The role of the bone in Complex Regional Pain Syndrome 1 – A systematic review

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:10.1002/ejp.2116.

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Acknowledgements:

The authors would like to thank Dr. Sabine Klein, Universitätsbibliothek Medizin, University of Zurich, for her support in the literature search.

Abstract

The aim of this systematic review was to appraise and analyze the knowledge on bone-related biochemical and histological biomarkers in Complex Regional Pain Syndrome 1 (CRPS 1). A total of 7 studies were included in the analysis (biochemical analyses n=3, animal study n=1, histological examination n=3). Two studies were classified as having low risk of bias and five studies with a moderate risk of bias. Biochemical analysis indicated an increased bone turnover with increased bone resorption (elevated urinary levels of deoxypyridinoline) and bone formation (increased serum levels of calcitonin, osteoprotegrin and alkaline phosphatase). The animal study reported an increased signaling of proinflammatory tumor necrosis factor four weeks post-fracture, which did however not contribute to local bone loss. Histological examination from biopsies revealed thinning and resorption of cortical bone, rarefication and reduction of trabecular bone and vascular modification in the bone marrow in acute CRPS 1 and replacement of the bone marrow by dystrophic vessels in chronic CRPS 1. The limited data reviewed revealed certain potential bone-related biomarkers in CRPS. Biomarkers hold the potential to identify patients that may benefit from treatments that influence bone turnover. Thus, this review identifies important areas for future research in CRPS1 patients

Key words

CRPS, Complex regional pain syndrome, bone, biomarker, osteoimmunology

Statements and Declaration

Funding sources: No funding was received for this study.

Conflict of interest: The authors declare that they have no competing interests.

Introduction

Complex Regional Pain Syndrome (CRPS) is a painful and often debilitating condition that usually develops distally after limb injury [Merskey and Bogduk 1994]. Characteristic symptoms occur within 4-6 weeks after the initiating event and typically exceed the expected clinical course in magnitude and duration [Merskey and Bogduk 1994, Brunner et al. 2017]. Clinical manifestations are multifaceted and include sensory, vasomotor, sudomotor, motor and trophic changes. The diagnosis is based on the presenting signs and symptoms using revised Budapest Criteria [Harden et al. 2010].

Current research indicates that CRPS represents a multimechanistic condition including aberrant pro-inflammatory response to tissue injury, disturbances in sympathetically-mediated vasomotor control, and maladaptive peripheral and central neuronal plasticity [Marinus et al. 2011].

CRPS is also considered as a multisystem disorder involving the skin, soft tissue, blood, peripheral, autonomous as well as the central nerve system and the bone [Knudsen et al. 2019, Varenna and Crotti 2018].

In 1900, the German surgeon Paul Sudeck (1866-1945) mentioned for the first time a potential connection between pathological bone remodeling and the today known CRPS [Sudeck 1900]. Using the X-ray examination, that was only recently introduced at the time, Sudeck suggested that CRPS may be caused by an exaggerated "acute inflammatory bone atrophy" after limb trauma. Later, the use of 3-phase bon scintigraphy (TPBS) was proposed as diagnostic tool [Simon and Carlso 1980] and the therapeutic use of bone targeting drugs such as strontium ranelate, calcitonin, bisphosphonates further suggested a potential role of the bone in the pathogenesis and pathophysiology of CRPS [Varenna and Crotti 2018].

Nevertheless, the focus of further CRPS research was not on bone but on better accessible skin or blood samples.

Biomarkers serve as indicators of normal or pathological processes and of responses to therapeutic interventions [Biomarkers Definitions Working Group 2001]. A valid biomarker potentially helps to improve diagnosis, prognosis and monitoring of diseases. Lately, the identification of biomarkers in CRPS has received increased attention. In a comprehensive review, Bharwani et al. summarized and discussed in detail potential biomarkers of inflammation as well as immune dysregulation of the condition [Bharwani et al. 2019]. In the field of CRPS research, several biomarkers have emerged as being potentially useful, including CGRP, TNF-α, Il-6, tryptase, the density of mast cells, SIL-2R, and microRNA. Despite these promising findings, it remains a challenge to determine the role of these markers in the clinical diagnosis and management of CRPS.

In another recent systematic review, Andronic et al. reviewed potential biomarkers of the skin associated with CRPS 1 [Andronic et al. 2022]. The biomarkers identified reveal a complex interplay of pathophysiological processes in CRPS, including inflammation characterized by elevated levels of interleukins and TNF-α, as well as disruptions in the regulation of the vascular system (such as alterations in ET-1/NOx and the presence of hypoxia with elevated lactate levels). There is also evidence of small fiber neuropathy and heightened sensitivity. At the skin level, studies have shown significant loss of neurites, heightened expression and abnormal migration of mast cells, and increased expression of α1-adrenoceptors on keratinocytes.

Until now, evidence regarding bone related biomarkers in CRPS 1 has not been assessed systematically. The aim of this study was to systematically appraise and analyze the knowledge on bone related biochemical and histological biomarkers in CRPS 1.

Methods

The systematic review was conducted in accordance with the recommendations by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement, see S1 Table) [Salameh et al. 2020].

Search strategy

We performed a comprehensive electronic search in MEDLINE (OvidSP), Embase (Elsevier), Google scholar, Scopus and Web of Science from inception to February 2022 to identify relevant studies. The terms for the search strategies were identified through discussion between an information specialist and the review team, by scanning the background literature, and by browsing the MEDLINE Thesaurus (MeSH). Medical Subject Headings terms and free-text terms were used as the search strategy. The search was performed by a professional information specialist. To ensure the completeness of the literature search, the reviewers, experienced clinicians and researchers in the field of CRPS, screened bibliographies of all included studies and retrieved review articles in an additional hand search and all potentially eligible references were included in the full text review (inclusion and exclusion criteria applied).

Study selection and main outcome variables

The eligible criteria for inclusion in the current systematic review were: (1) Human and animal studies. (2) Established diagnosis of CRPS 1 of the upper or lower extremity. Because the diagnostic criteria changed over time and we aimed at providing a comprehensive overview of the literature, we included all studies that assessed CRPS 1 patients. The diagnostic criteria for each study are reported in the baseline table. (3) Reporting of any biochemical or histological biomarkers related to bone tissue. (4) minimum level V case

series studies using Oxford Centre for Evidence Based Medicine Levels [Oxford Centre for Evidence-Based Medicine], (5) articles in English, German or French.

We excluded (1) case reports, reports, brief communications, systematic reviews, unpublished articles, abstracts, conference proceedings, or letters, (2) therapeutic studies investigating the efficacy of bone medications in CRPS 1 (strontium ralenate, calcitonin, bisphosphonates), (3) diagnostic studies based on imaging (X-ray, MRI, TPBS), (4) studies including patients with CRPS 2, stroke-related CRPS, and patient age < 18 years.

Risk of bias assessment

The quality of all studies was assessed using the MINORS criteria (methodological items for non-randomized studies) [Slim et al. 2003]. Depending on the score achieved, studies were qualified as either having low risk of bias (≥ 75%), moderate risk of bias (50% ≤ score < 75%) or high risk of bias (< 50%).

Analysis

Citations from the initial search results of each database were exported to EndNote (version X9.2, Clarivate Analytics, Philadelphia, PA, USA), and duplicates were removed. The titles and abstracts were screened and reviewed by two authors (G.K. and F.B.). Then, full texts of potential studies were retrieved and independently reviewed in detail for inclusion based on the pre-determined criteria. Discrepancies between two authors were resolved by discussion, and a third author (M.M.W.) was referred if consensus could not be reached. One author (G.K.) extracted the data from the included studies into a piloted sheet, and another author (F.B.) crosschecked the extracted data. The following data were extracted:

study characteristics (author, year, study design) and subjects' characteristics (sample size, age, localization, symptom duration, diagnostic criteria, outcome and biomarkers).

The analysis was performed using R software (version 3.6.1).

Results

The flow of study selection

The search across the databases resulted in 7194 references (Figure 1). After removing duplicates, the search retrieved 4275 potentially relevant references. Based on title and abstract, 4092 were excluded, and 48 articles were reviewed in full text. Finally, 7 studies were included in the current review. The main reasons for exclusion are displayed in Figure 1.

Study characteristics

The study characteristics are summarized in Table 1. There were six human studies (biochemical analyses n=3 [Kramer et al. 2014, Oehler et al. 2019, Sawicki et al. 1992], histological examinations n=3 [Arlet et al. 1981, Basle et al. 1983, Rohner 1985]) and one animal study [Sabsovich et al. 2008]. The studies were published between 1958 and 2018. In the human studies, two studies followed a prospective [Kramer et al. 2014, Sawicki et al. 1992] and four studies a retrospective design [Oehler et al. 2019, Arlet et al. 1981, Basle et al. 1983, Rohner 1985], respectively. The sample size of the included human studies ranged from 5-23 patients with a median age of 47 years (range 18-72 years) and a median disease duration of 6.6 months (range 1-48 months). In two studies the diagnosis of CRPS was based on the current revised Budapest criteria [Kramer et al. 2014, Oehler et al. 2019]. In the remaining four studies, the diagnosis was based on predefined clinical manifestations including sensory, sudomotor, vasomotor, motor and trophic changes without consideration of specific diagnostic criteria [Sawicki et al. 1992, Arlet et al. 1981, Basle et al. 1983, Rohner 1985]. In two of these studies, conventional x-rays [Sawicki et al. 1992, Arlet et al. 1981] and TPBS [Sawicki et al. 1992] were also used.

Quality assessment

All included studies followed a non-randomized design. Three studies were comparative. The corresponding control groups consisted of healthy controls [Kramer et al. 2014, Sawicki et al. 1992], fracture patients [Kramer et al. 2014] and the unaffected, contralateral lower limb [Oehler et al. 2019].

The average score of the MINORS tool was 73.3% (range 69-79%) (Table 2). Three studies were classified as having low risk of bias [Kramer et al. 2014, Oehler et al. 2019, Sawicki et al. 1992] and four studies with a moderate risk of bias [Arlet et al. 1981, Basle et al. 1983, Rohner 1985, Sabsovich et al. 2008].

Biochemical analysis

In three studies, potential biomarkers for bone remodeling were investigated [Kramer et al. 2014, Oehler et al. 2019, Sawicki et al. 1992].

In a prospective study, Kramer et al analyzed osteoprotegrin (OPG) serum levels in acute CRPS patients with a median disease duration of 12 weeks (IQR 8-24 weeks) and two control groups (asymptomatic patients, patients after uncomplicated fractures) [Kramer et al. 2014]. In addition, the correlation with radiotracer uptake in TPBS was assessed. OPG in CRPS patients was significantly increased by comparison with both control groups (controls $p=0.04$, fracture patients $p=0.001$). For the CRPS-affected hand, a significant correlation between OPG and TPBS of the carpal bones in phase III was detected indicating an enhanced osteoblastic activity ($r=0.391$, $p=0.03$). The authors proposed a possible contribution of bone turnover to CRPS pathophysiology and proposed OPG as a potential biomarker for acute CRPS.

In a retrospective observation by Oehler et al [Oehler et al. 2019], bone remodeling processes were studied in 14 women with a disease duration of 10.1 months (range 0-111 months) using biochemical analyses (calcium, phosphate, 25-hydroxyvitamin D, bone alkaline phosphatase,

parathyroid hormone, osteocalcin, urinary levels of deoxypyridinoline (DPD)) and by using high-resolution peripheral quantitative computed tomography (HR-pQCT). Urinary DPD levels were elevated indicating increased bone resorption. The altered bone microstructure measured by HR-pQCT encompassed a decrease in both cortical and trabecular thickness and a lower trabecular number on the affected tibiae. The authors concluded that increased bone resorption potentially correlates with the detected CRPS-mediated bone atrophy.

In a prospective study, Sawicki et al [Sawicki et al. 1992] compared the serum level of calcitonin, phosphate and total alkaline phosphatase activity in acute (n=8) and chronic (n=5) CRPS 1 patient (the actual disease duration was not reported) to an age-matched control group (n=30). Compared to the control patients, calcitonin level and total alkaline phosphatase activity were elevated in acute CRPS 1 and were normal in the chronic CRPS I.

Histological analysis

Three studies examined bone microstructure by histologic analyses of biopsies of CRPS patients.

In 1981, Arlet et al evaluated biopsies of the femur or the tibia of 16 CRPS patients (acute n=3, chronic n=13) [Arlet et al. 1981]. The biopsies showed an altered microstructure of the bone including thinning of cortical bone, increased cortical reabsorption, rarefaction of trabecular bone, stasis and fibrosis of bone marrow.

In a study in seven patients with acute CRPS of the upper and the lower extremity, Basle et al. found vascular modifications in the bone marrow with venous dilatation, thickening of arteriolar walls with reduction of diameter [Basle et al. 1983]. As a result, the bone marrow exhibited reduced islets of bone marrow, adipocytes necrosis and increased plasma exudation. The bone revealed a degeneration of osteocytes, a demineralization and trabecular atrophy, followed by irregular trabecular remodeling.

In the oldest study from 1958, Rohner et al differentiated between dystrophic changes in acute (<3 months, n=3) and atrophic changes in chronic CRPS (>3 months, n=2) [Rohner 1985]. The dystrophy was characterized by vascular modification of the bone marrow including arterial muscular hyperplasia with consecutive stenosis, epitheloid appearance of muscle cells, venous dilatation and an increased bone resorption. In chronic CRPS, an atrophy of the bone marrow with replacement by dystrophic vessels and hyperplastic adipocytes was observed.

Animal study

In the animal study (tibial fracture model, hindlimb casting for four weeks), post-fracture changes in cytokine tumor necrosis factor (TNF) expression and content in skin, nerve, and bone in rats tibia fracture were analyzed (n=24). Further, the effects of TNF antagonist on the development of nociceptive, vascular, and bone changes after the fracture were evaluated [Sabsovich et al. 2008].

Increases in TNF expression were observed in skin, nerve and bone, and pre-emptive soluble TNF receptor type 1 (sTNF-R1) treatment inhibited the development of pain behavior after fracture. There was no significant difference in bone microarchitectural parameters between the sTNF-R1 and saline treatment cohorts at 4 weeks after fracture. There was no significant difference in bone microarchitectural parameters between the sTNF-R1 and saline treatment cohorts at 4 weeks after fracture and therefore no effect of TNF signaling on regional trabecular bone loss after fracture has been shown. The authors concluded, that increased TNF signaling in the hindlimb is an important mediator of chronic regional nociceptive sensitization after fracture, but does not contribute to the hindlimb warmth, edema, and bone loss observed in this CRPS 1 model.

Discussion

Main findings

The limited data reviewed identified three potential bone-related biochemical biomarkers in CRPS 1: Elevated urinary levels of DPD indicated an increased bone resorption. Simultaneously, increased serum levels of calcitonin, OPG (acute phase) and alkaline phosphatase revealed an enhanced bone formation [Kramer et al. 2014, Oehler et al. 2019, Sawicki et al. 1992]. For other bone metabolism-associated factors such as calcium, phosphate, 25-hydroxy-vitamin D, bone alkaline phosphatase, parathyroid hormone [Oehler et al. 2019] and phosphorus [Sawicki et al. 1992] no differences were observed. Histological examination showed resorption of cortical bone, rarefication of trabecular bone and vascular modification of the bone marrow in acute CRPS 1 [Basle et al. 1983, Rohner 1985] followed by replacement of the bone marrow by dystrophic vessels in chronic CRPS 1 [Arlet et al. 1981, Rohner 1985].

Implementing a tibia-fracture model, one animal study reported an increased signaling of proinflammatory TNF four weeks post-fracture. Although pre-emptive soluble TNF receptor type 1 (sTNF-R1) treatment inhibited the development of pain behavior after fracture, it was not associated with local bone loss. Thus, TNF seems to play a role in central pain sensitization processes but not in bone turnover [Oehler et al. 2019].

Results in the light of the existing literature

Although diagnostic tools such as TPBS and bone targeted pharmacological therapeutic compounds like strontium ranelate, calcitonin and bisphosphonates suggest a potential role of the bone in the pathophysiology of CRPS 1 [Varenna and Crotti 2018], little research has been conducted to gain a better understanding of the underlying bone-related processes.

The biomarkers and histological changes summarized in the current systematic review support the role of increased bone turn over in particular during the early stages of CRPS 1. A series of protein or protein derivative biomarkers released during bone remodeling by osteoblasts and osteoclasts have been described as potential bone turnover markers [Greenblatt et al. 2017]. The total alkaline phosphatase (ALP) levels has been the first marker for bone remodeling [Hlaing and Compston 2014]. Only the bone-specific isoform of the alkaline phosphatase reflects bone anabolic activity. The bone specific ALP correlates positively with the number of osteoblasts released into the circulation and thus with bone formation. In CRPS 1 patients one study observed an increased ALP in acute CRPS 1 [Sawicki et al. 1992] that was no longer increased during later stages in another study [Oehler et al. 2019].

Type 1 collagen is the most abundant protein in the bone and its extensive network contribute to the overall biomechanical structure of the bone [Greenblatt et al. 2017]. Deoxy-pyridinoline (DPD) is predominantly found in bone, and is therefore considered a specific marker of bone turnover. During bone resorption, DPD is liberated and subsequently cleared renally [Apone et al. 1997]. Increased urinary DPD levels were observed in one retrospective study in patients with CRPS 1 of a median duration of 10 months [Oehler et al. 2019].

Osteoprotegerin (OPG) is a soluble RANKL decoy receptor that is predominantly produced by osteoblasts, which prevents osteoclast formation and osteoclastic bone resorption by inhibiting the RANKL–RANK interaction [Nobuyuki et al. 2021]. In one study, high OPG serum levels in acute CRPS patients was observed [Kramer et al. 2014], indicating that not only bone turnover is increased but also osteoclast formation suppressed.

The current understanding of the role of the bone in the pathophysiology of CRPS 1 is still very limited.

In an expert opinion from 2018, Varenna and Crotti considered the involvement of the bone as a cardinal feature of CRPS pathogenesis and described the underlying pathophysiology based on the existing knowledge [Varenna and Crotti 2018]. According to their model, direct

injury to the bone first results in a local release of pro-inflammatory mediators such as TNF, IL-1, IL-6, substance P and Calcium Gene Related Peptide [Coderre and Bennett 2010] followed by a deranged capillary permeability with
edema, consequent hypoxia and acidosis [Varenna and Zucchi 2015]. Subsequently, a circulatory disturbance develops in the bone marrow, with necrosis of adipocytes, venous dilatation and thickening of the arteriolar walls followed by bone demineralization [Arlet et al. 1981].

Recently, the newly emerging field of osteoimmunology has received increasing attention in research. Multiple studies have demonstrated the intricate interactions between the immune system and the skeletal system in both normal and pathological conditions.

According to a recent extensive review of the literature [Yang and Liu 2021], T cell subsets, including Th1, Th2, Th17, and Treg, in bone re-pair and regeneration has been extensively studied. Th17 cells, also known as osteoclastogenesis-supporting T cells, secrete IL-17 which upregulates RANKL expression, and elicits the pro-duction of pro-inflammatory cytokines, such as TNF-α and IL-1, from innate immune cells. These cytokines further stimulate the differentiation of osteoclast precursor cells and impair osteoblast function. On the other hand, Th1, Th2, and Treg cells regulate osteoclastogene-sis by secreting cytokines such as INF-γ, IL-4, CTLA-4, and IL-10, respectively. B cells play an antagonistic role in the regulation of RANKL, by secreting OPG, and they promote osteoclast apoptosis by secreting TGF-β.

Understanding the interplay between the immune system, the skeletal system, and the development of CRPS is vital to comprehend the relationship between the immune system, the skeletal system, and the development of this condition. This understanding is necessary to advance new treatment options. A recent review article by Duda et al. provides a com-prehensive overview of the cellular and humoral aspects of the early stages of fracture healing [Duda et al. 2023]. This early phase is a complicated interaction of cellular and molecular activities that are orchestrated by the immune system, including an initial clotting and pro-

inflammatory phase, followed by an anti-inflammatory and angiogenesis phase. During this stage, the immune system triggers a series of responses to clean up the injury site, remove debris, and facilitate tissue regeneration. In relation to CRPS, the local hypoxia, pro-inflammatory processes, and angiogenesis that occur during this phase are of significant interest, as they are believed to play a crucial role in the initiation and persistence of the condition [Bruehl 2010].

Strength and limitations

The strength of this study includes the application of robust systematic review methodology according to the current recommendations. The limitations of our study are two-fold. First, due to the small number of included articles the findings of this systematic review are therefore of exploratory nature. Second, only two studies applied the current diagnostic criteria for the diagnosis of CRPS 1 and therefore, a misdiagnosis cannot be completely ruled out.

Implication for practice

The results of this review do not have a direct impact on clinical practice. Clinicians should continue consider the use of bone-targeted medications in treating CRPS 1. To date, bisphosphonates show the best evidence, in particular in reducing pain [Wertli et al. 2014]. However, due to the heterogeneity of the available studies the routine use of bisphosphonates in the treatment of CRPS 1 remains controversial and further studies are needed to determine their effectiveness [Chevreau et al. 2017]. Bone turnover markers can offer prognostic information and – due to the rapid response to changes in bone physiology – may be helpful to assess patient response to therapies. However, various factors such as comorbidities, preanalytical and individual factors may influence the level of bone turnover markers and thus limit the clinical usefulness [Greenblatt et al. 2017] .

Implication for research

Urinary DPD, serum OPG, and alkaline phosphatase hold the potential to predict development of CRPS or the degree of bone affection during the disease. Possibly, they play a role in identifying patients who would benefit from bone-targeted medications, in particular bisphosphonates. However, the results of this systematic review reveal a need for additional bone-related biomarkers to improve the diagnosis and therapy of CRPS 1. Especially, potential biomarkers from pathways influencing bone metabolism such as the nuclear factor (NF)-κB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) and the wnt/β-catenin [Kobayashi et al. 2016] must be further explored in CRPS 1. Further, osteoblasts secrete type 1 collagen as an intact molecule containing the N- and C-terminal propeptides into the extracellular space. Thus, Nand C-terminal propeptides of type 1 collagen (PINP and PICP) levels are markers of type 1 collagen secretion by osteoblasts. Serum PINP is a useful indicator of disease activity in Paget's disease of bone, in bone metastases of osteoblastic nature, and in the follow-up of treatment of osteoporosis [Koivula and Risteli 2012]. Osteocalcin (OC) is secreted by mature osteoblasts and mainly incorporated into the organic matrix that will later ossify into bone. Thus, OC is widely considered a bone formation marker [Delmas et al. 1986]. No study has assessed the utility of P1NP and OC in CRPS 1 patients.

Conclusion

Based on the available studies, bone-related biomarkers may identify patients with increased bone turnover in CRPS. In order to improve the diagnosis and develop novel therapeutic approaches in the management of CRPS further high-quality research identifying relevant biomarkers and validating the currently known candidate biomarker are needed.

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Table 1: Characteristics of the included studies

Author, year	Study design	Sample size	Age (median)	Localisation	Symptom duration (months)	Diagnostic criteria	Outcome	Biomarker
Arlet, 1981 [17]	Retrospective, human	16	46	LE	20	Clinical, radiological, positive bone scintigraphy	Histological examination	Thinning of cortical bone, lacunae of cortical reabsorption, rarefaction of trabecular bone, stasis and fibrosis of bone marrow
Basle, 1983 [18]	Retrospective, human	7	45	UE, LE	3	Clinical and radiological	Histological examination	• Vascular modification: Venous dilatation, thickening of arteriolar walls with reduction of diameter • Bone marrow: Dissociated through plasma exudation, reduced islets of bone marrow, adipocytes necrosis • Bone: Vascular modification lead to degeneration of osteocytes and bone demineralization and trabecular atrophy, followed by irregular trabecular remodeling (increased secretion of osteoid by osteoblasts and increased osteoclastic bone resorption)
Krämer, 2014 [14]	Prospective, human	23	50	UE	3	Budapest	Biochemical analysis (Serum OGP level)	Compared to controls OPG was significantly increased in CRPS patients.
Oehler, 2018 [15]	Retrospective, human	14	50	LE	10.1	Budapest	Biochemical analyses (calcium, phosphate, 25-hydroxyvitamin D, bone alkaline phosphatase, parathyroid hormone, osteocalcin, urinary levels	Biochemical: Elevated urinary levels of DPD HR-pQCT: Significantly lower values of cortical bone mineral density and cortical thickness in the affected tibiae. Trabecular

							of DPD), DXA, HR-pQCT	number and thickness tended to be lower in affected tibiae.
Rohner, 1958 [19]	Retrospectiv	5	43	UE, LE	48	Clinical	Histological examination	Acute CRPS (<3 months): Dystrophy • Vascular modification of the bone marrow: Arterioral muscular hyperplasia with consecutive stenosis, epitheloid appearance of muscle cells, venous dilatation • Increased bone resorption Chronic CRPS (>3 months): Atrophy • Vessels and hyperplastic adipocytes replace atrophic bone marrow
Sawicki, 1992 [16]	Prospective, human	13	48	NR	NR	Clinical	Biochemical analysis (Calcium, phosphate, alkaline phosphatase, calciurie)	Osteocalcin level and alkaline phosphatase activity were elevated in the acute and normalized in chronic CRPS indicating increased bone turnover in acute CRPS.
Sabsovich, 2008 [20]	Animal	21	NA	Hindpaws (rats)	1	NA	Biochemical (TNF), Microcomputed tomography (µCT)	Increased TNF signalling in the hindlimb did not contribute to local bone loss four weeks post-fracture.

Abbreviations: UE: Upper Extremity, LE: Lower Extremity, NR: Not reported, NA: not applicable, DPD, Deoxypyridinoline, DXA: Dual energy X-ray absorptiometry, HR-pQCT: High-Resolution Peripheral Quantitative Computed Tomography, OGP: Osteoprotegerin, TNF: Tumor Necrosis Factor

Table 2: Individual risk of bias assessment using the MINORS tool [13]

	Arlet, 1981	Basle, 1983	Krämer, 2014	Oehler, 2018	Rohner, 1958	Sawicki, 1992	Sabsovich, 2008
Clearly stated aim	2	2	2	2	2	2	2
Inclusion of consecutive patients	2	2	2	2	2	2	2
Prospective data collection	2	2	2	2	2	2	2
Endpoints appropriate to study aim	2	2	2	2	2	2	2
Unbiased assessment of study endpoint	1	1	1	1	1	1	1
Follow-up period appropriate to study aim	2	2	2	2	2	2	2
<5% Loss to follow-up	0	0	0	0	0	0	0
Prospective calculation of study size	0	0	0	0	0	0	0
Adequate control group	NA	NA	2	2	NA	2	NA
Contemporary Groups	NA	NA	2	2	NA	2	NA
Baseline equivalent groups	NA	NA	2	2	NA	2	NA
Adequate statistical analysis	NA	NA	2	2	2	2	NA
Total score (…/24) (%)	11/16 (69%)	11/16 (69%)	19/24 (79%)	17/24 (79%)	11/16 (69%)	19/24 (79%)	11/16 (69%)

Abbreviations: NA: not applicable (non-comparative study), 0: not reported, 1: reported but inadequate, 2: reported and adequate

Figure 1

- FIGURE 1 PRISMA flowchart of study selection