



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

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Abstract

Complex regional pain syndrome (CRPS) is characterized by continuous pain that is often accompanied by sensory, motor, vasomotor, sudomotor, and trophic disturbances. If left untreated, it can have a significant impact on the quality of life of patients. The diagnosis of CRPS is currently based on a set of relatively subjective clinical criteria: the New International Association for the Study of Pain clinical diagnostic criteria for CRPS. There are still no objective laboratory tests to diagnose CRPS and there is a great need for simple, objective, and easily measurable biomarkers in the diagnosis and management of this disease. In this review, we discuss the role of inflammation in the multi-mechanism pathophysiology of CRPS and highlight the application of potential biomarkers of inflammation in the diagnosis and management of this disease.

Key Points

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

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

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

1 Introduction

Complex regional pain syndrome (CRPS) is a painful disease of the extremities that is usually initiated by tissue damage, e.g., following fracture or surgery [1, 2]. It is characterized by continuous pain that is disproportionate to the

inciting event, and which can be accompanied by sensory, motor, vasomotor, sudomotor, and trophic disturbances [3]. The incidence of CRPS has been reported to vary between 5.5 and 26.2 per 100,000 person-years and women are reported to be affected more often than men [1, 2].

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

If left untreated, CRPS can lead to a debilitating loss of function of the affected extremity and can have a significant social impact on the life of patients [7]. It is therefore important that this disease is diagnosed early and treated with appropriate mechanism-based therapies. However, early diagnosis and therapy selection are often hampered by the aforementioned lack of objective tests. Currently, physicians have to rely on subjective symptoms reported by patients and relatively subjective signs observed during physical examination for diagnosis and management of CRPS. This

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subjectivity of symptoms and signs, which is often accompanied by a discrepancy between the symptoms and signs, leads to various diagnostic and therapeutic challenges for clinicians, such as delayed diagnosis and inappropriate selection of therapies. To make these matters more complicated, CRPS is a disease with a heterogeneous clinical presentation and there may be various disease subtypes with their own specific phenotype [8–10]. These matters therefore not only complicate diagnosis of this disease but also the selection of therapies based on the underlying pathophysiological mechanisms as, at present, these underlying mechanisms are also deduced from the relatively subjective, and often discrepant, symptoms and signs.

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

2 Pathophysiology of Complex Regional Pain Syndrome (CRPS)

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

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

In CRPS, neurogenic inflammation, neuroinflammation, and dysregulation of the immune system have all been implicated as a source of inflammation. Peripheral neurogenic inflammation has long been implicated in the pathophysiology of CRPS [16]. In peripheral neurogenic inflammation, primary afferent sensory neurons release neuropeptides that cause cutaneous vasodilation (mainly through calcitonin gene-related peptide [CGRP]), changes in vascular permeability (mainly through substance P [SP]), increased protein extravasation, and increased leukocyte recruitment [17, 18].

Weber et al. [19] conducted a study using transcutaneous electrical stimulation via intradermal microdialysis capillaries and found significantly increased axon reflex vasodilation in the affected extremity of CRPS patients compared with healthy controls. This study further found increased protein extravasation in the affected extremity of these CRPS patients [19]. These findings suggest an increased release of neuropeptides such as CGRP and SP by activated sensory neurons in CRPS patients and thus point towards facilitated neurogenic inflammation in CRPS [19]. This increased neuropeptide release may also account for symptoms such as allodynia, hyperalgesia, edema, vasodilation, and trophic abnormalities that are seen in CRPS patients [19, 20].

Besides neurogenic inflammation, recent studies have provided evidence supporting a role for neuroinflammation in CRPS [21, 22]. Neuroinflammation refers to inflammation occurring within the nervous system (central nervous system and/or peripheral nervous system) that is characterized by glial cell activation leading to an increased production of pro-inflammatory cytokines and chemokines [23]. Neuroinflammation can be initiated by various forms of trauma and surgery, and it has also been suggested that it can be caused by increased neuronal activity of primary afferent nerve fibers and/or higher-order neurons [23, 24]. This latter phenomenon has been coined ‘neurogenic neuroinflammation’ and has also been implicated in CRPS [20, 24]. Neuroinflammation can lead to various adverse effects, such as a transition from acute to chronic pain and maintenance of chronic pain [23]. This chronic pain is established via central sensitization, which is induced and maintained by central cytokines, chemokines, and glia-produced mediators [23]. Central sensitization is characterized by pain hypersensitivity and manifests clinically as dynamic tactile allodynia, secondary punctuate and/or pressure hyperalgesia, temporal summation, and sensory after sensations [25]. Symptoms of central sensitization have also been described in CRPS [25–28] and have been attributed to a sensitization of the nociceptive system due to ongoing pain and, therefore, to continuous nociceptive input [11, 29]. As neuroinflammation seems to drive central sensitization, and studies now suggest neuroinflammation may play a role in CRPS, it is possible that part of the symptoms of CRPS that are attributed to central sensitization can be caused not only by continuous nociceptive input but also by neuroinflammation [21–23]. Neuroinflammatory findings in CRPS are new and need to be studied in further detail, yet neuroinflammation represents an interesting therapeutic target in patients with symptoms and signs of central sensitization.

Although peripheral neurogenic inflammation has long been implicated in CRPS, it is only in recent years that evidence to support involvement of the immune system in CRPS has grown. Until recently, the involvement of the

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

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

3 CRPS: Subtypes and Phenotypes

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

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

The hypothesis that phenotypical characterization in CRPS can be used to assess the underlying pathophysiological mechanisms of disease, and consequently to select targeted therapies, was presented in an article by Birkle and Schlereth in 2015 [9]. The authors described two phenotypes of CRPS: the “peripheral inflammatory phenotype” reflecting clinical symptoms and signs that are generated by inflammation, and the “central neuroplasticity phenotype” reflecting clinical symptoms and signs generated by the central nervous system (e.g., mechanical allodynia) [9]. The problem with phenotypical characterization is, however, that clinicians are still dependent on relatively subjective symptoms and signs to characterize the subtype of CRPS that a patient may have. Another issue to take into consideration is the large amount of clinical overlap between the various phenotypes and subtypes, which could create confusion when determining underlying mechanisms of disease [10]. In addition, phenotypical characterization can also misguide clinicians to a certain degree. For example, a clinician may disregard inflammation as an underlying pathophysiological

mechanism in a patient with cold-type CRPS; however, we now know from a study in blister fluid that a subgroup of cold CRPS patients also suffers from inflammation [44]. These issues highlight the need for simple, objective, and easily measurable biomarkers in the diagnosis and management of CRPS.

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

4 Definition of a Biomarker and Criteria for CRPS

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

that can be used in the third situation is applicable in the management of this disease.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5 Potential Biomarkers of Neurogenic Inflammation and Neuroinflammation

5.1 Local Biomarkers

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

Jung et al. [22] conducted an explorative study in which they studied the correlation between peripheral metabolites in blood and urine, central neurometabolites using proton magnetic resonance spectroscopy (¹H-MRS), and

neuroinflammation using [¹¹C]-(R)-PK11195 PET. The authors found statistically significant positive correlations between the levels of lipid 13a and lipid 09 relative to total creatine and neuroinflammation in certain brain regions of CRPS patients [22]. They further found that peripheral pH, glucose, CO₂, basophil, and creatinine levels were associated with an increase or decrease of the level of neuroinflammation in the brain of CRPS patients [22]. The authors suggest that characterization of peripheral and central metabolites may help us to understand the role of neuroinflammation in the pathophysiology of CRPS. They further suggest that central lipid levels may be used as a biomarker for neuroinflammation.

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

5.2 Systemic Biomarkers

Four studies measured venous blood levels of the soluble neuropeptide CGRP in CRPS patients [32, 46–48]. Blair et al. [46] conducted a study in which they measured CGRP levels in the blood of CRPS patients and healthy controls: blood CGRP

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

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

6 Potential Biomarkers of Immune Dysregulation in CRPS

6.1 Local Biomarkers

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

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

6.2 Systemic Biomarkers

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

Circulating T lymphocyte subsets represent another example of potential cellular biomarkers in CRPS. To our knowledge, three studies have determined T lymphocyte subsets in venous blood of CRPS patients [31, 62, 66]. These studies, however, show conflicting results when it comes to alterations in circulating T lymphocyte subsets. For example, while Kaufmann et al. [66] found significantly lower absolute numbers of cytotoxic CD8⁺ T lymphocytes in CRPS patients than in healthy controls, Ribbers et al. [31] observed no difference in the absolute numbers of this subset, and Ritz

et al. [62] found no difference in the percentage of this subset between CRPS patients and healthy controls. Interestingly, Kaufmann et al. [66] did not find any correlation between CD8⁺ T lymphocytes and pain, as measured on a Visual Analog Scale ranging from 0 to 10 in CRPS patients. Based on these conflicting results, we cannot draw a conclusion on whether T lymphocyte subset measurement in peripheral blood can currently be used as a biomarker for diagnosis and/or management of CRPS. Yet the existence of many different T lymphocyte subsets, including different types of T helper subsets, clearly warrants further study into the relation between T lymphocytes and CRPS [67, 68].

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

Autoantibodies represent another example of soluble biomarkers. Autoantibodies are commonly used as biomarkers for diagnosis and monitoring of inflammatory diseases and autoimmune diseases [76]. Multiple studies have described various autoantibodies in CRPS [34–37]. One study identified autoantibodies against autonomic nervous system structures in a small subset of CRPS patients: five of 12 CRPS patients were positive for these autoantibodies [34]. Another study found that ~ 30 to 40% of their CRPS patient group had autoantibodies against an autonomic nervous system autoantigen [35]. In a follow-up study, this group aimed to identify the antigens targeted by these autoantibodies and found agonistic autoantibodies against the β_2 -adrenergic receptor and/or the muscarinic-2 receptor in a subset of their CRPS patients [36]. A third study found a higher prevalence

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

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

application of miRNA profiling could be useful in the diagnosis as well as the management of CRPS; however, research on this subject is still in its initial phase and more research is needed to validate current results.

7 Biomarkers in CRPS: Limitations, Considerations, and Recommendations for Future Research

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

First, acquisition of the marker is of great importance: is it acquired locally or systemically? Some of the markers mentioned earlier are acquired in blister fluid and/or skin biopsies while others are measured in venous blood. The techniques for skin blisters and skin biopsies are considered to be of a higher risk to the patient and more invasive than a venipuncture and require a close follow-up to assess healing of the damaged skin. The skin blister technique is also time consuming and requires pain physicians to have access to materials and devices not usually available in routine practice [33]. Second, is it simple to obtain in routine practice?

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

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

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

In general, a marker that can be measured in venous blood is easier to obtain in routine practice than a marker that needs to be measured in skin biopsies or blister fluid. Furthermore, certain techniques require training. Pain physicians would thus need to be trained in obtaining skin biopsies and inducing artificial skin blisters. Third, is the biomarker easy to measure? This question is largely dependent on whether an affiliated laboratory has the facilities to determine the aforementioned markers; for example, an enzyme-linked immunosorbent assay (ELISA) is a relatively simple technique to quantify substances such as autoantibodies, while a cell-based assay is a more complex technique that is often not routinely available. Lastly, and most importantly, does it pose a minimal risk to the patient? Patients with CRPS have continuing pain of the affected limb that can be worsened by touch or any form of contact (allodynia and/or hyperalgesia). Performing a skin biopsy or inducing a skin blister in the affected limb can temporarily increase the pain a patient is experiencing. In contrast, venipuncture is usually conducted in the contralateral limb, or, in the case of the lower limb, in an arm, thereby avoiding the painful limb.

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

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

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

non-invasive monitoring of inflammatory markers that warrants further exploration in CRPS.

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

8 Conclusions

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

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

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

Compliance with Ethical Standards

Conflict of interest K. D. Bharwani, W. A. Dik, M. Dirckx, and F. J. P. M. Huygen declare that there is no conflict of interest regarding the publication of this paper.

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