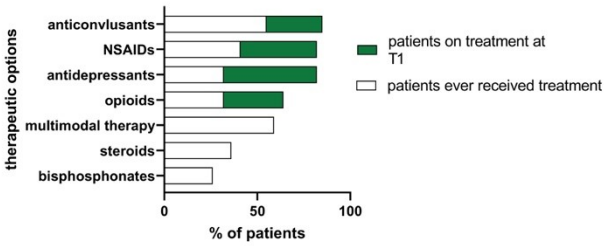


Figure 13. Different treatments during follow-up
Shown is the percentage of patients that received a certain treatment at follow-up (T1, green bar) or had ever received it (green bar). All patients had physiotherapy or occupational therapy (not shown).

Opioids were part of the treatment regimen in 64 %. At follow-up, 15 patients (38.5%) did not fulfil the Budapest criteria anymore. These patients had a lower disease severity (4.7 vs. 9.9, $p<0.001$) and lower mean pain (2.3 vs. 5.0, $p<0.001$) than those still fulfilling the criteria at follow-up. In general, pain intensity ratings including mean, current and maximum pain as well disease severity improved significantly during follow-up (**Table 12**) but patients were not free of pain and symptoms. A significant reduction in mean pain ($\Delta \geq 2$) was more likely to occur in patients with higher pain intensities. Even in the group of patients not fulfilling the Budapest criteria any longer (n=15) four patients had pain intensities higher than three. The detailed illustration of the single CRPS severity score items shows significant improvement in reported symptoms as well as observed signs including allodynia and skin color asymmetry (**Figure 14**). At the same time, it is visible that many symptoms (e.g. decreased range of motion) persist and motor symptoms

worsen. Similarly, the neuropathic pain character as described by the NPSI and psychological measurements are unchanged.

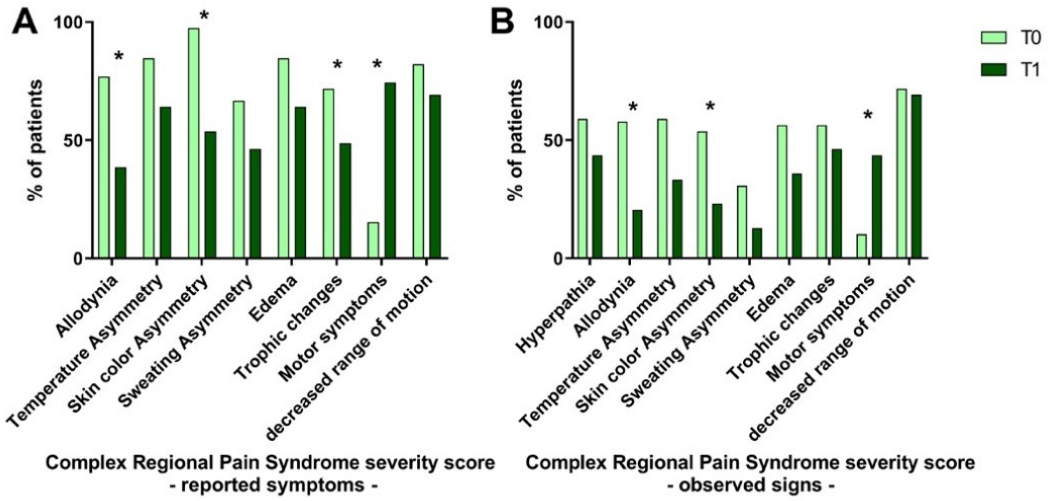


Figure 14. Detailed illustration of the CRPS severity score in patients at baseline (T0) and at follow-up (T1) shows a mixed picture of symptom amelioration but also stagnation and aggravation
Frequency of symptom report (A) and observation of clinical signs (B) as documented for the CRPS severity score at baseline (T0, light green) and follow-up (T1, dark green). *p<0.05, McNemar test

Contralateral hyperalgesia (as defined above) occurred in four patients at baseline and in eleven patients at follow up. In detail, nine patients developed, and two patients lost contralateral hyperalgesia. Patients with contralateral hyperalgesia at baseline did not have higher pain intensities (4.3 vs. 3.9, p=0.810) or severity scores (8.8 vs. 7.8, p=0.637) at follow-up. Warm CRPS was the most frequent temperature phenotype at baseline (n=23). At follow-up, the majority of patients (n=18) still described the affected extremity as warm. However, there was an increased proportion (n=14) with an indifferent temperature phenotype. In seven cases this indifferent phenotype had developed in cases initially classified as warm CRPS and in six cases in patients with cold CRPS. Interestingly, the frequency of lower back pain increased significantly during follow-up. While at baseline only nine patients reported to have lower back pain it were 26 patients at follow-up (McNemar test: p<0.001).

Table 12. Patient and disease characteristics of the follow-up cohort (n=39) in comparison to the complete CRPS cohort (n=199)

Variables	Follow-up cohort (n=39)	Full cohort (n =199)	p value (full vs. follow-up)
Age (range)	50.7 (22; 73)	51.3 (18; 91)	0.784
Sex f/m (%)	74.4/25.6	76.9/23.1	0.734
Disease duration (mean ± SD, years): T0 at T1	**1.9 ± 2.5 (0;12)** 4.3 ± 2.7 (1.3; 14.9)	**0.9 ± 2.0**	**0.027**
Time to T1 (mean ± SD, years)	2.5 ± 0.9 (0.8; 4.3)	/	/
Affected extremity upper/lower (%)	84.6/15.4	86.3/13.7	0.764
Type I/type II (%)	84.6/15.4	87.9/12.1	0.567
Max pain (numeric rating scale): T0 T1	7.7 ± 2.1 **6.3 ± 3.1*** (p=0.009)	7.2 ± 2.0	0.205
Mean pain (numeric rating scale): T0 T1	5.3 ± 2.0 **4.0 ± 2.4*** (p<0.001)	5.1 ± 2.0	0.573
Current pain (numeric rating scale): T0 T1	4.7 ± 2.2 **3.4 ± 2.5*** (p=0.004)	4.3 ± 2.4	0.326
CRPS severity score: T0 T1	10 ± 3 **8 ± 4*** (p=0.002)	11.0 ± 3	0.103
Neuropathic pain symptom inventory (n=34): T0 T1	0.43 ± 0.22 0.41 ± 0.24 (p=0.689)	0.38 ± 0.22	0.217
Beck depression inventory II (n=38): T0 T1	14 ± 11 12.3 ± 11.8 (p=0.319)	13 ± 11	0.777
State-Trait anxiety inventory, trait version (n=30): T0 T1	41 ± 14 42 ± 14 (p=0.737)	43 ± 13	0.999

Patient and disease characteristics of patients included in the follow up investigation. T0 is the timepoint of baseline participation, T1 represents the timepoint of follow-up investigation. Baseline characteristics (T0) of the follow up cohort are compared to the complete CRPS study cohort (n=199) to prove comparability. T0 vs. T1 paired t-test; comparison to full cohort independent t-test; printed boldly when $p < 0.05$

Disease duration at baseline might be an important factor influencing observations during follow-up. Therefore, the follow-up cohort (n=39) was divided in cases with short disease duration (≤12 months = acute, n=21) and long disease duration (>12 months = chronic, n=18) at baseline (**Table 13**). Baseline patient and disease characteristics were comparable between the two groups. Patients with acute CRPS at baseline had improved pain intensity ratings as well as improved disease severity after follow-up. In chronic cases, there was no improvement and disease characteristics were stable.

Table 13. Patient and disease characteristics of the follow up cohort depending on disease duration

Variables	Disease duration ≤ 12 months "acute"	Disease duration > 12 months "chronic"	# p-value comparison acute vs. chronic at T0	*p-value T0 vs. T1 (acute)	*p-value T0 vs. T1 (chronic)
n	21	18			
Age (range)	51.1 (22; 69)	50.3 (22; 73)	0.762	/	/
Sex f; m (%)	71.4; 28.6	77.8; 22.2	0.651	/	/
Disease duration at T0 (years)	**0.35 ± 0.31**	**3.7 ± 2.80#**	**<0.001**	/	/
Time between T0-T1 (years)	2.4 ± 1.0	2.6 ± 0.73	0.616	/	/
Affected extremity upper vs. lower (%)	85.7; 14.3	83.3; 16.7	0.837	/	/
Type I/type II (%)	81.0/19.0	88.9/11.1	0.493	/	/
Maximum pain (0-10): T0 T1	**7.8 ± 1.8** **6.0 ± 3.4***	7.5 ± 2.4 6.8 ±2.8	0.650	**0.024**	0.200
Mean pain (0-10): T0 T1	**5.5 ± 2.0** **3.8 ± 2.2***	5.1 ± 1.9 4.2 ± 2.6	0.572	**0.004**	0.056
Current pain (0-10): T0 T1	**4.8 ± 2.3** **3.1 ± 2.4***	4.6± 2.1 3.7 ± 2.7	0.771	**0.014**	0.144
CRPS severity score: T0 T1	**10 ± 3** **8 ± 4***	10 ± 3 8 ± 4	0.518	**0.006**	0.154
Neuropathic pain symptom inventory: T0 T1	0.40 ± 0.23 0.37 ± 0.24	0.46 ± 0.21 0.47 ± 0.23	0.351	0.451	0.810
Beck depression inventory II: T0 T1	12 ± 11 14 ± 14	16 ± 10 12 ± 11	0.329	0.865	0.093
State-Trait anxiety inventory, trait version: T0 T1	40 ± 15 43 ± 17	45 ± 12 42 ± 13	0.228	0.154	0.051

Shown are patient and disease characteristics of the follow up cohort (n=39) divided in two groups depending disease duration. Long lasting (>12 months) CRPS was seen in 18 patients, short lasting CRPS (<12 months) in 21 patients. Baseline characteristics (T0) of both groups were compared with independent t-tests. Two additional analyses compared baseline (T0) and follow up (T1) characteristics in each group with paired t-tests.

3.4.2. QST and sensory profiles

Sensory disturbances persisted during follow-up. At baseline, sensory profiles were characterized by pressure pain hyperalgesia (z-score pressure pain threshold affected vs. unaffected: 2.03 vs. -0.59; T(38)= -3.35, p=0.002) and hypoesthesia towards non-painful warm (z-score warm detection threshold: -0.86 vs. 0.03; T(38)=3.66, p=0.001), cold (z-score cold detection threshold: -1.43 vs. -0.50; T(38)=4.08, p<0.001) and mechanical stimulation (z-score mechanical detection threshold: -0.36 vs. 0.51; T(38)=2.90, p=0.006) (**Figure 15A**). The sensory profile at follow-up was similar with the exception that cold detection thresholds were no longer different between affected and unaffected extremity (**Figure 15B**).

For direct comparison of sensory profiles from the affected limb at baseline and at follow up a repeated-measurement analysis of variance (rm-ANOVA) was calculated for each QST parameter. “Baseline vs. follow-up” and “affected vs. unaffected limb” served as within -subject factor. A significant within-subject factor “follow up” was seen for cold pain threshold (F(1)=5.55, p=0.024), mechanical detection threshold (F(1)=4.92, p=0.033) and mechanical pain threshold (F(1)=8.02, p=0.007). The clinically more relevant interaction between both factors describing a significant difference between unaffected and affected extremity depending on baseline or follow-up was significant for cold detection threshold (F(1)=7.24, p=0.011). However, if correcting for multiple comparison this difference is no longer significant and the clinical relevance is questionable. In summary, sensory profiles with a focus on side differences (as done in the previous chapters) are similar at baseline and follow up with the tendency towards lower cold pain thresholds at follow-up (**Figure 15C+D+E**). Two separate analyses were carried out to look into factors influencing sensory profiles. By using “acute vs. chronic” as between subject factor the impact of disease duration at baseline was analyzed. This between subject factor was insignificant in all QST parameters indicating that disease duration at baseline had no significant impact on the development of sensory profiles during follow-up. Further it was of interest, if patients still reporting clinically relevant pain (NRS >3) at follow-up had different sensory profiles than those with lower pain intensities. This between subject factor “relevant pain intensity at follow-up” was also insignificant in all QST parameters.

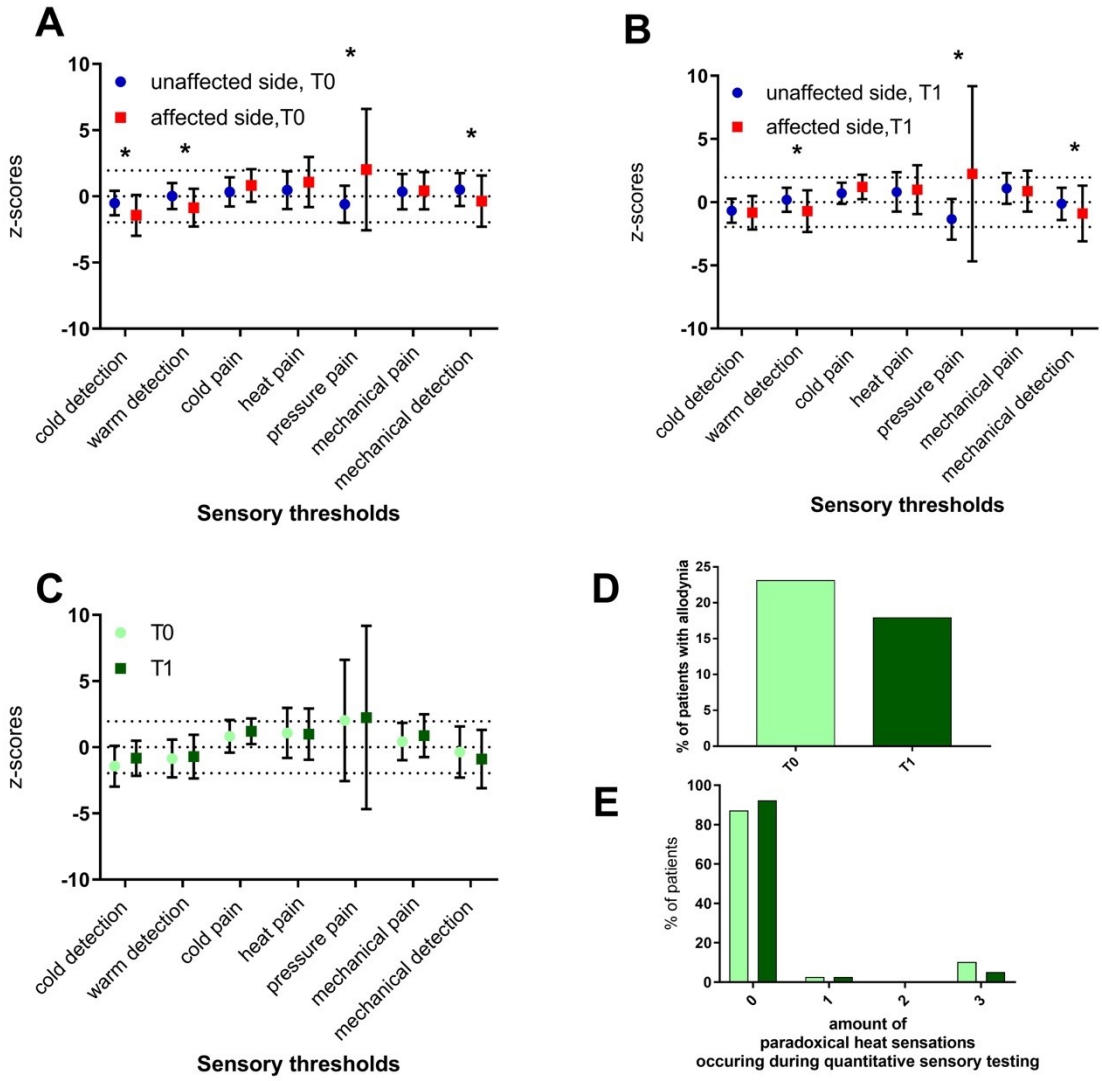


Figure 15. Sensory profiles at baseline (T0) and at follow-up (T1) are similar in 39 CRPS patients

Detection and pain thresholds of (**A**) the unaffected (blue) and affected extremity (red) at baseline (T0), (**B**) of the unaffected (blue) and affected extremity (red) at follow-up (T1) and (**C**) of the affected extremities at baseline (T0, light green) and follow-up (T1, dark green). Shown are mean z-scores (±SD) of the seven included QST tests. Dotted lines at -1.96 and 1.96 mark the 95%-confidence interval referring to the reference mean. (**D**) illustrates the frequency of allodynia at T0 and T1, (**E**) the amount of paradoxical heat sensations. *p<0.05, paired t-test (A, B), repeated-measurement ANOVA for each parameter (C), McNemar test (D, E)

4. Discussion

4.1. Summary of all results

In a sample of 199 CRPS cases with mainly acute CRPS and moderate disease severity, sensory profiles were characterized by lowered thresholds for pressure and cold pain (gain of function) and increased thresholds for mechanical detection and thermal detection (loss of function). Allodynia was more often seen on the affected extremity. Anxious and depressive symptoms were common and seen in 50% and 38% of patients, respectively.
Five subgroups based on sex, the temperature phenotype, the existence of nerve lesions (CRPS type I and II), the affected extremity and hyperalgesia on the contralateral extremity were investigated. Female and male patients differed in their sensory function with female patients being more pressure pain sensitive. Differences in disease characteristics were seen between warm and cold CRPS patients with higher disease severity scores in warm cases. Patients with a nerve injury (type II CRPS) had a more pronounced neuropathic pain character. The most prominent finding was seen in patients with contralateral hyperalgesia where higher pain intensities accompanied the affection of the contralateral extremity. Similarly, patients with contralateral hyperalgesia were more sensitive to mechanical pain stimulation.
In a second step, a subset of 105 acute, upper extremity CRPS patients was compared to fracture controls recovering from upper extremity fracture. Pain intensities were higher and of stronger neuropathic intensity in CRPS patients. Mechanical pain and pressure pain hyperalgesia were more commonly seen in CRPS than fracture patients with all other sensory parameters being similar between these patient groups. Furthermore, depressive symptoms were not seen in fracture controls but in 35% of CRPS patients.
In step three, I investigated longitudinal outcomes in 39 CPRS patients. Disease severity and pain intensity improved during a mean follow-up interval of 2.5 years. In contrast, pressure pain hyperalgesia persisted, and motor symptoms increased from baseline to follow-up. The improvement was mainly seen in patients with short disease duration at baseline. The prevalence of contralateral hyperalgesia increased from baseline to follow-up.

4.2. Sensory function in CRPS

The CRPS-affected extremity exhibited signs of hyperalgesia (pressure and cold stimuli, allodynia) as well as hypoesthesia (thermal and mechanical detection). The occurrence of hyperalgesia (gain of sensory function) and hypoesthesia (loss of sensory function) in parallel has been reported as CRPS typical phenotype before [11]. A loss of function phenotype is predominantly seen in patients with peripheral nerve injury [11] or polyneuropathy [64]. A gain of function phenotype was found in small fiber neuropathy [64]. Sensory dysfunction in CRPS therefore combines sensory features seen in nerve injury and small fiber neuropathy patients. In fact, peripheral nerve injury as seen in type II CRPS but also small fiber damage are both involved in CRPS pathology [65].

Even though frequently observed, abnormal QST values do not necessarily coincide with CRPS. As illustrated, the majority of individual QST values is within the 95%-confidence interval (**Figure 3D**). It is the comparison of the affected and unaffected extremity that points towards the sensory imbalance.

To better estimate the clinical impact of sensory disturbances, sensory observations and clinical disease characteristics have to be linked. In my study, clinically relevant mean pain at follow-up (>3 on a 0-10 NRS) did not have a significant impact on sensory profiles. This is in accordance with findings that QST and sensory profiles were not able to clearly differentiate between patients with painful and non-painful neuropathy [64]. Apparently, sensory disturbances cannot be explained by pain alone. In a comparison of painful conditions including arthritis and CPRS, sensory disturbances were rarely seen in arthritis but common in CRPS [13]. This further supports that an additional pathology explaining sensory abnormalities is needed. Mechanisms behind sensory abnormalities are manifold: Hyperalgesia can be explained either by central or peripheral sensitization. Presumably both play a role in CRPS [9]. Neurogenic inflammation resulting in peripheral sensitization was shown to be involved in CRPS pathology [66]. Here, hyperalgesia on the contralateral extremity was a frequent phenomenon. Similar to the spreading of pain and hyperalgesia to more distant areas, this indicates central sensitization [21].

Further mechanisms have to be considered when trying to explain the observed hypoesthesia. Here, higher pain intensities were correlated with higher warm and mechanical detection thresholds. Even though correlations were weak, pain induced

hypoesthesia as seen in chronic pain patients [67] is a possible mechanism. Alternatively, the observed loss of function or hypoesthesia can be caused by nerve fiber damage. CRPS type II is an example for large nerve fiber damage and is associated with stronger loss in mechanical detection [11]. However, the role of minor nerve injuries. i.e. small fiber damage is not completely clear [11] even though reduced small fiber density has been shown in skin biopsies of CRPS patients [65]. Higher thresholds for warm and cold detection could indeed point towards small fiber dysfunction.

4.3. Relevance of CRPS subgroups

Disease phenotypes are heterogenous and treatment responses in CRPS are mixed. A profound knowledge and exact description of subgroups is important for the development of future interventional trials investigating differential treatment response. My thesis explored five subgroups in terms of sensory function, disease and patient characteristics. Comparable to other studies and as typically seen in chronic pain disease the cohort was dominated by female patients [29, 30]. It remains unclear whether female patients are more susceptible to CRPS or if a higher number of wrist fractures in women mediates the higher prevalence [9]. Patient and disease characteristics including measures of anxiety and depressiveness were similar in female and male patients, but male patients were younger. This is in contrast to earlier findings where male CRPS patients had higher levels of depressiveness [30]. Sensory profiles differed even after z-transformation that corrects for general sex differences. Female patients were more sensitive to pressure pain stimuli and had lower thresholds for non-painful mechanical detection. The finding of pressure pain hypersensitivity reproduces very recently published findings on sex differences in sensory function in CRPS [31]. The authors proposed that this observation is of limited clinical relevance and a consequence of generally lowered perception thresholds in women [31]. Here, the finding of lower pressure pain thresholds was not linked to higher CRPS severity scores or higher pain intensity indeed questioning the clinical relevance. However, evident sex differences cannot be ignored. A growing body of literature comprising of endocrinological, genetic, immunological and social studies underpins the relevance of sex differences in pain disease. Many pathological mechanisms leading to the observed differences are being discussed. Estrogen as primary

female sex-hormone seems to be involved in the perception of pain as shown for migraine [68]. Sex hormones can also influence neuroimmunity which in turn has an impact on pain perception [69, 70]. The role of immune cells such as microglia in pain and sex differences has become more prominent in recent years. As one example, animal models showed responses to microglia-inhibiting drugs in male rodents only [70]. On a genetic level, genetic sequencing could reveal interactions between sex and genes that interfere with opioid receptors [71-73]. Beside biological evidence, psychological observations can also explain sex-differences in pain disease [74]. It is known that pain perception and coping strategies are different between women and men [30, 75]. Catastrophizing as important aspect of coping with pain is more often seen in female patients [76]. In addition, social aspects e.g. the belief in certain forms of masculinity or femininity can influence the perception of pain [75, 77]. In summary, future studies should account for sex differences in CRPS and monitor treatment response to allow for individualized treatment.

The presence of a nerve lesion defines CRPS type II. Here, the presence of a nerve lesion had no impact on sensory profiles. More pronounced hypoesthesia as seen in a prior study could not be reproduced. Similarly, patient and disease characteristics did not differ with the exception of a more pronounced neuropathic pain character in type II CRPS patients probably reflecting the nerve injury. The separation of type I and type II CRPS is part of the updated disease definition [32]. However, in the past the clinical relevance of this separation has been debated. A circumstance that is referred to in the mentioned disease definition. In fact, there has been evidence for [78] and against clinically relevant differences [11]. When discussing the relevance of the type I/type II differentiation it is important to acknowledge the finding that habituation to therapeutic spinal-cord-stimulation is influenced by the CRPS type [79]. This raises the general question of treatment response in different subgroups which should be assessed in further interventional trials.

The majority of patients included in the study had a warm CRPS temperature phenotype. Patients with warm CRPS had more often short lasting CRPS. This fits the assumption that warm CRPS is more common at early disease stages [33]. The temperature phenotype was also discussed in a recently published cluster analysis [55]. There, patients with mainly inflammatory symptoms were labeled as peripheral phenotype whereas those with

e.g. motor symptoms and allodynia were labeled as central phenotype. Bringing the temperature phenotype into play, warm CRPS is more common in the peripheral phenotype which was associated with higher CRPS severity scores [55]. In line with this observation, warm CRPS patients had higher disease severities. The observation of higher mechanical detection thresholds in cold CPRS patients confirm previous findings [34]. Lower skin temperatures causing less receptor activation and signal conduction could explain this finding mechanistically.

Differences in disease characteristics depending on the identity of the affected extremity have not been systematically addressed so far. CRPS of the upper extremity was more common in the presented data set which is a typical finding [9]. Despite a younger age of patients with lower extremity CRPS, patient and disease characteristics were comparable. Similarly, sensory profiles did not differ between upper and lower extremity cases. The only difference regards paradoxical heat sensations that were more frequently documented on the lower extremity. This, however, is a known finding from healthy subjects [80] and of minor clinical importance. In summary, no large differences were seen in CRPS of the upper and lower extremity. This rejects the hypothesis that obvious differences in hand and foot, e.g. cortical representation or use in daily life, affect disease characteristics. If confirmed in other study populations, these results may justify the pooling of upper and lower extremity CRPS in studies facilitating the recruitment for future trials. In terms of treatment strategies this subgroup is special as physiotherapy and occupational therapy of course adopt to the affected extremity. In how far medical treatment should be extremity-specific needs further evaluation.

The affection of the contralateral extremity, here defined as hyperalgesia of the contralateral limb, was seen in about 30% of the patients. Patients with hyperalgesia on the contralateral limb had higher pain intensities, a more pronounced neuropathic pain character and were also more sensitive to mechanical pain on the affected side. General hypersensitivity possibly explained by central sensitization could be assumed [38]. There is also morphological evidence that CRPS might affect both extremities as reduced intraepidermal nerve fiber densities were not only seen in the CRPS extremity but also on the contralateral side [81]. However, it is unclear whether these small fiber abnormalities are caused by CRPS or predispose for the onset of CRPS after a trauma [81]. During my longitudinal observation contralateral hyperalgesia was seen at baseline

and at follow-up but occurred more frequently at follow-up. In nine patients, contralateral hyperalgesia developed as new feature. However, in the cross-sectional analysis of the large cohort no difference in disease duration was seen. As far as sensory abnormalities on the contralateral extremity are concerned, an increase was noted in an earlier longitudinal observation of 19 patients potentially indicating that CRPS is first limited to one extremity and spreads over the course of the disease. This was interpreted as part of a general pronociceptive pain modulation in CRPS [39]. An affection of the contralateral extremity might therefore also occur in early disease stages. In clinical practice, patients are sometimes confronted with denied compensation claims when not only the originally injured extremity, but both are affected by CRPS. In this context, the finding that almost one third of the 199 patients showed signs of contralateral hyperalgesia is especially important. In how far clinical decision making should acknowledge this subgroup is an open question. As higher pain intensities and contralateral hyperalgesia were correlated, it might be possible that intensified treatment could help limiting the spread of symptoms to the contralateral extremity. In addition, higher disability ratings with regard to the functionality of the upper extremity support the clinical importance of contralateral hyperalgesia.

In summary, important differences were found in the explored subgroups and regarded the sensory function depending on sex and the presence of contralateral hyperalgesia. Interestingly, disease severity and pain intensities were comparable in most subgroups with the exception of the temperature phenotype and contralateral hyperalgesia subgroup. In how far treatment responses depend upon these groups needs further investigation in future trials. However, the observed similarities should also trigger new approaches based on molecular biomarkers [27] or phenotyping algorithms [55] to better capture the diversity of the clinical picture.

4.4. Psychological impairment in CRPS

Psychosocial factors are important in CRPS [27]. In order to account for this, my study measured anxiety and depressiveness with the State-trait anxiety inventory and the Beck depression inventory II, respectively. Here, 38% of CRPS patients were above the cut-off for mild depression and 50% above the threshold indicating anxiety. Interestingly, no

fracture control reached the cut-off for mild depression and anxiety scores were lower than in CRPS patients. A causality cannot be demonstrated with this study design, but the finding underpins that psychological factors are important already in acute, short lasting CRPS. So far, no causal psychological risk factors have been described [27]. However, higher levels of post-traumatic stress [82] or the inability to describe feelings, i.e. alexithymia [83], have been reported in CRPS patients. Psychological factors can also influence the disease course. Pain-related fear, anxiety and perceived disability were found to be associated with a worse disease outcome [49]. In the follow-up investigation stable anxiety and depressiveness scores were noted even though disease severity improved. This discrepancy could partially be explained by a shift in disease burden that is no longer captured by the CRPS severity score. The severity score focusses on core signs and symptoms of CRPS that improve in many cases. However, CRPS often becomes a chronic condition potentially leading to social distress e.g. job-loss or isolation. This might influence depressiveness and anxiety scores. Another explanation would of course be, that these scores are already elevated before the onset of CRPS and represent preexisting conditions or indicate vulnerability.

4.5. Sensory function in fracture patients

Accurate distinction between patients with normal healing after a fracture or CRPS is important and can be of help in avoiding delayed diagnosis [43, 44]. In my study, CRPS patients – in contrast to fracture controls – were characterized by higher pain intensities, a neuropathic pain character, pressure and mechanical hyperalgesia as well as psychological disease burden.

However, similarities of fracture and CRPS patients complicate diagnostics [84]. Fracture controls were not free of symptoms with some cases reporting pain intensities as high as 10 on a 0-10 NRS. In addition to high pain ratings in some cases, the fractured extremity was more sensitive to cold pain. This finding of sensory abnormality is supported by literature [45-47]. Furthermore, some symptoms of CRPS such as edema can occur in fracture patients [47].

Similarities between fracture controls and CRPS patients could be explained by inflammatory processes involved in fracture healing [85]. Indeed, increased levels of

immune-mediators have been found in skin biopsies of hands following surgery and immobilization [47]. This parallels in part with findings of increased inflammatory markers in the skin or skin blisters of CRPS patients [14, 15]. Similarities could further be explained by immobilization during fracture treatment. In an experimental setting, immobilization of the forearm alone was inducing CRPS-like symptoms e.g. increased skin temperature or hypersensitivity to mechanical and cold stimuli [46].

Even if difficult in some cases, a differentiation between the two groups is possible and has been demonstrated in another study population [84]. The frequency of symptom report and symptom observation, respectively, was clearly higher in CRPS patients of my study. With these symptoms being part of the diagnostic criteria, the result was expected. Further, a pronounced neuropathic pain component was noted which is in accordance with earlier findings [86]. However, ratings of the NPSI in CRPS patients were even higher than in patients with diabetic polyneuropathy, a definite neuropathic disease. This is surprising, because the labeling of CRPS as "neuropathic disease" is under debate. It emphasizes that an additional pathology is needed to explain the observed differences. Activation of neurogenic inflammation perpetuating pain is one possible explanation. Especially in neuropathic pain diseases disturbances of barriers e.g. in the myelin layer are assumed to play a role in pathogenesis [87-89]. Injury to barriers could explain edema in CRPS. Data from microRNAs in CRPS patients also point towards an involvement of barrier disruption [51].

It is the strength of the Budapest criteria and the CRPS severity score to combine different symptom categories adequately reflecting the multidimensional clinical picture of CRPS. In addition, assessing the pain characteristics by using standardized questionnaires as the NPSI can help in discriminating fracture and CRPS patients. Beside an early detection of CRPS patients, preventive strategies detecting patients at risk for CRPS are needed. A recent study addressing chronic postoperative pain developed a predictive model enabling future studies on measures preventing postoperative chronic pain [90]. This encouraging result could help designing new studies targeting CRPS risk factors.

4.6. Residual state and sensory scar theory

Especially in cases with acute CRPS an improvement in pain and overall disease severity as visualized by lower pain intensities and lower CRPS severity scores was noted. This supports that significant symptom amelioration is more frequently seen in early disease stages [48]. However, an interesting discrepancy could be observed. The clinical improvement was contrasted by stable psychological parameters, neuropathic pain symptoms and sensory dysfunction. Intuitively, one might assume that improvements in disease severity go along with a normalization in sensory dysfunction. My results and the literature suggest otherwise when stating that sensory profiles are similar over the disease course [20], and sensory dysfunction is an ongoing phenomenon [39]. The observation of ongoing sensory disturbances might indicate sustained pathological processes in the peripheral or central nervous system. It is likely that motor symptoms like tremor and dystonia that were more frequent at follow-up, originate from the CNS. Similarly, hyperalgesia of the contralateral extremity that was more frequent at follow-up underpins the relevance of central nervous alterations over the disease course. A finding that is supported by another longitudinal observation [39]. My study supports that CRPS signs and symptoms improve over the disease course and that this is especially true in patients with acute CRPS. However, in many cases the CRPS diagnostic criteria are still fulfilled. Patients that did not fulfil the Budapest criteria anymore at follow-up (n=15) had lower pain intensities. But in four of the fifteen cases mean pain intensities were still higher than three (>3 NRS). One longitudinal study confirmed that after one year only 5.4% of patients were free of symptoms even if one quarter did not fulfill the Budapest diagnostic criteria anymore [48]. This emphasizes that treatment might still be necessary even though the diagnosis of CRPS cannot be made any longer. Of the 39 patients included 18 already suffered from long lasting (>12 months) CRPS at baseline. As the diagnosis is often delayed [43] patients with chronic CRPS are common in specialized clinics. The presented results support that significant improvement more likely occurs in early disease stages [48]. However, a tendency to improved pain intensities was also seen in chronic cases. This underlines that patients with long-lasting CRPS should not be considered “lost cases” and treatment efforts are important. The additional finding that the prevalence of back pain markedly increased from baseline to follow-up visualizes the need for a holistic approach in pain treatment as chronic pain might not be limited to a single body site.

Mechanistically, a prolonged malposition due to protective pain behavior might explain the significant increase.

Taking together the variable clinical development and the persistence of sensory abnormalities, a residual state must be assumed in many cases. Further research is needed to understand if sensory abnormalities are reversible or if permanent damage occurred that could be described as a sensory scar.

4.7. Methodical considerations

4.7.1. Measuring pain as subjective sensation

Pain is a subjective sensation perceived by an individual. So far, pain intensity ratings are an important clinical but also scientific tool to quantify pain. However, pain ratings can be biased in many ways [91]. Beside the pain intensity, the descriptive pain character can be explored. In a clinical setting this can help in finding the cause of the pain. In research, questionnaires as the NPSI can enable the quantification of pain characteristics hence making them comparable. One challenge of modern pain research is the development of reliable pain biomarkers. Such biomarkers can help in quantifying the analgesic effects of novel drugs or enable clinicians to accurately capture pain in infants or the elderly [92]. Fortunately, research on pain biomarkers is developing and promising approaches involve neuroimaging but also stem-cell based molecular research [92]. Studying the sensory function and finding causal links to pain could help establishing sensory biomarkers. QST was developed to minimize biases but remains a psychophysical testing method and correlations to clinical pain are not always clear [93]. The interpretation of the presented results should consider this circumstance especially when it comes to the rather large variances of QST data. Furthermore, statistical comparisons of e.g. pain intensities and according conclusions always have to be checked with individual patient reports before being back-translated into clinical practice.

4.7.2. QST in chronic pain disease

QST is a widely accepted tool in pain research. The measurements are carried out by trained investigators in certified laboratories and follow a standardized protocol [80] improving the reliability and comparability of the results. The method´s ability to detect deviations in somatosensory function relative to an age- and sex-matched healthy population allows for the description of sensory disturbances not only in pain disease but also neuropathic diseases [12, 64]. The use of different stimuli including temperature stimulation, mechanical stimulation and stimulation of deep tissue nociceptors covers all relevant entities of the somatosensory nervous system and different nerve fiber qualities [80]. When addressing the time course of a disease it is important to acknowledge that data based on a one-day interval showed good test-retest reliability and interobserver reliability [94]. I analyzed single QST items as done in many other studies before. Recent research broad to light a new approach by calculating algorithms based on QST data. In one study, sorting algorithms based on sensory data were used to allocate patients with sensory abnormalities to three different disease groups [95]. This new approach illustrates the variety of research possibilities granted by QST. However, using the conventional way of comparing single QST items made the results of my study comparable to the existing literature.

Despite the numerous strengths, QST has limitations. The integration into clinical practice can be cumbersome as QST measurements in order to capture the whole somatosensory function take time. Future studies could focus on finding "variables of interest" that e.g. predict disease outcomes in specific diseases. With regard to CRPS, pressure pain thresholds have repeatedly been shown to be specific and have been able to differentiate CRPS patients from those with peripheral nerve injury [11] or limb pain of other genesis [96]. This might help developing shorter, disease adopted QST protocols of course keeping in mind the loss of information that would result from such a shortening. Despite the quantitative assessment, QST is a psychophysical testing method and relies on the cooperation of the patient or study participant [54]. The follow-up data on sensory profiles shown above demonstrated a discrepancy between stable sensory findings and improved clinical CRPS severity as already shown before [20, 39]. Explanations of this observation can be manifold. A recent publication titled "No pain, still gain (…)" [93] discussed the mismatch of pain reports and sensory profiles. It was observed that

hyperalgesia and allodynia during QST also occur in patients without pain report. This brings up the question if the stimuli used to evoke pain in QST actually match with those causing pain in daily life. Proposed explanations for this mismatch included the difficulty of separating painful sensations and dysesthesia as a patient which could blur the results. Further explanatory approaches regarded differences in spontaneous and evoked pain but also sensory abnormalities as precursor for clinical pain [93]. A critical commentary set out the perspective that a new mechanism-based approach is needed to better understand the actual stimuli of clinical pain [97].

4.8. Strengths and limitations

This study has several strengths. The large sample size of the analyzed cohort (n=199) and the multicentric patient recruitment make the results reliable. Compared to other longitudinal studies the follow-up sample size of 39 is noteworthy. Importantly, the same study protocol and methods have been used at baseline and at follow-up instead of using a retrospective chart review. Further, the follow-up cohort included patients with chronic CRPS at baseline allowing for longitudinal observation of a cohort rarely represented in longitudinal studies. QST as cornerstone of this work is an accepted tool in pain research and was carried out by trained investigators.

The following limitations have to be considered when interpreting the results. The study design was observational and non-interventional so causality cannot be proven. Due to this observational character there were differences in subgroup sizes complicating the analysis. However, the observed differences in group size were “CRPS-typical” and in line with earlier study populations. The follow-up period was non-standardized but individual treatment followed national guidelines. Of the 74 patients suitable for follow-up participation only 39 could be recruited implicating a risk of selection bias.

4.9. Future directions of CRPS research

Future studies should investigate differential treatment response in the described subgroups and the role of sensory abnormalities in treatment response. The resolution of pain and the mechanisms behind are an important field of modern pain research. My results described the discrepancy between clinical improvements and stable sensory

dysfunction. It is important to better understand the pathological background of this observation e.g. by investigating skin biopsies and small nerve fibers. Pressure pain sensitivity has repeatedly been described as CRPS typical sensory abnormality. A focus on deep tissue sensitivity and according stimulation might prove as promising clinical tool in detecting CRPS patients. Beside experimentally inducing pressure pain with pressure pain algometers, examinations closer to daily life experiences (e.g. recording painful sensations when shaking hands or lifting heavy weights) could overcome the gap between clinical and experimental pain.

Interventional trials should also address the question if certain treatments influence sensory function and if treatment responses are in special cases linked to changes in sensory function. Importantly, if sensory disturbances are found to be independent, they should not be pathologized but rather be accepted if patients are not impaired and report well-being.

Even though I have described distinctive features separating fracture and CRPS patients the search for diagnostic and risk factors remains important. Ideally, preventive measures are developed for fracture patients at high risk for CRPS. At least, a profound understanding of risk factors could lead to a closer monitoring of patients at risk and allow for early diagnosis and adequate treatment initiation. Clinical decision making could be supported by algorithms that include sensory parameters but also additional clinical or psychological aspects.

5. Summary / Zusammenfassung

Complex regional pain syndrome (CRPS) presents with a variable clinical picture. This heterogeneity is clinically challenging and requires the description of distinctive disease phenotypes.

My thesis explored five disease subgroups in 199 CRPS patients. In general, the sensory function of the affected extremity was characterized by hypersensitivity to pressure and mechanical pain as well as hyposensitivity to non-painful stimulation. The explored subgroups were depending on sex, the presence of a nerve lesion, the temperature phenotype, the affected extremity and the affection of the contralateral extremity. The most prominent difference was seen between patients with and without hyperalgesia on the contralateral extremity. If contralateral hyperalgesia was present, pain intensity ratings were higher and the CRPS affected extremity was more sensitive to mechanical pain possibly indicating an overall hypersensitivity in these patients. A hypersensitivity to mechanical and pressure pain was also more frequently seen in female than in male patients matching earlier findings.

As fractures are the most common cause of CRPS another focus of the presented work was the comparison of fracture and CRPS patients. An adequate separation of both groups is especially important for prompt treatment initiation in CRPS. Here, CRPS patients were characterized by higher pain intensity, a neuropathic pain character and signs of mild depressiveness as well as anxiety. CRPS patients were more sensitive to mechanical and pressure pain. Based on the results, exploring sensory function and addressing pain characteristics in fracture patients with abnormal pain intensities might prove promising in detecting CRPS patients at an early disease stage.

In addition, longitudinal outcomes of 39 CRPS patients were described. In general, a clinical improvement was noted during a mean follow-up interval of 2.5 years. This was contrasted by a stagnation of sensory disturbances. This discrepancy could be the consequence of ongoing pathological processes. As discussed, methodical reasons could also explain the observed mismatch as experimentally induced pain as used in QST does not necessarily match with clinical pain especially in chronic pain disease. The observed clinical improvement is promising and future studies with a focus on the mechanisms of pain resolution are needed for individualized pain treatment.

Das komplexe regionale Schmerzsyndrom verursacht neben medizinischen auch psychologische und soziale Probleme. Diese Multidimensionalität ist typisch für chronische Schmerzerkrankungen und findet auch in dem klinisch sehr variablen Bild des CRPS einen Widerhall. Die klinische Heterogenität verlangt dabei nach einer Einteilung in Untergruppen zur genaueren Beschreibung individueller Krankheitsverläufe.

Meine Arbeit untersuchte in 199 Patient*Innen mit überwiegend kurzer CRPS-Krankheitsdauer fünf Untergruppen. Der Schwerpunkt lag dabei unter anderem auf der Charakterisierung der sensorischen Profile. So konnte gezeigt werden, dass die betroffene Extremität typischerweise empfindlicher auf schmerzhafte Druckreize und mechanische Reize reagiert. Dieser Überempfindlichkeit steht eine herabgesetzte Sensibilität für nicht-schmerzhafte mechanische Reize gegenüber. Die untersuchten Untergruppen richteten sich nach dem Geschlecht, dem Vorhandensein einer Nervenläsion, dem Temperaturphänotyp, der betroffenen Extremität und der Affektion der kontralateralen Extremität durch das CRPS. Der wichtigste Unterschied betrifft Patient*Innen mit Hyperalgesie an der kontralateralen Extremität, da diese mehr Schmerzen berichteten und empfindlicher auf mechanischen Schmerz reagierten. Ein weiterer Unterschied zeigte sich zwischen weiblichen und männlichen Probanden. Entsprechend früherer Beobachtungen hatten Patientinnen niedrigere Druckschmerzschwellen. Da sich ein CRPS meist infolge einer Fraktur entwickelt analysierte ich in einem zweiten Teil der Arbeit systematisch Unterschiede zwischen Fraktur- und CRPS-Patient*Innen. Klinisch ist eine exakte Differenzierung etwa bei der Einleitung einer frühzeitigen Therapie relevant. Hier konnte gezeigt werden, dass CRPS-Patient*Innen stärkere Schmerzen erleiden, die dabei eine ausgeprägte neuropathische Komponente besitzen. Weiterhin waren psychische Symptome häufiger bei CRPS-Patient*Innen zu finden. Die sensorischen Profile zeigten vor allem eine deutlichere Druckschmerzempfindlichkeit bei CRPS-Patient*Innen.

Bei chronischen Erkrankungen wie dem CRPS sind longitudinale Beobachtungen wichtig. In einer Kohorte von 39 Patient*Innen zeigten sich klinische Verbesserungen wie etwa eine Abnahme der Schmerzintensität. Bemerkenswert ist dabei die Stabilität der sensorischen Profile und auch der Schmerzcharakteristik, die trotz der klinischen Befundverbesserung unverändert blieben.

6. References

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Appendix

I. Abbreviations

Abbreviations in alphabetic order

ANOVA	Analysis of variance
CDT	Cold detection threshold
CNS	Central nervous system
CPT	Cold pain threshold
CRPS	Complex regional pain syndrome
CSS	CRPS severity score
FC	Fracture control
HPT	Heat pain threshold
ICD	International classification of diseases
MDT	Mechanical detection threshold
MPS	Mechanical pain sensitivity
MPT	Mechanical pain threshold
NPSI	Neuropathic pain symptom inventory
NRS	Numeric rating scale
pDN	Painful diabetic polyneuropathy
PC	Principal component
PCA	Principal component analysis
PPT	Pressure pain threshold
QST	Quantitative sensory testing
RM-ANOVA	Repeated measurements analysis of variance
SD	Standard deviation
TSL	Temperature threshold limen
VDT	Vibration detection threshold
WDT	Warm detection threshold
WUR	Wind-up ratio

II. List of figures

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V. Author contribution

I recruited patients for the follow-up investigation, performed the QST measurement in this cohort (n=39) and did the clinical examination. I am a trained QST-researcher (DFNS-certified). The study was supervised by Professor Heike Rittner. I also prepared and analyzed the data of the fracture control cohort [51] and of the large CRPS cohort [63]. A third publication based on the follow-up is in preparation. Results were presented in talks at the Würzburger Anästhesie Tage 2019 and 2020 and as a poster at the meeting of the German Neuroscience Society 2019.