Systemic cytokine level differences in patients with chronic muscoloskeletal spinal pain compared to healthy controls ans its association with pain severity : a systematic review

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SYSTEMIC CYTOKINE LEVEL DIFFERENCES IN PATIENTS WITH CHRONIC MUSCULOSKELETAL SPINAL PAIN COMPARED TO HEALTHY CONTROLS AND ITS ASSOCIATION WITH PAIN SEVERITY: A SYSTEMATIC REVIEW

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Funding Source: There was no funding provided for this review from any source.

Conflict of Interest: The authors have no relevant financial or non-financial conflict of interest.

Financial Disclosure: Amber Billens is appointed on a Starting Grant from the Special Research Fund (BOF) from Ghent University, Belgium, grant number BOF/STA/201909/054 received by Jessica Van Oosterwijck. The other authors have nothing to disclose.

Running Head: Systemic Cytokines in Chronic Spinal Pain

PROSPERO Registration Number: CRD42021246647

ABSTRACT

Objective: Although there has been increasing interest in the role of systemic cytokines in chronic spinal pain (CSP), the evidence on their potential contribution is still unclear. Therefore, the current study systematically reviewed the evidence on systemic cytokine level differences between people with CSP compared to healthy controls (HCs) and the potential associations with pain severity.

Methods: An electronic search was conducted on PubMed, Web of Science and Embase. All included studies were classified as observational studies, exploring the comparison between a CSP group and a HC group, and the association between systemic cytokine levels and pain severity.

Results: Nine articles were included with a total sample of 400 CSP patients suffering from chronic whiplash associated disorder (CWAD) or chronic low back pain (CLBP). In CLBP, moderate evidence was found for elevated tumor necrosis factor (TNF) α, interleukin (IL) 6, IL-1 receptor antagonist (IL-1RA), and soluble TNF receptor (sTNF-R) type 2, for normal interferon (IFN) γ and IL-2 levels, and for reduced IL-10 levels. No association was found between pain severity and these cytokines in CLBP. In CWAD, moderate evidence was found for elevated CRP and evidence for changes in TNF-α was inconclusive. Evidence for the association between pain severity and CRP was limited, and there is probably no association between pain severity and TNF-α with limited evidence in CWAD.

Conclusions: Moderate evidence indicates the presence of systemic inflammation in CSP. Evidence regarding the association between pain severity and systemic cytokines is inconclusive and limited.

Keywords: cytokines, neck pain, low back pain.

INTRODUCTION

Chronic musculoskeletal pain is one of the most common chronic pain conditions and can be described as pain arising from muscles, tendons, joints and ligaments, which has been present for at least three months [1, 2]. In acute musculoskeletal pain, the initial source of nociception can be resolved [3]. However, persistent pain, resulting from ongoing input to actual or impending tissue damage, can be characterized by non-specific and nociplastic pain, indicating altered function of pain related sensory pathways in the peripheral and central nervous system despite the lack of clear evidence of actual or impending tissue damage [4, 5].

Chronic spinal pain (CSP), of which the prevalence has been shown to increase with age, is the most frequent form of musculoskeletal pain and is situated in the cervical, thoracic or lumbar region [6-11]. The annual prevalence of CSP among the adult population ranges from 12.1% to 71.5% for neck pain, 15% to 34.8 % for thoracic pain and 15% to 45% for low back pain (LBP) [10, 12-14]. CSP is associated with higher disability, morbidity, reduced quality of life, loneliness, fatigue and enormous socioeconomic costs [12, 14-16]. Although the number of evidence-based diagnostic techniques for the identification of CSP has increased in order to reduce its prevalence and impact, CSP continues to be the main reason for persistent pain in human adults [17, 18].

Central hyperexcitability is responsible for common pain symptoms such as mechanical hyperalgesia, allodynia and widespread pain in chronic musculoskeletal pain [19, 20]. The cytokine signaling system is proposed as the underlying mechanism of central hyperexcitability [21-24]. Cytokines modulate perceived pain and contribute to the persistence of pain by acting in the spinal cord and brain [19]. Under physiological conditions, the blood-brain barrier prevents cytokine transportation to the brain [25, 26]. However, its permeability can be

modulated by cytokine levels, transporting the overflowing cytokines from the systemic circulation through the blood-brain barrier to the brain [25, 26]. Besides the blood-brain barrier, systemic cytokines can enter the brain at the circumventricular organ at the base of the 4th ventricle as well [26]. Cytokines entering the brain ultimately cause aberrant glial activation, resulting in persistent pain [19, 27]. In the spinal cord, the circulating cytokines affect the dorsal root ganglia through fenestrated vasculature that lies outside the blood-brain barrier and results in aberrant glial activation in the spinal cord [21].

Systemic cytokine assessment has been used to monitor pain severity for predicting recovery in CSP. To clarify the cytokine role in CSP, previous systematic reviews investigated the systemic cytokines in musculoskeletal spinal pain [28-30]. However, based on these studies, it is difficult to distinguish between acute spinal pain and CSP with regard to cytokine levels, and their potential relationship with clinical pain severity. This is mainly due to the use of mixed populations, including both patients with acute and chronic spinal pain in these studies [28-30]. Additionally, it has been reported that there are differences in the association between acute and chronic inflammatory processes and clinical symptoms in acute and chronic spinal pain as well as different cytokine mechanisms such as inflammatory/anti-inflammatory ratio in acute and chronic spinal pain [24, 31, 32].

Patients with acute LBP or WAD may recover and these patients might have shown different levels of cytokines during acute pain compared to patients who developed chronic pain [22, 32]. Besides different amounts of cytokines during the acute and chronic phase, cytokines may have different roles, i.e.inflammatory and antiinflammatory, in acute and chronic pain [33, 34]. Elevated serum cytokine levels have only been shown to be positively associated with disc and local muscle degeneration in CLBP, but not in acute LBP [35, 36]. Furthermore, it has been

observed that acute and chronic spinal pain have different prognoses and responses to treatment [24]. Considering these differences, this study aimed to specifically focus on cytokines in CSP. Further understanding of the cytokine role in CSP may facilitate and improve the approach of CSP. This systematic review aimed to address two questions in particular: 1) Do differences exist in systemic cytokines (O) in people suffering from CSP (P) compared to healthy people (C)? 2) Are there any associations between systemic cytokines (E) and pain severity (O) in patients with CSP (P)?

METHODS

Protocol registration

This systematic review was conducted following the Preferred Reporting Items for Sytematic Reviews and Meta-Analysis (PRISMA) guidelines [37]. The protocol was pre-registered on PROSPERO (Registration number: CRD42021246647).

Information sources and search strategy

To identify relevant studies, the existing literature up to the 20th of December 2021 was systematically searched in the following medical databases: PubMed (www.ncbi.nlm.nih.gov/pubmed), Web of Science (www.webofknowledge.com/), and Embase (www.embase.com).

A search strategy was composed using a combination of index terms (in PubMed and Embase) and keywords for population, intervention and outcome, all derived from the PECO approach for aim 1 and 2: different CSP populations (Population), systemic cytokines (Exposure), healthy controls (Comparison), and pain (Outcome). The search strategy for each database can be found in Appendix 1.

Eligibility criteria

Studies had to meet the following criteria to be included in this review: 1) studying human adults (>18 years); 2) studying CSP located in the muscles, bones, joints or tendons with spontaneous or evoked pain in the affected region more than 3 months [2]. 3) evaluating systemic cytokine level; 4) written in English; 5) full-text report of orginal analytical studies; and 6) studies both comparing cytokine levels between patients with CSP and healthy participants (case-control study design), and studying the association between cytokines and pain severity within the CSP group (cross-sectional analysis).

The exclusion criteria were: 1) studies on children/adolescents (<18 years); 2) studies on patients with acute musculoskeletal spinal pain (≤3 months); 3) studies on patients with other types of musculoskeletal pain than CSP; 4) studies involving CSP of non-musculoskeletal origin; 5) animal studies; and 6) short reports i.e. case reports, abstracts, etc. and secondary research i.e. letters to the editor, reviews, meta-analysis etc. 7) studies in languages other than English.

Study selection

After deduplication in Endnote, all articles were transferred to Rayyan (https://rayyan.qcri.org), where remaining duplicates were removed. Two reviewers (K.C. and A.B) used the screening tool Rayyan to allow for blinded screening. A first screening was carried out to examine the fulfillment of the predefined inclusion criteria for all articles based on title and abstract. If any of the inclusion criteria were not fulfilled, the article was excluded. During a second screening, the full-text of the remaining articles were screened by the same two reviewers to ensure fulfillment of the inclusion criteria. Screening results from the two reviewers were compared

and in case of disagreement, a decision was made after discussion between the two authors. When no consensus could be made, a third independent reviewer (MM) was consulted to make the final decision.

Data extraction

Data extraction was performed by one reviewer (KC) and then independently verified in a blinded way by two reviewers (AB and KDM) for all included studies and systematically summarized in an evidence table (Table 1). The presented data included

1) publication (author and year of publication); 2) participants (sample size, gender, mean age); 3) pain severity assessment; 4) cytokines assessment (outcome, laboratory techniques, blood fraction); and 5) results.

Risk of bias in individual studies

In this systematic review, the included articles that comprised a patient group and control group were identified as a case-control design. The risk of bias (RoB) was independently assessed by two reviewers (KC and AB) using the Newcastle-Ottawa Scale (NOS) with eight items [38]. Potential RoB was evaluated using a star system which differentiated between three types of bias (selection, comparability, and exposure/outcome). An article could achieve a maximum of nine stars indicating high quality and no RoB. After rating, both reviewers compared the results and analyzed the differences. Inconsistencies were resolved during a consensus meeting, and when no consensus could be achieved, the decisive opinion of a third researcher (MM) was solicited.

Level of evidence and strength of conclusion

A level of evidence between A1 (systematic review of at least 2 independently conducted studies of evidence level A2) and D (expert opinion) was assigned to each included article, taking into account the study design and RoB, following the EBRO-guidelines of the Dutch Institute for Healthcare Improvement (CBO). A strength or level of conclusion (LoC) ranging between 1 (1 A1 or at least 2 independent A2 studies) and 4 (expert opinion) was assigned, considering the level of evidence and the consistency of the results (Table 2) [39].

RESULTS

Study selection

The systematic database search resulted in a total of 5658 records (i.e. Web of Science, n= 1875; Pubmed, n=1913; Embase, n=1870). Following deduplication, a total of 4181 articles were screened of which 4172 articles were excluded as they did not fulfill the eligibility criteria. Hence, 9 articles were included for synthesis in this systematic review. The entire selection process is presented in the flowchart of Figure 1.

Study characteristics

Key data from the included articles are shown in Table 1. Sample size comprised a total of 400 CSP patients with a mean age ranging from 30 to 46 years and 321 HCs with a mean age ranging from 30 to 45 years. All included studies compared the cytokine level between **a** HC and patient group and studied the correlation between pain severity and cytokines in the patient group.

In three studies, the patient and HC group were age and gender matched [40-42]. In two studies, there were no significant differences for age and gender between patients and HCs [43, 44]. In one study, the patient and HC group were gender-matched and there were no significant

differences for age between groups [45]. Although three studies provided information on age and gender for both groups, they did not examine whether these differences were significant or not [22, 24, 46].

Of the included studies, two investigated chronic Whiplash Associated Disorder (CWAD), accounting for 41 subjects [22, 41] and seven studies focused on chronic low back pain (CLBP), resulting in 359 subjects [24, 40, 42-46].

Pain severity was evaluated using a McGill Pain Questionnaire-Short Form (MPQ-SF) in one study [40] a Visual Analog Scale (VAS) in five studies [24, 41, 42, 45, 46] a Numeric Rating Scale (NRS) in two studies [43, 44] and pressure, cold and heat pain threshold (HPT) assessment in two studies [22, 41].

Plasma was extracted from blood in two studies [24, 40], whole blood was used in one study [46] and serum was obtained after blood extraction in six studies [22, 41-45]. A total of 47 cytokines were evaluated: tumor necrosis factor (TNF) α (8/9 studies, 88.88%), interleukin (IL) 6 (5/9 studies, 55.55%), IL-1β (5/9 studies, 55.55%), C-reactive protein (CRP) (2/9 studies, 22.22%), interferon (IFN) γ (3/9 studies, 33.33%), IL-2 (3/9 studies, 33.33%), IL-10 (3/9 studies, 33.33%), soluble TNF receptor (sTNF-R) type 2 (2/9 studies, 22.22%), IL-1 receptor antagonist (IL-1RA) (2/9 studies, 22.22%), and remaining cytokines (sTNF-R, IL-4, IL-8, IL-16, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage migration inhibitory factor (MIF), C-C motif chemokine ligand (CCL)1, CCL2, CCL3, CCL7, CCL8, CCL11, CCL13, CCL15, CCL17, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, C-X-C motif chemokine ligand (CXCL)1, CXCL2, CXCL5, CXCL6, CXCL9,

CXCL10, CXCL11, CXCL12, CXCL13, CXCL16 and C-X3-C motif chemokine ligand 1 (CX3CL1)) were analyzed in only one study.

Risk of bias and level of evidence

The RoB and level of evidence for included studies are shown in Table 3. There was a 91.11% agreement between both assessors prior to the consensus meeting. After the interrater comparison, consensus was reached for all items. A mean of 7 stars was attained, varying between studies with low to moderate RoB. Studies mostly lost points due to the presence of selection bias. Since all studies had a case-control design, all studies were assigned a level of evidence B.

Synthesis of Results

TNF-α

Comparison

Eight studies about TNF-α, six examining patients with CLBP [24, 42-46] and two studying patients with CWAD [22, 41] were included.

CLBP: Five studies found higher TNF-α levels in patients with CLBP compared to HCs [24, 42, 43, 45, 46]. One study, performed in CLBP with modic changes 1 (MC1) (oedema type) or modic changes 2 (MC2) (fatty type), found no differences for serum TNF-α between patients with CLBP and HCs [44].

Moderate evidence indicates the presence of elevated TNF-α levels in CLBP (LoC 2).

CWAD: One study found higher levels of TNF-α in serum in CWAD patients compared to HCs [41]. Similarly another study did not find any differences between CWAD patients and HCs regarding to TNF-α in serum [22].

There is inconclusive evidence for altered TNF-α levels in CWAD (LoC 3).

Relationship

CLBP: Five studies found no significant correlations between pain severity and TNF-α levels in CLBP [42-46]. One study found a very strong significant positive correlation between pain severity and TNF-α levels, meaning that more severe pain was associated with higher levels of TNF-α in CLBP [24].

Moderate evidence indicates that there is probably no association between pain severity and TNF-α levels in CLBP (LoC 2).

CWAD: One study found no significant correlation between pain severity and TNF-α levels [41].

There is probably no association between pain severity and TNF-α levels in CWAD with limited evidence (LoC 3).

IL-6

Comparison

IL-6 levels were assessed in five studies examining patients with **CLBP** [24, 40, 43, 44, 46]. Of these, higher levels of IL-6 were found in plasma in one [24], in serum in two [43, 44] and whole blood in one [46] in patients with CLBP compared to HCs. One study did not find IL-6 level differences in plasma in patients with CLBP compared to HCs [40].

Moderate evidence indicates the presence of elevated IL-6 levels in CLBP (LoC 2).

Relationship

Three out of five studies did not find any significant correlations between pain severity and plasma IL-6 [24], serum IL-6 [43] or whole blood IL-6 [46], Moderate significant positive correlation was found between pain severity and plasma IL-6 in one studie [40], and serum IL-6 in one studie [44] in CLBP.

There is inconclusive evidence for the relation between pain severity and IL-6 levels in CLBP (LoC 2).

IL-1β

Comparison

Five studies about IL-1β of which four were performed in CLBP [24, 43, 44, 46] and one in CWAD [22] were included.

CLBP: Two studies found no differences for IL-1β in serum in patients with CLBP compared to HCs [43, 44], while significantly higher levels of IL-1β were found in plasma in one studie [24] and in whole blood in one studie [46].

There is inconclusive evidence for alterations in IL-1β levels in CLBP (LoC 2).

CWAD: One study found no differences in IL-1β in serum between CWAD patients and HCs [22].

There is limited evidence indicating normal IL-1β level in serum in CWAD (LoC 3).

Relationship

CLBP: In one study, a strong significant positive correlation was found between pain severity and plasma IL-1β levels, meaning that more severe pain was associated with higher levels of plasma IL-1β in CLBP [24]. Two other studies performed in CLBP did not find any significant associations between pain severity and serum IL-1β levels [44], and whole blood IL-1β levels [46].

There is probably no relation between IL-1β levels and pain severity in CLBP, although no firm conclusions can be made as the current evidence is conflicting (LoC 2).

CRP

Comparison

CRP was assessed in two studies evaluating **CWAD** patients; both studies reported higher CRP levels in serum in CWAD compared to HCs [22, 41].

Moderate evidence indicates elevated CRP levels in serum CWAD patients (LoC 2).

Relationship

One study reported a moderate significant negative correlation between pressure pain thresholds (PPTs) at the Tibialis Anterior muscle and CRP levels in CWAD, indicating that higher serum CRP levels were associated with lower PPTs [22]. This study also reported a moderate significant positive correlation between cold pain thresholds (CPTs) over the mid to lower regions of the cervical spine and CRP levels, indicating that higher CRP levels are associated with higher CPTs [22]. One study indicated that a correlation between PPTs at the cervical spine (C2) and serum CRP levels in CWAD approached significance [41].

There is limited evidence for a relation between pain severity and serum CRP levels in CWAD (LoC 3).

IFNγ and IL-2

Comparison

Three studies evaluated IFNγ and IL-2 in patients with CLBP and reported no differences for IFNγ levels in serum [44], plasma [24] or whole blood [46] between patients with CLBP and HCs.

Moderate evidence indicates normal IFNγ and IL-2 levels in patients with CLBP (LoC 2).

Relationship

No significant associations were reported between pain severity and serum [44], plasma [24] or whole blood [46] IFNγ and IL-2 levels in CLBP [24, 44, 46].

Moderate evidence indicates that there is probably no association between pain severity and IFNγ and IL-2 levels in CLBP (LoC 2).

IL-10

Comparison

Three studies assessed IL-10 levels in patients with **CLBP** [24, 44, 46]. Lower levels of IL-10 was found in plasma in one studie [24] and in serum in one studie [44], while one study found no significant differences in IL-10 in serum in CLBP patients compared to HCs [44].

Moderate evidence indicates the presence of decreased IL-10 levels in CLBP (LoC 2).

Other cytokines

Four studies on cytokines (other than the aforementioned) were performed in CLBP patients [24, 43, 44, 46].

Comparison

Two studies assessed IL-1RA and sTNF-R2 in patients with CLBP [24, 46]. Higher levels for both cytokines were found in plasma in one [24], and in whole blood in one [46] in CLBP patients compared to HCs. sTNF-R in serum was also assessed in another study, in which no difference was found for this cytokine between CLBP patients and HCs [43]. One study measured the following cytokines: IL-4, IL-8, IL-16, GM-CSF, MIF, CCL1, CCL2, CCL3, CCL7, CCL8, CCL11, CCL13, CCL15, CCL17, CCL19, CCL20, CCL21, CCL22, CCL 23, CCL24, CCL25, CCL26, CCL27, CXCL1, CXCL2, CXCL5, CXCL6, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL16 and CX3CL1 in serum in CLBP patients with MC1 or MC2 [44]. This study found higher levels of MIF, CCL27 and CX3CL1 in serum in MC1 and MC2 in patients with CLBP, but only CCL20 levels in serum in MC1 were significantly higher in CLBP patients when compared to HCs [44]. The levels of the other cytokines were not significantly different between the CLBP group and HC group [44].

There is moderate evidence for elevated IL-1RA and sTNF-R2 levels in CLBP (LoC 2). Furthermore, limited evidence indicate the presence of elevated CCL20 levels in CLBP patients with MC1 (LoC 3). Limited evidence regarding the other above mentioned cytokines indicates their levels are normal in CLBP (LoC 3).

Relationship

Two studies reported that pain severity was not significantly correlated with plasma or whole blood IL-1RA and sTNF-R2 levels in CLBP [24, 46]. One study in CLBP reported a moderate positive correlation between pain severity and serum CCL22, CCL20, CCL27, and CX3CL1 levels, and a weak significant positive correlation between pain severity and CCL26, CCL19, CXCL13 and MIF levels [44].

Moderate evidence indicates that there is probably no relation between pain severity and plasma or whole blood IL-1RA and sTNF-R2 levels in CLBP (LoC 2). Limited evidence was

found for the relation between pain severity and CCL22, CCL20, CCL27, CX3CL1, CCL26, CCL19, CXCL13, and MIF in CLBP (LoC 3).

DISCUSSION

To the best of our knowledge, this is the first systematic review that summarizes the evidence concerning 1) potential differences in altered systemic cytokine levels in patients with CSP compared to HCs while differentiating between CWAD and CLBP, and 2) potential associations between systemic cytokine levels and pain severity in patients with CSP. In CLBP, moderate evidence indicates the presence of elevated TNF-α, IL-6, IL-1RA, and sTNF-R2, normal IFNγ and IL-2, and reduced IL-10 levels. Evidence on alterations in IL-1β levels in CLBP is inconclusive. In CWAD, moderate evidence indicates the presence of elevated serum CRP levels, limited evidence points to normal serum IL-1β levels, and findings regarding changes in serum TNF-α levels are inconclusive. Studying whether systemic cytokine levels are associated with pain severity, moderate evidence in CLBP showed that TNF-α, sTNF-R2, IFNγ, IL-2 and IL-1RA levels are not associated with pain severity. Furthermore, limited evidence supports a relation between serum CCL22, CCL20, CCL27, CX3CL1, CCL26, CCL19, CXCL13, and MIF levels and pain severity in CLBP. Evidence on the relation between IL-1β and IL-6 levels and pain severity in CLBP is inconclusive. In CWAD, limited evidence indicates serum CRP levels to be associated with pain severity, while serum TNF-α levels do not seem to be associated with pain severity.

Moderate evidence points towards higher systemic (serum, plasma or whole blood) TNF-α levels in CLBP. However, the current evidence also indicates that TNF-α levels are probably not related to pain severity in CLBP. Evidence regarding altered serum TNF-α levels in CWAD is inconclusive, furthermore, limited evidence indicates the lack of a relation with pain severity

in this population. Elevated serum TNF-α levels have been observed in various musculoskeletal pain conditions including knee osteoarthritis and upper limb overuse injuries [47, 48]. In line with these findings, most studies included in this review found elevated TNF-α levels in CLBP, indicating the presence of systemic low-grade inflammation. Only two studies included in this review, performed by Sterling et al. [22] in CWAD and Gjefsen et al. [44] in CLBP, did not find elevated serum TNF-α levels in the CSP populations. In the study of Sterling et al. [22), anti-inflammatory medication usage by some participants (19%) 7 days prior to testing may have affected the results. Gjefsen et al. [44] only provided information about the control group regarding potential confounders such as BMI, comorbidities or smoking that may have influenced the results of the control and patient groups. In the literature, Van den Berg et al. [28] Farrell et al. [30] and Lim et al. [29] previously performed a review about the role of cytokines in spinal pain, but included studies examining both acute and chronic pain conditions. Van den Berg et al. [28] and Lim et al. [29] found conflicting and consistent evidence, respectively, for an association between elevated levels of serum or plasma TNFs (TNF-α, TNF, sTNF-R1) and pain severity in patients with non-specific LBP. Furthermore, Farrell et al. [30] concluded in their review that levels of whole blood or serum TNF-α and sTNF-R2 were elevated in patients with chronic neck pain of traumatic, non-traumatic and mixed nature, although the level of evidence for this conclusion was low. TNF-α in peripheral blood plays an essential role in the maintenance of chronic pain [25]. Increased TNF-α in peripheral blood can alter the permeability of the blood-brain barrier. Subsequently, endothelial cells of the brain respond to cytokine signals in the peripheral blood and secrete cytokines in the brain that contribute to persistent pain, as is also found in nociplastic pain [25]. It is interesting to note that elevated plasma levels of TNF-α have previously been associated with higher pain severity in patients with neuropathic, nociceptive and mixed pain for longer than 6 months [49] whereas the current review did not find any evidence for associations between TNF-α levels and pain

severity in CSP. However, it is noteworthy that the only study evaluating TNF-α in plasma, performed by Teodorczyk-Injeyan et al. [24], found enhanced TNF-α levels to be associated with higher pain severity in CLBP. The other studies assessed TNF-α in serum or in whole blood and did not find any associations between TNF-α levels and pain severity [41-46]. Plasma cytokines have been shown to accurately reflect cytokine levels in the human body due to cytokines being more stable in plasma than in serum [50]. It has been proposed that plasma is a better matrix than serum for the evaluation of cytokines in clinical or research analyses [50]. However, in the study performed by Theodorczyk-Injeyan et al. [24], which assessed TNF-α in plasma, data were not stratified based on age due to a relatively narrow age range and small sample size. Therefore, age-related degenerative changes may have affected these results. Hence, the results of the study performed by Theodorczyk-Injeyan et al. [24] need to be confirmed by future studies.

Elevated plasma and serum IL-6 has been previously shown in various chronic musculoskeletal disorders such as fibromyalgia, chronic pain patients, and patients with postoperative pain compared to healthy volunteers, and is found to be associated with pain severity in these populations [49, 51, 52]. Considering CSP, higher systemic (serum and plasma) IL-6 has been associated with higher pain severity in non-specific LBP and with reduced pain relief in non-specific LBP in previous reviews performed by Lim et al. [29] and Van den Berg et al. [30]. Since both acute and chronic non-specific LBP were included in these reviews and the results were not reported seperately for each population, no direct conclusion can be drawn from these studies on the relationship between IL-6 and pain severity in chronic non-specific LBP as IL-6 exhibits different characteristics during the acute and chronic phase [33, 34]. During acute pain, serum IL-6 contributes to the anti-inflammatory response with (in)direct analgesic effects on the nociceptors, whereas in chronic pain IL-6 contributes to a pro-inflammatory response

resulting in enhanced neuronal activity which elicits pain hypersensitivity [33, 34]. This review showed moderate evidence for elevated IL-6 levels, and inconclusive evidence regarding potential associations between IL-6 levels and pain severity in CLBP. IL-6 is a proinflammatory cytokine that may act on the peripheral and central nervous system, and modulates pain perception. de Goeij et al. [53] reported that systemic inflammation via experimentally induced increases in plasma TNF-α, IL-6, IL-10 and IL-1RA levels were accompanied by changes in pain perception and resulted in reductions in PPTs, electrical pain thresholds and cold pressure pain tolerance. The inconclusive evidence for the association between IL-6 and pain severity may be a result of the studies in which blood was collected at different times throughout the day. IL-6 concentrations change during the day and peak in the evening. IL-6 assessment performed at earlier times of day may provide more reliable results [43]. Blood samples were taken at somewhat earlier times of the day in the study performed by Heffner et al. [40] which found a moderate positive correlation between plasma IL-6 and pain severity compared to studies performed by Teodorczyk-Injeyan et al. [24, 46] which found no association between plasma and whole blood IL-6 and pain severity. However, Kraychete et al. [43] and Gjefsen et al. [44] who found no association between serum IL-6 and pain severity, and moderate positive correlations between IL-6 and pain severity did not provide any specific information on when the blood was drawn.

This review showed inconclusive and limited evidence supporting changes in IL1-β levels of CLBP or CWAD patients, and for a potential association between IL1-β and pain severity in CLBP. In line with these results, Lim et al. [29] previously reported conflicting evidence for an association between systemic IL-1β and pain severity in a mixed population of acute and chronic non-specific LBP. Farrell et al. [30] reported a low level of evidence for increased

serum and whole blood IL-1β levels in patients with chronic neck pain of traumatic, non-traumatic and mixed nature.

CRP contributes differently to acute and chronic pain. In acute LBP and WAD, elevated serum CRP indicates a normal inflammatory response that results in recovery and is expected to resolve within 6 months [22, 32]. In contrast, elevated serum CRP in chronic LBP and WAD indicates systemic inflammation and ongoing tissue healing [22, 32]. However, up to now, reviews included both acute and chronic spinal pain without making a differentiation, demonstrating increased serum CRP levels and an association between elevated serum CRP and higher pain severity in chronic neck pain of traumatic, non-traumatic and mixed nature [30]. In this review, moderate evidence supporting the presence of elevated CRP levels in CWAD was found, whereas limited evidence was found to support an association between CRP levels and pain severity in CWAD. Patients with nociplastic pain report a regional, rather discrete pain distribution and show pain hypersensitivity (mechanical, heat or cold hypersensitivity) at the local pain area [4]. Two studies were included in this review that assessed PPTs at both a local painful and remote body site (i.e. cervical region and tibialis anterior muscle, resp.), and CPT and HPT at the painful region (cervical region) in CWAD [22, 41]. In one study, higher CRP levels were associated with higher CPTs indicating reduced cold hypersensitivity [22]. In the second study, the correlation between serum CRP levels and PPTs in the painful region approached significance [41]. However, in the first study, higher serum CRP levels were significantly associated with lower PPTs measured at the remote areas indicating mechanical hypersensitivity [22]. Although these two studies demonstrated increased pain hypersensitivity in local pain areas and remote regions, it cannot be directly concluded that CRP levels have an effect on nociplastic pain. More research is needed on this topic.

This review provided moderate evidence for the presence of increased sTNF-R2 and IL-1RA levels and reduced IL-10 levels in CLBP, while the changes in sTNF-R2 and IL-1RA levels did not seem to be associated with pain severity. sTNF-R2 and IL-1RA are specific cytokine receptors at the cellular level that block TNF-α and IL1-β mediated cellular changes, respectively. sTNF-R2 and IL-1RA might be increased as a result of higher TNF-α and IL-1β in CSP and may indicate the effort to suppress higher TNF-α and IL-1β levels. Lower IL-10 levels, which is an anti-inflammatory cytokine, may indicate suppression of the anti-inflammatory profile in CLBP, yet more research is needed to support this hypothesis.

There is limited evidence for increased levels of serum CCL22, CCL20, CCL27, CX3CL1, CCL26, CCL19, CXCL13, and MIF in CLBP and their association with pain severity. Normal levels of IFNγ and IL-2 are not associated with pain severity in CLBP. In the systematic review of Farrell et al. [30], limited evidence for increases in whole blood IL-1RA and CCL-3 were found in chronic neck pain of traumatic, non-traumatic and mixed nature.

This review had some limitations. To examine potential differences in cytokine levels in CSP, patients needed to be compared with a HC group. Therefore, only case-control studies were included in this review. Subsequently, the LoC could be moderate at most, as case-control studies receive a level of evidence B. In this review, the inclusion of studies was limited to articles written in English, so related articles written in other languages may have been missed. The differences in systemic cytokine level between males and females could not be investigated as none of the included studies compared on the basis of gender. As females and males demonstrate differences in immune response to the chronic pain [54], it would be interesting

to investigate gender role on systemic cytokine in chronic spinal pain as well. Further research is needed in this area.

This review had some strengths as well. First, this review was pre-registered in the PROSPERO database, which avoids unplanned duplication and reduces potential publication bias. Secondly, in most studies, there were no significant differences between control and patient groups for age and gender, both of which can affect systemic cytokines. Third, the majority of the included studies had low to moderate RoB.

Future studies should investigate the effects of cytokine levels on pain hypersensitivity, such as mechanical, hot and cold pain hypersensitivity at local and remote regions of the primary pain site, to provide insights on the effects of cytokine levels on nociplastic pain in CSP.

Conclusion

As a conclusion, moderate evidence indicates enhanced TNF-α, IL-6, IL-1RA, and sTNF-R2 levels, reduced IL-10 levels and normal IFNγ and IL-2 levels in CLBP indicating the presence of systemic inflammation. However, these cytokine levels are not associated with pain severity in CLBP, although evidence on IL-6 is inconclusive. Moderate evidence indicates enhanced CRP levels in CWAD, and an association between CRP and pain severity, though evidence is limited. In addition, in CWAD there is limited evidence indicating normal IL-1β levels, and findings regarding changes in TNF-α levels are inconclusive. Limited and inconclusive evidence exists regarding changes in the level of other cytokines and their association with pain severity.

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Table Titles

Table 1: Characteristics of included studies and summary of findings table

Table 2: EBRO classification of study results and recommendations

Table 3. The risk of bias results for included studies

Figure Titles

Figure 1: Flowchart of the selection process

Table 1: Characteristics of included studies and summary of findings table

Publication	Sample (n) Gender (F/M) Age in years (mean and SD or median and range)	Pain severity assessment	Cytokines assessment			Results[a] Differences in concentrations in mg/l for CRP and in pg/ml for all other cytokines (mean/geometric mean and SD/SEM or percent or mean)
			Outcome	Laboratory Techniques (Assay)	Blood Fraction	
Gjefsen et al. (2021)	**Patient Group** Chronic LBP with MC1 or MC2 n=83 <u>MC1</u> n=46 F/M= 69.6%/30.4% 42.1±8.3 y <u>MC2</u> n=37 F/M=51.4%/48.6% 45.8±9.0 y	NRS	TNF-α IFN-γ IL-1β IL-2 IL-4 IL-6 IL-8 IL-10 IL-16 GM-CSF MIF CCL1	40-plex Pro Human Chemokine multi-bead	Serum	**Comparison** <u>IL-6</u> **MC1>CG (0.28±0.02) (p<0.001*)** **MC2>CG (0.31±0.07) (p<0.001*)** <u>IL-16</u> **MC1>CG (0.36±0.09) (p<0.001*)** **MC2>CG (0.34±0.02) (p<0.001*)** <u>MIF</u> **MC1>CG (0.96±0.58) (p<0.001*)** **MC2>CG (1.23±0.80) (p<0.001*)** <u>CCL20</u> **MC1>CG (0.36±0.01) (p<0.001*)**

[a]Only cytokines reported in the results section of the referred article are mentioned.

			CCL2			**MC2>CG (0.30±0.12) (p=0.01*)**
			CCL3			CCL27
			CCL7			**MC1>CG (0.44±0.13) (p<0.001*)**
	Control Group		CCL8			**MC2>CG (0.40±0.21) (p<0.001*)**
	Sex and age-matched		CCL11			CX3CL1
	healthy controls		CCL13			**MC1>CG (0.33±0.06) (p<0.001*)**
	n=50		CCL15			**MC2>CG (0.21±0.07) (p<0.001*)**
	F/M=56%/44%		CCL17			Other cytokines
	44±9.9 y		CCL19			TNF-α
			CCL20			MC1>CG (0.1±0.22) (p>0.05)
			CCL21			MC2>CG (0.04±0.18) (p>0.05)
			CCL22			IFN-γ
			CCL23			MC1>CG (0.16±0.00) (p>0.05)
			CCL24			MC2>CG (0.12±0.02) (p>0.05)
			CCL25			IL-1β
			CCL26			MC1>CG (0.15±0.08) (p>0.05)
			CCL27			MC2>CG (0.07±0.24) (p>0.05)
			CXCL1			IL-2
			CXCL2			MC1>CG (0.17±0.05) (p>0.05)
			CXCL5[b]			MC2>CG (0.15±0.04) (p>0.05)

[b] Excluded from the analysis as more than half of the samples were below the limit of quantification

			CXCL6			IL-4
			CXCL9			MC1>CG (0.16±0.00) (p>0.05)
			CXCL10			MC2>CG (0.16±0.00) (p>0.05)
			CXCL11			IL-8
			CXCL12			MC1>CG (0.09±0.01) (p>0.05)
			CXCL13			MC2>CG (0.08±0.03) (p>0.05)
			CXCL16			IL-10
			CX3CL1			MC1>CG (0.14±0.10) (p>0.05)
						MC2>CG (0.10±0.10) (p>0.05)
						GM-CSF
						MC1>CG (0.27±0.31) (p>0.05)
						MC2>CG (0.10±0.22) (p>0.05)
						CCL1
						MC1>CG (0.09±0.10) (p>0.05)
						MC2<CG (0.12±0.14) (p>0.05)
						CCL2
						MC1>CG (0.11±0.04) (p>0.05)
						MC2>CG (0.12±0.05) (p>0.05)
						CCL3
						MC1>CG (0.13±0.03) (p>0.05)

						MC2>CG (0.12±0.01) (p>0.05) <u>CCL7</u> MC1>CG (0.14±0.01) (p>0.05) MC2>CG (0.12±0.01) (p>0.05) <u>CCL8</u> MC1>CG (0.10±0.02) (p>0.05) MC2>CG (0.15±0.03) (p>0.05) <u>CCL11</u> MC1>CG (0.10±0.01) (p>0.05) MC2>CG (0.12±0.05) (p>0.05) <u>CCL13</u> MC1>CG (0.07±0.03) (p>0.05) MC2>CG (0.19±0.10) (p>0.05) <u>CCL15</u> MC1>CG (0.25±0.07) (p>0.05) MC2>CG (0.10±0.25) (p>0.05) <u>CCL17</u> MC1>CG (0.18±0.07) (p>0.05) MC2>CG (0.34±0.04) (p>0.05) <u>CCL19</u>

						MC1>CG (0.20±0.06) (p>0.05) MC2>CG (0.16±0.05) (p>0.05) CCL21 MC1>CG (0.14±0.00) (p>0.05) MC2>CG (0.14±0.05) (p>0.05) CCL22 MC1>CG (0.24±0.07) (p>0.05) MC2>CG (0.29±0.05) (p>0.05) CCL23 MC1>CG (0.10±0.22) (p>0.05) MC2>CG (0.18±0.19) (p>0.05) CCL24 MC1>CG (0.08±0.02) (p>0.05) MC2>CG (0.10±0.03) (p>0.05) CCL25 MC1>CG (0.17±0.05) (p>0.05) MC2>CG (0.16±0.11) (p>0.05) CCL26 MC1>CG (0.23±0.03) (p>0.05) MC2>CG (0.23±0.10) (p>0.05)

						CXCL1 MC1>CG (0.05±0.03) (p>0.05) MC2>CG (0.06±0.04) (p>0.05) CXCL2 MC1<CG (0.14±0.11) (p>0.05) MC2>CG (0.10±0.06) (p>0.05) CXCL6 MC1>CG (0.16±0.09) (p>0.05) MC2>CG (0.18±0.10) (p>0.05) CXCL9 MC1>CG (0.17±0.09) (p>0.05) MC2>CG (0.07±0.17) (p>0.05) CXCL10 MC1>CG (0.09±0.12) (p>0.05) MC2>CG (0.13±0.03) (p>0.05) CXCL11 MC1>CG (0.10±0.13) (p>0.05) MC2>CG (0.08±0.12) (p>0.05) CXCL12 MC1>CG (0.10±0.05) (p>0.05)

						MC2>CG (0.10±0.08) (p>0.05) CXCL13 MC1>CG (0.24±0.03) (p>0.05) MC2>CG (0.19±0.14) (p>0.05) CXCL16 MC1>CG (0.16±0.13) (p>0.05) MC2>CG (0.21±0.15) (p>0.05) **Relationship** **In Patient Group** Moderate positive correlations between: Pain severity and IL-6 (r=0.41, **p<0.001***), Pain severity and CCL22 (r=0.41, **p<0.000***) Pain severity and CCL20 (r=0.41, **p<0.000***), Pain severity and CCL27 (r=0.45, **p<0.000***)

						Pain severity and CX3CL1 (r=0.44, **p<0.000***) Pain severity and IL-16 (r=0.49, **p<0.000***) Weak positive correlations between: Pain severity and CCL26 (r=0.31, **p<0.000***) Pain severity and CCL19 (r=0.30, **p=0.001***) Pain severity and CXCL13 (r=0.32, **p<0.000***) Pain severity and MIF (r=0.38, **p<0.000***)
Heffner et al. (2011)	**Patient Group** Chronic LBP n=25 F/M=60%/40% 30.8±11.4 y	MPQ-SF	IL-6	ELISA	Plasma	**Comparison** IL-6 PG>CG (0.1±0.4) (p=0.67)

	Control Group Age and gender matched individuals without chronic pain n=25 F/M=60%/40% 30.8±11.4 y					**Relationship** **In Patient Group** Moderate positive correlation between: Pain severity and IL-6 (r=0.46, **p=0.02***)
Kraychete et al. (2010)	**Patient Group** Individuals with herniated lumbar intervertebral disc disease n=23 F/M=48%/52% 42.8±7.0 y **Control Group** Healthy subjects n=10	NRS	IL-1β IL-6 TNF-α sTNF-R	Quantitative sandwich enzyme immunoassay technique	Serum	**Comparison** IL-1β PG>CG (0.0±0.2) (p=1.00) IL-6 **PG>CG (3.2±2.6) (p=0.01*)** TNF-α **PG>CG (4.0±1.8) (p=0.01*)** sTNF-R PG<CG (9.0±14.0) (p=0.87) **Relationship**

	F/M=40%/60% 39.5±4.5 y					**In Patient Group** No significant correlations between: <u>Pain severity and TNF-α</u> (r=0.28, p=0.18) <u>Pain severity and IL-6</u> (r=0.32, p=0.13)
Sterling et al. (2013)	**Patient Group** Moderate/severe disability WAD n=20 F/M=75%/25% 39.5±9.5 y **Control Group** Asymptomatic controls n=18 F/M=77.7%/22.3% 40.1±9.6 y	PPTs over the C5 and bilateral TA CPT and HPT over mid to lower range of cervical spine	TNF-α IL-1β CRP	ELISA	Serum	**Comparison** <u>TNF-α</u> PG>CG (0.29±0.6) (p>0.05) <u>IL-1β</u> PG>CG (0.4±0.3) (p>0.05) <u>CRP</u> **PG> CG (2.9±0.24) (p=0.01*)** **Relationship** **In Patient Group (Moderate/Severe Disability WAD)** Moderate negative correlation between:

						PPTs at TA and CRP (r=-0.55, **p=0.001***) Moderate positive correlation between: CPT and CRP (r=0.42, **p=0.01***)
Sterling et al. (2016)	**Patient Group** Chronic WAD n=21 F/M=71.4%/28.6% 44.4±11.1 y **Control Group** Asymptomatic controls n=21 F/M=71.4%/28.6% 44±11 y	VAS PPTs over C2 and bilateral TA CPT and HPT over C5 spinous process	TNF-α CRP	ELISA	Serum	**Comparison** TNF-α **PG>CG (1.23±1.14) (p=0.046*)** CRP (mg/l) **PG>CG (0.7±1.45) (p=0.04*)** **Relationship** **In Patient Group** No significant correlations between: PPTs/CPT/HPT and TNF-α (r and p value not mentioned) PPTs/CPT/HPT and CRP (r and p value not mentioned) PPTs at C2 and CRP

						(r = 0.371, p=0.098)
Teodorczyk-Injeyan et al. (2019)	**Patient Group** Chronic LBP n=25 F/M=56%/44% 36.5±11.1 y **Control Group** Asymptomatic participants n=24 F/M=62.5%/37.5% 35.2±10.4 y	VAS	TNF-α IL-1β IL-6 IL-2 IFNγ IL-1RA sTNFR2 IL-10	ELISA	Plasma	**Comparison** <u>TNF-α</u> **PG>CG (993±52) (p=0.0001*)** <u>IL-1β</u> **PG>CG (942±50) (p=0.001*)** <u>IL-6</u> **PG>CG (2,917±147) (p=0.0003*)** <u>IL-2</u> PG<CG (21±03) (p=mentioned as not significant, exact p value not given) <u>IFNγ</u> PG>CG (576±191) (p=mentioned as not significant, exact p value not given) <u>IL-1RA</u> **PG>CG (875±180) (p=0.006*)** <u>sTNFR2</u> **PG>CG (480±21) (p=0.0001*)**

						IL-10 **PG<CG (272±45) (p=0.03*)** **Relationship** **In Patient Group** Very strong positive correlation between: Pain severity and TNF-α (r=0.83, **p<0.001***) Strong positive correlation between: Pain severity and IL-1β (r=0.72, **p<0.001***) No significant correlations between: Pain severity and IL-6 (r=0.22, p value mentioned as not significant, exact p value not given) Pain severity and IL-2 (r=0.29, p value mentioned as not significant, exact p value not given) Pain severity and IFN γ

						(r=0.23, p value mentioned as not significant, exact p value not given)
Teodorczyk-Injeyan et al. (2021)[c]	**Patient Group** Chronic LBP n=25 F/M=56%/44% 36.5±11.1 y **Control Group** Asymptomatic participants n=24 F/M= F/M=62.5%/37.5% 35.2±10.4 y	VAS	TNF-α IL-1β IL-6 IL-1RA IL-2 IFNɣ sTNFR2 IL-10	ELISA	Whole blood	**Comparison** TNF-α **PG>CG (1000) (p= 0.0001*)** IL-1β **PG>CG (1100) (p=0.0001*)** IL-6 **PG>CG (3000) (p=0.003*)** IL-2 PG<CG (21) (p value mentioned as not significant, exact p value not given) IL-1RA **PG>CG (875) (p=0.006*)** IFNγ PG>CG (576) (p value mentioned as not significant, exact p value not given)

[c] This study compared baseline cytokine level between groups. However, baseline IFNγ, IL2 and IL-1RA levels in t the study performed by Teodorczyk-Injeyan et al. (2019). Since cytokine values were shown in the figures, the diffe PG and CG were approximated in the table. Besides, correlation analyses were done on the outcomes post manual t

						sTNFR2 **PG>CG (400) (p=0.0001*)** IL-10 **PG<CG (200) (p=0.03*)** **Relationship** **In Patient Group** No significant correlations between: Pain severity and TNF-α (r=0.13, p value mentioned as not significant, exact p value not given) Pain severity and IL-1β (r=0.17, p value mentioned as not significant, exact p value not given) Pain severity and IL 2 (r=0.09, p value mentioned as not significant, exact p value not given) Pain severity and IFNɣ (r=-0.26, p value mentioned as not significant, exact p value not given)

						Pain severity and IL-6 (r=0.18, p value mentioned as not significant, exact p value not given)
Wang et al. (2008)	**Patient Group** Chronic LBP n=120 F/M= 43.3%/56.7% 46.6±10.9 y **Control Group** n=120 F/M= 43.3%/56.7% 45.4±11.4 y	VAS	TNF-α	Bio-plex	Serum	**Comparison** TNF-α **PG>CG (45.3%)** (p value mentioned as significant, exact p value not given) **Relationship** **In Patient Group** No significant correlation between: The course of pain severity and TNF-α (r and p value not mentioned)
Wang et al. (2010)	**Patient Group** Chronic LBP - depression n=29 F/M=58.62%/41.38%	VAS	TNF-α	Bio-plex	Serum	**Comparison** TNF-α **PG(Chronic LBP - depression)>CG**

	44.69 (24 to 68) y Chronic LBP + depression n=29 F/M=58.62%/41.38% 45.31 (20 to 69) y **Control Group** Healthy controls n=29 F/M= 58.62%/41.38% 40.72 (23 to 66) y					**(2.48) (p=0.004*)** **PG(Chronic LBP + depression)>CG (2.41) (p=0.002*)** **Relationship In Patient Group (Whole Patient Group)** No significant correlation between: <u>Pain severity and TNF-α</u> (exact r and p value not mentioned)

n: number. F: Female. M: Male. IR: Interquartile Range. SD: Standard Deviation. SEM: Standard Error of the Mean. PG: Patient Group. CG: Control Group. y: Years. mg/l: Milligram per Liter. pg/ml: Picogram per Milliliter. LBP: Low Back Pain. WAD: Whiplash Associated Disorder. MC: Modic Changes. MC1: Oedema Type Modic Changes. MC2: Fatty Type Modic Changes. ELISA: Enzyme Linked Sandwich Immunoassay. NRS: Numeric Rating Scale. VAS: Visual Analog Scale. MPQ-SF: McGill Pain Questionnaire-Short Form. PPTs: Pressure Pain Thresholds. CPT: Cold Pain Threshold.

HPT: Heat Pain Threshold. TA: Tibialis Anterior. C2 and C5: Cervical Spinous Process 2 and 5. IL: Interleukin. TNF-α: Tumor Necrosis Factor-α. CXCL5: C-X-C Motif Chemokine Ligand 5. GM-CSF: Granulocyte-macrophage Colony-stimulating Factor. MIF: Macrophage Migration Inhibitory Factor. IFNγ: Interferon Gamma. CRP: C-reactive Protein. IL-RA: IL Receptor Antagonist. sTNFR2: Soluble TNF Receptor Type 2. CCL: C-C Motif Ligand. CX3CL1: C-X3-C Motif Chemokine Ligand 1.

Table 2: EBRO classification of study results and recommendations

	Classification of the study results
A1	Systematic review of at least 2 independent from each other conducted studies of evidence level A2
A2	Randomized double-blinded comparative clinical research of good quality and efficient size
B	Comparative research, but not with all characteristics as mentioned for A2 (this includes patient–control research and cohort research)
C	Non-comparative research
D	Expert opinion
	Level of conclusion per outcome
1	1 A1 or at least 2 independent A2 studies
2	1 A2 or at least 2 independent B studies
3	1 B or C study or conflicting evidence
4	Expert opinion

EBRO, Evidence-Based Richtlijn(guideline)Ontwikkeling

Table 3. The risk of bias results for included studies

Study	Year	Design	Selection				Comparability		Exposure		Total Stars	Risk of bias (low/moderate/ high)	Level of evidence
			1	2	3	4	1a	1b	1	2			
Gjefsen et al(48).	2021	Case control study	*	*	/	*	*	*	*	*	8/9	Low	B
Heffner et al(44).	2011	Case control study	/	*	*	/	*	*	**	*	8/9	Low	B
Kraychete(47) et al.	2010	Case control study	*	*	/	*	*	/	**	*	7/9	Low	B
Sterling et al(23).	2013	Case control study	/	*	*	*	*	*	**	*	8/9	Low	B
Sterling et al(45).	2016	Case control study	/	*	*	*	*	/	**	*	7/9	Low	B
Teodor et al(25).	2019	Case control study	/	*	*	*	*	/	**	*	7/9	Low	B
Teodor et al(50).	2021	Case control study	/	*	/	*	*	/	**	*	6/9	Moderate	B
Wang et al(46).	2008	Case control study	*	*	/	*	*	*	**	*	8/9	Low	B
Wang et al(49).	2010	Case control study	*	*	/	*	*	*	**	*	8/9	Low	B

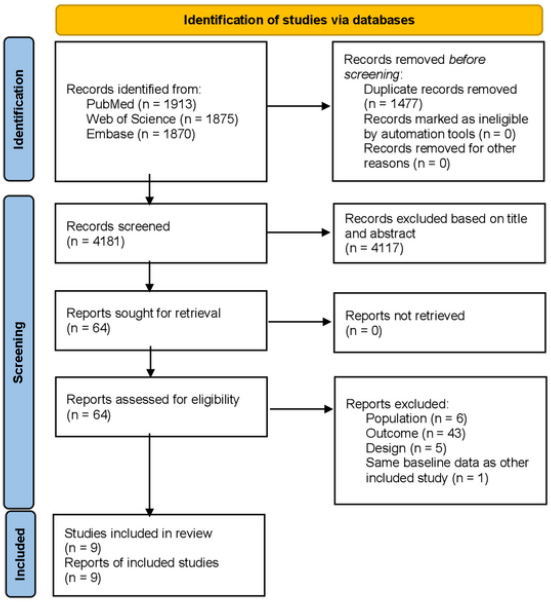
Results of the risk of bias assessment using the Newcastle Ottawa Scale. For "selection", a maximum of 4 stars could be obtained, for "comparability" 2 stars and for "exposure" 3 stars. Total score range was 0-9: low risk of bias was a score of 7-9, moderate risk of bias was a score of 4-6 and high risk of bias was a score of 0-3[36].

Selection: 1. Is the case definition adequate?: a = yes, with independent validation *, b = yes, e.g. record linkage or based on self reports, c = no description; **2.** Representativeness of the cases: a = consecutive or obviously representative series of cases *, b = potential for selection biases or not stated; **3.** Selection of controls: a = community controls *, b = hospital controls , c = no description; **4.** Definition of controls: a = no history of disease (endpoint) *, b = no description of source

Comparability: 1. Comparability of cases and controls on the basis of the design or analysis: a = the study controls for the most important factor (age/gender/body mass index) *, b = the study controls for any additional factor (exercise regularity, caffeine, smoke) *

Exposure[d]: 1. Ascertainment of exposure: a = validated measurement tool **, b = non-validated measurement tool, but the tool is available or described *, c = no description of the measurement tool; **2.** Same method of ascertainment for cases and controls: a = yes *, b = no. **3.** Non-response rate, a = same rate for both groups *, b = non respondents described, c = rate different and no designation

[d]The third item of 'exposure' was not used as all cytokines were derived once for all participants in the included studies.



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

34x49mm (600 x 600 DPI)

APPENDIX 1

PUBMED

((((“Spine”[Mesh] OR “Sacrum”[Mesh] OR “Coccyx”[Mesh] OR “Lumbosacral Region”[Mesh] OR “Back”[Mesh] OR “Neck”[Mesh] OR spine[TIAB] OR spinal[TIAB] OR sacrum[TIAB] OR coccyx[TIAB] OR “lumbosacral region”[TIAB] OR “low back”[TIAB] OR lowback[TIAB] OR back[TIAB] OR neck[TIAB] OR lumb*[TIAB] OR thoracic[TIAB] OR “thoracic back”[TIAB] OR “middle back”[TIAB] OR “upper back”[TIAB] OR cervical[TIAB]) AND (pain[TIAB] OR ache*[TIAB])) OR (“Low Back Pain”[Mesh] OR “Sciatica”[Mesh] OR “Neck Pain”[Mesh] OR “low back pain”[TIAB] OR “low backpain”[TIAB] OR “lower back pain”[TIAB] OR “lower backpain”[TIAB] OR LBP[TIAB] OR sciatica[TIAB] OR “neck pain”[TIAB] OR neckpain[TIAB] OR “spinal pain”[TIAB] OR lumbalgia[TIAB] OR “lumbar pain”[TIAB] OR “lumbar back pain”[TIAB] OR “lumbar spine pain”[TIAB] OR “lumbar region pain”[TIAB] OR backache*[TIAB] OR “back ache*”[TIAB] OR “low back ache*”[TIAB] OR “low backache*”[TIAB] OR lumbago[TIAB] OR dorsalgia[TIAB] OR “discogenic pain”[TIAB] OR “thoracic pain”[TIAB] OR “thoracic back pain”[TIAB] OR “upper back pain”[TIAB] OR cervicalgia[TIAB] OR “cervical pain”[TIAB])) AND (“Chronic Pain”[Mesh] OR chronic pain[TIAB] OR chronic[TIAB])) AND ("Cytokines"[Mesh] OR "Calcitonin Gene-Related Peptide"[Mesh] OR "Substance P"[Mesh] OR "Neuropeptides"[Mesh] OR “Tumor Necrosis Factor-alpha”[Mesh] OR cytokine*[TIAB] OR “calcitonin gene-related peptide*”[TIAB] OR CGRP[TIAB] OR “substance P”[TIAB] OR neuropeptide*[TIAB] OR interleukin*[TIAB] OR interferon*[TIAB] OR chemokine*[TIAB] OR “inflammatory biomarker*”[TIAB] OR inflammatory[TIAB] OR lymphokine*[TIAB] OR “tumor necrosis factor-alpha”[TIAB] OR “tumour necrosis factor-alpha”[TIAB] OR “tumor necrosis factor*”[TIAB] OR “tumour necrosis factor*”[TIAB] OR TNF[TIAB] OR “TNF alpha”[TIAB] OR TNFα[TIAB] OR “erythrocyte sedimentation rate”[TIAB] OR ESR[TIAB]) NOT (rat[TIAB] OR rats[TIAB] OR rodent*[TIAB] OR mice[TIAB] OR mouse[TIAB] OR rabbit*[TIAB] OR animal*[TIAB] OR "Spondylitis, Ankylosing"[Mesh] OR "Arthritis, Rheumatoid"[Mesh] OR "Infections"[Mesh] OR "Spondylarthropathies"[Mesh] OR "Neoplasms"[Mesh])

WEB OF SCIENCE

((((“spine” OR “spinal” OR “sacrum” OR “coccyx” OR “lumbosacral region” OR “low back” OR “lowback” OR “back” OR “neck” OR “lumb*” OR “thoracic” OR “thoracic back” OR “middle back” OR “upper back” OR “cervical”) AND (“pain” OR “ache*”)) OR (“low back pain” OR “low backpain” OR “lower back pain” OR “lower backpain” OR “LBP” OR “sciatica” OR “neck pain” OR “neckpain” OR “spinal pain” OR “lumbalgia” OR “lumbar pain” OR “lumbar back pain” OR “lumbar spine pain” OR “lumbar region pain” OR “backache*” OR “back ache*” OR “low back ache*” OR “low backache*” OR “lumbago” OR “dorsalgia” OR “discogenic pain” OR “thoracic pain” OR “thoracic back pain” OR “upper back pain” OR “cervicalgia” OR “cervical pain”)) AND (“chronic pain” OR “chronic”)) AND (“cytokine*” OR “calcitonin gene-related peptide*” OR “CGRP” OR “substance P” OR “neuropeptide*” OR “interleukin*” OR “interferon*” OR “chemokine*” OR “inflammatory biomarker*” OR “inflammatory” OR “lymphokine*” OR “tumor necrosis factor-alpha” OR “tumour necrosis factor-alpha” OR “tumor necrosis factor*” OR “tumour necrosis factor*” OR “TNF” OR “TNF alpha” OR “TNFα” OR “erythrocyte sedimentation rate” OR “ESR”) NOT (“rat” OR “rats” OR “rodent*” OR “mice” OR “mouse” OR

"rabbit*" OR "animal*" OR "ankylosing spondylitis" OR "rheumatoid arthritis" OR "infection*" OR "spondylarthropathie*" OR "neoplasm*")

EMBASE

((((spine OR spinal OR sacrum OR coccyx OR 'lumbosacral region' OR 'low* back' OR lowback OR back OR neck OR lumb* OR thoracic OR 'thoracic back' OR 'middle back' OR 'upper back' OR cervical) NEAR/3 (pain OR ache*)):ti,ab,kw) OR 'low back pain'/exp OR 'low backpain':ti,ab,kw OR 'lower back pain'/exp OR 'lower backpain':ti,ab,kw OR 'lbp':ti,ab,kw OR 'sciatica'/exp OR 'neck pain'/exp OR neckpain:ti,ab,kw OR 'spinal pain'/exp OR lumbalgia:ti,ab,kw OR 'lumbar pain':ti,ab,kw OR 'lumbar back pain':ti,ab,kw OR 'lumbar spine pain':ti,ab,kw OR 'lumbar region pain':ti,ab,kw OR 'backache'/exp OR 'backache*':ti,ab,kw OR 'back ache*':ti,ab,kw OR 'low back ache*':ti,ab,kw OR 'low backache*':ti,ab,kw OR lumbago:ti,ab,kw OR dorsalgia:ti,ab,kw OR 'discogenic pain'/exp OR 'thoracic pain':ti,ab,kw OR 'thoracic back pain':ti,ab,kw OR 'upper back pain'/exp OR 'cervicalgia'/exp OR 'cervical pain'/exp) AND (chronic:ti,ab,kw OR 'chronic pain'/exp) AND (cytokine*:ti,ab,kw OR 'calcitonin gene related peptide*':ti,ab,kw OR cgrp:ti,ab,kw OR 'substance p'/exp OR neuropeptide*:ti,ab,kw OR interleukin*:ti,ab,kw OR interferon*:ti,ab,kw OR chemokine*:ti,ab,kw OR 'inflammatory biomarker*':ti,ab,kw OR inflammatory:ti,ab,kw OR lymphokine*:ti,ab,kw OR 'tumor necrosis factor-alpha':ti,ab,kw OR 'tumour necrosis factor-alpha':ti,ab,kw OR 'tumor necrosis factor*':ti,ab,kw OR tnf:ti,ab,kw OR 'tnf alpha':ti,ab,kw OR tnfα:ti,ab,kw OR 'erythrocyte sedimentation rate'/exp OR esr:ti,ab,kw) NOT ('rat'/exp OR rats:ti,ab,kw OR rodent*:ti,ab,kw OR 'mice':ti,ab,kw OR 'mouse'/exp OR rabbit*:ti,ab,kw OR animal*:ti,ab,kw OR 'ankylosing spondylitis'/exp OR 'rheumatoid arthritis'/exp OR 'infection'/exp OR 'spondyloarthropathy"/exp OR 'neoplasm'/exp) NOT 'conference abstract':it