The role of inflammation in sciatica: the contradictory effect of macrophages

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Chapter 2

The contradictory effect of macrophage related cytokine expression in lumbar disc herniation: a systematic review

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"Luck is what happens when preparation meets opportunity." (Seneca)

Abstract

Purpose
Sciatic symptoms due to lumbar disc herniation are likely to be not solely caused by mechanical compression of the nerve root, but also by pain inducing elements from inflammatory processes. Key components in the inflammatory reaction are M1- and M2 macrophages, the M1 type being associated with pro-inflammatory processes and M2 with anti-inflammatory-processes.

Methods
The present systematic review summarizes all literature on associations between M1 and M2 macrophages and their related inflammation factors, and pain symptoms in lumbar disc herniation. Literature search was performed using an optimally sensitive search string. Studies were selected for inclusion by means of predefined in- and exclusion criteria and subsequently graded for risk of bias. A total of 14 studies were included. Overall risk of bias was moderate (8/14), three studies had a high- and three a low risk of bias.

Results
Regarding M1 related cytokines, high levels of TNF-α, TNFR1, IL-6, IL-8, IFN- γ, were all associated high VAS scores. In contrast, high levels of TNFR2 was associated with lower VAS scores. Moreover, no associations were found for IL-1a and IL-1β. Results regarding M2 related cytokines revealed the opposite: high levels of both IL-4 and IL-10 were associated with lower VAS scores. No associations were established for TGF-β. Moreover, presence of macrophages (CD68) was negatively associated with VAS scores.

Conclusion
while M1 related pro-inflammatory cytokines worsen pain symptoms, M2 related anti-inflammatory cytokines alleviate pain symptoms, Nevertheless, present evidence is limited and further research on the underlying pathophysiological mechanism in sciatica is required.

Introduction

At present, one of the most prevalent causes for physical disability is herniation of the intervertebral disc. When a disc herniates, it often causes compression of the nerve root, which leads to a radiating pain alongside the dermatome, often referred to as sciatica [1, 2]. At first sight, the cause for sciatica seems purely mechanical, but the observation of nerve root compression due to disc herniation in 20-76% of asymptomatic cases, suggests that mechanical compression is not the only factor at play [3-5].

Over the past two decades several researchers suggested that inflammation of the nerve root and/or disc plays a significant role in sciatica [4, 6-8]. It is hypothesized that nucleus pulposus (NP) material that herniates into the epidural space, induces a foreign-body reaction that involves macrophage infiltration [8]. These macrophages are not only suggested to play a role in resorption of herniated disc material [4, 5, 9] but also to, at least partially, play a role in inducing an inflammatory response. This could in turn cause pain. The different roles of macrophages are reflected in the contradictory views of experts and physicians. Any discrepancy in these roles may be dependent on the type of macrophage present in the disc material.

Present literature distinguishes M1 and M2 macrophages [10]. An M1 macrophage can differentiate from a monocyte if stimulated by Lipopolysaccharide (LPS) or Interferon-gamma (IFN-γ) or tumor necrosis factor (TNF), or granulocyte macrophage colony-stimulating factor (GM-CSF). M1 produces pro-inflammatory cytokines and products such as IL-1, IL-1α, IL-1β, IL-6, IL-8, IL-12, IL-18, IL-23 IL-27, TNF-α, and Bone morphogenic protein 2 (BMP-2) [10-12], the main focus of this type is microbicidal activity [11], and its expression profile is associated with exacerbation of pain symptoms [13]. On the contrary, if a monocyte is stimulated by IL-4, IL-10, IL-13, glucocorticoids or macrophage colony-stimulating factor (M-CSF), it differentiates into a M2 macrophage. This alternative type of macrophage excretes anti-inflammatory cytokines such as IL-1Ra, IL-10, and transforming growth factor-beta (TGF-β) [11], which are involved in multiple functions such as tissue repair and remodelling [10, 11, 14]. In contrast to the effect of M1 macrophages, M2 is believed to alleviate pain symptoms through resorption of herniated disc material [15]. During most inflammation processes, M1 or M2 macrophages occur sequentially [11]. However, depending on the disease and genetic predisposition, their ratios may vary widely [11]. Despite extensive research in the field of sciatica, the role of M1 and M2 macrophages remains to be elucidated. A better understanding of these processes could lead to improved prognostics and personalized treatment. The aim of the present study is therefore to systematically review all literature concerning the role of macrophages and their related pro- and anti-inflammatory cytokines and factors in lumbar disc herniation patients suffering from sciatic symptoms.

Materials and methods

Inclusion criteria
Studies with patients suffering from sciatica were to be included if the study analyzed the correlation between the presence of macrophages or their related cytokines and/or excretion factors, as verified by serum, CSF or disc material samples, and quantitatively measured clinical outcome parameters.

Search and selection
The electronic databases Medline (from 1960), EMBASE (from 1947) and Web of Science (from 1960) where searched up until February 2018. A search string in order to systematically explore all studies that included presence of macrophages or their related cytokines and factors was constructed and adapted per database. Eligible studies were selected on title and abstract by two independent review authors (ND and GL), with consensus meeting and referee (CVL) available, according to PRISMA guidelines. If the abstract alone did not provide sufficient information, the full paper was assessed. Afterwards, citation tracking was performed and further eligible studies were acquired.

The search strategy comprised strings for sciatica and macrophages, granulocyte macrophage colony stimulating factor (GM-CSF), interferon gamma (IFN-γ), tumor necrosis factor-α (TNF-α), tumor necrosis factor-beta (TNF-b), bone morphogenic protein 2 (BMP-2), tumor growth factor beta TGF-β, IL-1, IL-1α, IL-1Ra, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-18, IL-27. No restrictions on publication date were made and all articles were to be fully published in English. Conference proceedings were excluded. The included studies had to consist of a minimum of 10 patients suffering from acute, sub-acute or chronic pain in lumbar disc herniation. No restrictions were made on follow-up. Furthermore, studies were only included if clinical outcome was measured reporting a pain scale, the Straight Leg Raising test (SLR) or the Oswestry Disability Index (ODI). Pains scales are the Visual Analogue Scale (VAS) leg pain or a comparable scale like the Visual Rating Scale (VRS) for pain or the Numeric Rating Scale (NRS) for pain. The ODI scale evaluates functionality focussing on the leg and back. Additionally, studies were excluded if they failed to specify which cytokine or excretion factor was present, or if the cytokine or excretion factor was not measured in serum, CSF or disc material.

Quality assessment
Two authors (ND, GL) reviewed the methodological quality of all included articles individually, using an adjusted version of the scoring criteria by Cowley (supplementary Table 1) [16], in which a maximum of 10 points can be given. Risk of bias was deemed low if the Cowley score was ≥ 8, moderate between 5-7 and High risk of bias with a score of 0-4. Differences in quality assessment between the two reviewers were justified in a consensus meeting.

Data extraction
The primary outcome of the present study comprises associations between macrophage related parameters and pain symptoms. From each study, basic information was gathered concerning authors (sponsoring, affiliation), methods (study design, sample size and type of analysis), patients (source population, inclusion criteria, exclusion criteria, baseline characteristics, and diagnostic characteristics), treatments (interventions), outcome variables and results.

Results

Study selection

The search in the PubMed database yielded 305 results, EMBASE yielded 585 and Web of Science yielded 272 results. In total 1162 references were obtained. After removal of duplicates, 755 remained. After abstract and full text screening, fourteen articles met inclusion criteria. Subsequently, citation tracking was applied, which did not lead to any additional findings. Hence the final number of included articles was fourteen (Figure 1).

Risk of bias Assessment

Of the fourteen studies, three were scored to have low, eight to have moderate and three to have high risk of bias. Regarding individual categories: First, risk of population bias was generally moderate, all studies reported age and sex, whereas only five studies provided specific and explicit in- and exclusion criteria for lumbar disc herniation [13, 17-20]. Second, selection bias could be ruled out in six studies and was also regarded as generally moderate [13, 17, 18, 20-22]. Third, risk of outcome bias was generally considered moderate as well; most studies clearly defined outcome measures except for Schistad et al (2014). Here, the authors described IL-8 measurements in the method section but failed to elaborate on them in the results [18]. If studies failed to test parametric test assumptions for VAS scores, no points for statistical analyses were awarded [13, 18, 20, 21, 23-26]. None of the studies described clinical evaluation as independent of the treating physician. Fourth, the selected studies showed a low risk of attrition bias, as all of the fourteen selected articles were prospective studies. Eight studies had a follow-up period longer than six months in all described studies [13, 18, 20, 22-25, 27]. Finally, only five studies explicitly reported to have no conflict of interest [18-20, 23, 24]. An overview of the risk of bias scores is provided in Table 1.

Data extraction – macrophages and related cytokines and -factors

The reported methods of measuring macrophages, cytokines and excretion factors varied widely. Some authors histologically described their presence in nucleus pulposus material that was taken out during surgery, others looked at presence of macrophages and accompanying inflammatory factors in blood or cerebral spinal fluid. Moreover, the choice of parameter studied varied widely. Not all the parameters that are associated with M1 and M2 macrophages were reported in the studies that were eligible for this review. The histological parameter for macrophages, CD68 (surface marker), was reported in a few studies. M1 related factors that were encountered are: interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor receptor 1 / 2 (TNFR1 / TNFR2), and M1 related cytokines that were reported are: IL-1α, IL-1β, IL-6, IL-8, IL-12. M2 related factors that were reported in the articles are: tumor growth factor-beta, (TGF- β), and the M2 related cytokines that were reported are: IL-4 and IL-10.

Association between macrophage marker and pain

Two out of the four studies on CD68 [22, 27], found a negative association with pain scores during follow-up [22, 27-29] and one study found a negative association with Straight Leg Raising test [29], which means that patients with higher CD68 (macrophage) expression, had less pain and lower SLR scores.

Association between pro-inflammatory factors (M1) and clinical outcome (Table 2)
In studies examining the association of TNF-α with VAS pain or SLR or ODI, five out of six studies found a positive association [17, 19, 20, 24-26], which means that patients with higher TNF-α levels had higher pain scores. The only study that did not find such association had a high risk of bias [26]. In most studies, TNF-α association with clinical parameters was evaluated at baseline, but in follow-up data the association remained present [20, 25]. Both studies on TNFR1, a TNF-α receptor, found a positive association with pain scores, one at baseline [24], and both during follow-up [23, 25]. In contrast, the same studies found that TNFR2 had a negative association with pain scores, which means that patients with high levels of TNFR2 reported lower pain scores [23, 25]. Three out of five studies on IL-6 found a positive association with pain scores and ODI [13, 18, 19, 23, 26]. One of the studies that did not find an association had high risk of bias [26]. The other study that did not demonstrate an association between pain and IL-6 determined the IL-6 concentration in disc material, while the three studies that did find a positive correlation examined IL-6 in serum.

Two out of four studies on IL-8 found a positive association with pain scores and SLR [13, 17, 19, 26], ; one of these studies examined IL-8 in disc material [17], and the other in serum [13]. Two of four studies on IL-8 found no association with pain scores, SLR or ODI, one of these studies (high risk of bias [26]) examined IL-8 in CSF and the other study examined IL-8 in serum [19]. All three studies on IL-1β showed no association with pain scores and SLR [24, 26]. The IL-1beta expression was examined in disc material, CSF and serum. The study on IL-1a found no association with pain scores [17].

Two studies examined the association of IFN- γ with pain or SLR and did not find an association [21, 26]. However, one of these studies had a high risk of bias [26], and the other examined the association with several VAS cut-off scores, thereby inducing outcome bias [21].

Association between anti-inflammatory factors (M2) and clinical outcomes (Table 2)
Two studies examining IL-10 demonstrated different results [17, 19]. One study did not demonstrate an association with pain score or SLR [17]. The other study demonstrated a negative association: in patients with higher pain scores or ODI, the concentration of IL-10 in serum was lower as compared to patients with a low pain score or ODI [19].

One of the two studies on IL-4 found a negative association with pain scores at 12 months follow up [20]. The other study demonstrated no association with VAS or ODI [19]. The study on TGF-β found no association with pain or SLR [17].

Discussion

The present systematic review established associations between the presence of macrophages and their pro-, and anti-inflammatory cytokines with pain and/or disability in lumbar disc herniation. Because of the heterogeneity in outcome measures and data presentation, only a qualitative analysis was performed. Also, methodological quality of the studies varied widely. For the M1 related factors, literature presented moderate evidence for associations between high pain scores and high levels of TNF- α, TNFR1 and IL-6, limited evidence for associations between high pain scores and high levels of IL-8, no associations between pain related outcome measures and IL-1α , IL-1β or IFN-gamma, and moderate evidence for an association between low pain scores and high levels of TNFR2. In contrast, for the M2 related factors, evidence with moderate quality was found for an association between low pain scores and high levels of IL-4, limited evidence for an association between low pain scores and high levels of IL-10, no association was found with TGF-beta.

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Associations between inflammation markers and clinical outcomes

Primary outcome measures were sciatic symptoms, and cytokines and other macrophage related parameters. The present review specifically included studies that measured pain symptoms expressed by VAS- and/or ODI scores and/or SLR. These clinical symptoms were subsequently correlated to inflammatory parameters. The tissue or fluid in which these parameters were examined varied among studies. Some studies looked at mRNA [17, 23-25] or protein expression patterns [21, 23-25] in the nucleus pulposus or annulus fibrosis, while others measured concentrations of cytokines in CSF [26] or blood [13, 18-20, 26]. Other studies examined macrophage infiltration histologically [22, 27-29] in herniated disc tissue. The comparability of the studies included for review is therefore rather limited and these differences could hence have confounded our results.

The most convincing positive association between pain-related outcome measures and M1 excretion factors was provided by studies on TNF-α. Five out of six studies examining TNF-α expression patterns indicate that higher pain scores associate with a higher protein- and mRNA expression intensity in the nucleus pulposus [17, 23, 25], and with higher serum concentrations [19, 20, 26]. Of these studies, one had low risk of bias [20] and four had moderate risk of bias [19]. Only the study with lowest quality could not establish an association between inflammation and clinical outcome [26]. These findings suggest that lowering TNF-α levels with drugs such as TNF-α blockers may alleviate sciatic symptoms. The efficacy of TNF-α inhibitors has recently been reviewed by Williams et al. (2013) and Wang et al. (2014) [30, 31]. Both studies concluded that evidence supporting anti-inflammatory agents, as a means of therapy in sciatica, is present but not yet convincing. Combining these results with the findings from the present systematic literature review, we suggest the lack of convincing evidence of the previous reviews may be explained by the fact that TNF-α levels vary among patients, which diminishes the overall efficacy of TNF-α inhibitors when given to all sciatica patients instead of only the subpopulation with actual high TNF-α levels. In order to properly evaluate the efficacy of these inhibitors, an RCT that only includes patients with high TNF-α levels is needed.

Previous systematic reviews

Our findings are in agreement with previous systematic literature reviews and meta-analyses. Goupille et al. (1998) were the first to identify inflammatory mediators in disc herniation, and to suggest that inflammation is involved in sciatic symptom development [32]. However, the literature of 1998 failed to provide evidence for the suggested involvement. The present literature review is the first to specifically outline all established associations between different cytokines and other macrophage related inflammatory factors involved in sciatica on clinical outcomes.

Clinical implications and recommendations

Our current treatment for radicular pain is conservative care for a period of 8-12 weeks, if symptoms persist however, a surgical intervention is offered [33]. Unfortunately, even after surgery, some patients do not recover satisfactorily. As of today, it remains unclear why nerve decompression does not lead to pain reduction. Recent evidence by Lama et al. showed that in some discs cartilage fragments were found, and that in these discs only little swelling and infiltration of immune cells was present [34]. The authors suggested that cartilage fragments could interfere with the resorption process, which could be an explanation for the abovementioned variety in recovery rate. Presence of cartilage fragments in the intervertebral disc may be caused by a defect in the endplate. Moreover, endplate defects are known to increase permeability of the intervertebral disc, thereby increasing the risk of infection in the endplate. Pre-clinical findings suggest that when an inflammatory response is induced in mice discs by bacteria, increasing nerve outgrowth from the dorsal root ganglion into the disc consequently occurs [35]. Nerve outgrowth could subsequently lead to sensitization of the disc, thereby facilitating pain symptoms. Currently, the evidence for this theory is still limited and further exploration of these concepts is required.

Nevertheless, recent clinical studies are in line with the abovementioned findings and show that discs of some lumbar disc herniation patients were infected with Propionibacterium acnes or Staphylococcus Epidermidis [36, 37]. In addition, Dudli (2017) induced herniated disc samples with P. Acnes and found that 6 out 10 discs responded with excretion of pro-inflammatory cytokines [38]. This inflammatory response was associated with endplate defects on MRI, more often described as Modic changes [39, 40]. Likewise, others have associated the presence of Modic changes with less recovery after surgery [41], and a chronic inflammatory response. However, other factors than bacterial infection may induce a shift of macrophage differentiation towards M1 macrophages and induce the excretion of pro-inflammatory cytokines, like for instance endplate pathology, or an innate defect in macrophage differentiation [42].

Even though the abovementioned findings are still inconclusive; it has inspired the following theory (Figure 2): Patients that suffer from disc inflammation without any complicating factors such as bacterial infection, endplate pathology or immune defects will show a response that is dominated by M2 macrophages, which excrete anti-inflammatory cytokines such as IL-4 and IL-10. This type of inflammation will likely induce a resorption process and may thus be beneficial to the patient. On the contrary, if patients show an inflammation reaction of the disc with one of the abovementioned complicating factors, the reaction is likely to be mediated by M1 macrophages and pro-inflammatory products such as IL-6, IL-8, TNF-α and IFN-γ, originating from the disc and/or endplate. This type of inflammation, will likely aggravate the symptoms of the patients and could be recognised by presence of Modic changes on MRI.

As mentioned earlier, current evidence for this theory is limited, and the underlying pathofysiological mechanism should be further explored before this theory can be confirmed.. Hence we recommend that future studies focus on exploring the possible causes of macrophage differentiation towards M1 and the excretion of pro-inflammatory cytokines, and how the different causes affect symptoms of sciatica.

Moreover, despite the fact that inflammation has shown to play a significant role in sciatica, the impact of mechanical compression should not be forgotten. Inflammation only occurs in a fraction of the patients [43], indicating that for many, the pain has a mechanical origin that can be alliviated through decompression [33]. Nonetheless, in many cases both the mechanical and the inflammatory component will attribute to the sciatic symptoms. For such caseses, it remains difficult to define how much each component contributes to the experienced pain, since excision of the hernia will not only releave the compression, but also a part of inflammation, which was present in the herniated part. Likewise, anti-inflammatory agents are usualy studied in a population that contains both patients with and without inflammation. This attenuates the reported effect of the anti-inflammatory agents and prevents us from finding the real effect size of inflammation. This stresses the importance of taking inflammation into consideration in a study population. By doing so, steps can be made in delivering a more personalized treatment that takes the heterogeneity of sciatica into account.

Limitations of our study

Because this review only assessed Embase, Pubmed and Web of Science, relevant studies that are hidden elsewhere might have been missed. Furthermore, the criteria used for risk of bias assessment included arbitrary cut of points, such as: duration of follow up, exclusion criteria and validity of statistical analysis (Supplementary Table 1). Therefore, the risk of bias scores given to the evaluated studies may alter in a different review, which could lead to different qualities of evidence for the found associations. At last, this review was only able to include fourteen papers, and thus only has a limited amount of evidence to draw conclusions from. This illustrates that more studies on this topic are needed in order to validate the results from previous trials and further explore the role of inflammation in sciatica.

Conclusion

Cytokines excreted during the process of disc herniation in sciatica seem to have a contradictionary effect on pain symptoms. Pro-inflammatory cytokines worsen pain symptoms, while anti-inflammatory cytokines alleviate pain symptoms.

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Conflict of interest

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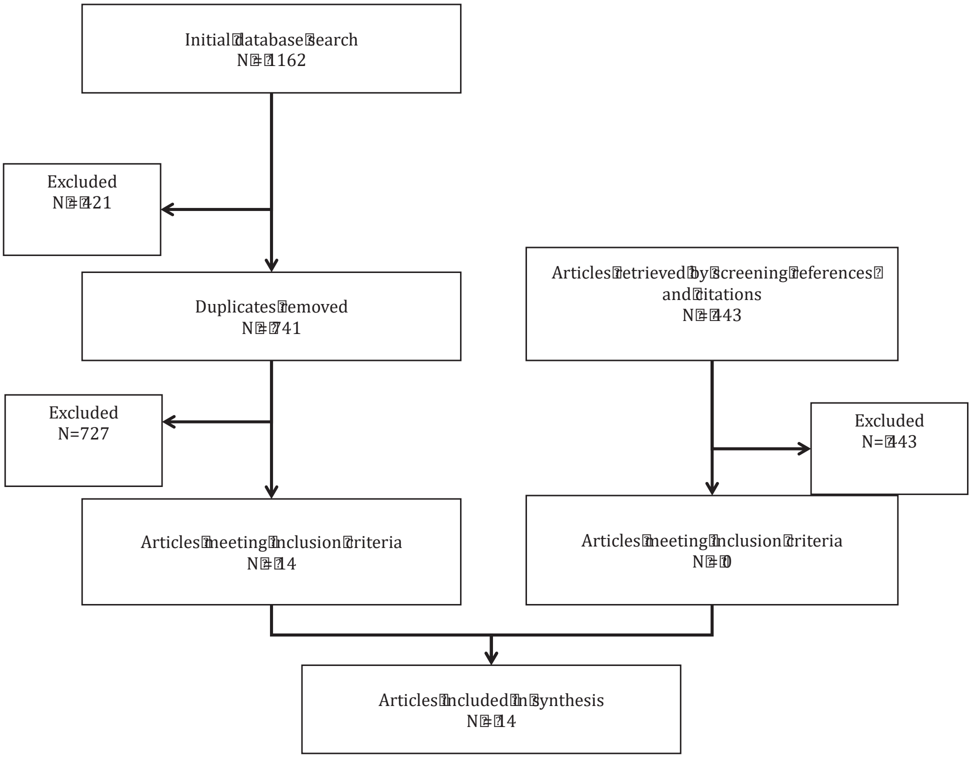
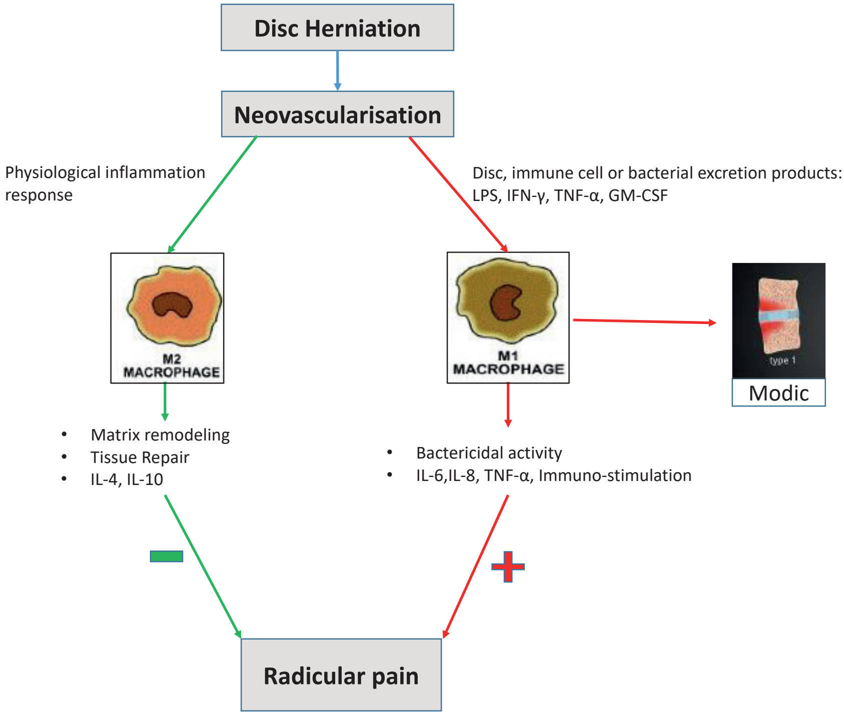
Appendix

Figure 1. Flow chart of the database search.



From the initial database search 1175 articles were obtained, of which 741 remained after removal of duplicates. 14 articles from the initial search met the inclusion criteria. After citation tracking, 443 articles were found. After the duplicates were removed and the inclusion criteria were applied, no articles were used from the citation tracking.

Figure 2. Proposed hypothesis



Disc inflammation with a M2 macrophage dominance is beneficial for the pain symptoms, whereas a dominance of M2 macrophages, likely induced by a bacterial infection and recognizable by Modic changes, will aggravate the pain symptoms.

Table 1. Overview of the Risk of Bias.
This table shows the overall risk of bias and the individual categories; Population bias (P), selection bias (S), outcome bias (o), attrition bias (A) and conflict of interest.

Study (year of publication)	**Score on risk of bias scale**	**P**	**S**	**O**	**A**	**I**
Ahn et al. 2002	6/10	3/3	1/1	1/3	1/2	0/1
Andrade et al. 2011	6/10	1/3	0/1	2/3	2/2	1/1
Andrade et al. 2013	6/10	1/3	0/1	2/3	2/2	1/1
Andrade et al. 2016	5/10	2/3	0/1	1/3	2/2	0/1
Brisby et al. 2002	4/10	1/3	0/1	2/3	1/2	0/1
Cuellar et al. 2000	5/10	2/3	1/1	1/3	1/2	0/1
Pedersen et al. 2015	8/10	3/3	1/1	2/3	2/2	0/1
Rothoerl et al. 1998 Acta Neurochirurgia	3/10	1/3	0/1	1/3	1/2	0/1
Rothoerl et al. 2002	5/10	1/3	0/1	2/3	2/2	0/1
Rothoerl et al. 1998 Spine	3/10	1/3	0/1	1/3	1/2	0/1
Schistad et al. 2014	8/10	3/3	1/1	1/3	2/2	1/1
Wang et al. 2016	6/10	3/3	0/1	1/3	1/2	1/1
Woertgen et al. 2000	5/10	1/3	1/1	1/3	2/2	0/1
Zu et al. 2016	8/10	3/3	1/1	2/3	2/2	1/1

Table 2 . An overview of the evidence on the associations between macrophage infiltration, M1-related or M2-related factors and the clinical outcomes.

Study	Cohort size (n)	Risk of bias	Specification	Clinical parameter	Association
CD68 macrophage marker					
Rothoerl et al. [28] (Acta Neurochirurgica)	179	3/10	Disc infiltration	Pre-op pain (VAS) Pre-op SLR	No No
Rothoerl et al. [27]	177	6/10	Disc infiltration	Pre-op pain (VAS) 7-month FU pain (VAS) Pre-op SLR	No Neg No
Rothoerl et al. [29] (Spine)	44	4/10	Disc infiltration	Pre-op pain (VAS) Pre-op SLR	No Neg
Woertgen et al. [22]	79	5/10	Disc infiltration	Pre-op SLR Pre-op pain (VAS) 6-month FU pain (VAS)	No No Neg
M1 expression profile					
TNF-α					
Ahn et al. [17]	23	5/10	mRNA expression NP	Pre-op pain (VAS) Pre-op SLR	Pos Pos
Andrade et al. [25]	15	8/10	Protein expression NP/AF mRNA expression NP/AF	Pre-op pain (VAS) 6-week FU pain (VAS) 12-month FU pain (VAS) Pre-op pain (VAS) 6-week FU pain (VAS) 12-month FU pain (VAS)	No/no Pos/no Pos/no No/no Pos/no Pos/no
Andrade et al. [24]	20	5/10	Protein expression mRNA expression	Pre-op pain (VAS) 6-month FU pain (VAS) VAS < 3.5 vs > 3.5	Pos Pos Pos
Brisby et al. [26]	39	3/10	CSF and serum concentration	Pre-op pain (VAS) Pre-op SLR	No No
Wang et al. [19]	138	6/10	Serum concentration	Pre-op pain (VAS) Pre-op ODI	Pos Pos
Zu et al. [20]	262	9/10	Serum concentration at baseline Serum concentration at 1-month FU Serum concentration at 12-month FU	12-month FU pain (VAS) 12-month FU pain (VAS) 12-month FU pain (VAS) 12-month FU ODI	Pos Pos Pos Pos

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Study	Cohort size (n)	Risk of bias	Specification	Clinical parameter	Association
TNFR1					
Andrade et al. [25]	15	8/10	Protein expression NP/AF	Pre-op pain (VAS) 6-week FU pain (VAS) 12-month FU pain (VAS)	Pos/pos Pos/no Pos/no
Andrade et al. [23]	20	5/10	Protein expression mRNA expression	Pre-op pain (VAS) 6-month FU pain (VAS) Pre-op pain VAS	Pos Pos Pos
TNFR2					
Andrade et al. [25]	15	8/10	Protein expression NP/AF	Pre-op pain (VAS) 6-week FU pain (VAS) 12-month FU pain (VAS)	No/neg No/neg No/neg
Andrade et al. [23]	20	5/10	Protein expression mRNA expression	Pre-op pain (VAS) 6-month FU pain (VAS) Pre-op pain VAS	Neg Neg Neg
IL-6					
Andrade et al. [24]	15	8/10	mRNA expression NP/AF protein expression NP/AF	Pre-op pain (VAS) 6-week FU pain (VAS) 12-month FU pain (VAS) Pre-op pain (VAS) 6-week FU pain (VAS) 12-month FU pain (VAS)	No/no No/no No/no No/no No/no No/no
Brisby et al. [26]	39	3/10	CSF and serum concentration	Pre-op pain (VAS) Pre-op SLR	No No
Pedersen et al. [13]	127	8/10	Serum concentration	12-month FU pain (VAS)	Pos
Schistad et al. [18]	54	7/10	Serum concentration	ODI baseline—1 year FU 12-month FU back pain (VAS) 12-month FU leg pain (VAS)	Pos Pos Pos
Wang et al. [19]	138	6/10	Serum concentration	Pre-op pain (VAS) Pre-op ODI	Pos Pos
IL-8					
Ahn et al. [17]	23	5/10	mRNA expression NP	Pre-op pain (VAS) Pre-op SLR	Pos Pos
Brisby et al. [26]	39	3/10	CSF concentration	Pre-op pain (VAS) Pre-op SLR	No No

Study	Cohort size (n)	Risk of bias	Specification	Clinical parameter	Association
Pedersen et al. [13]	127	8/10	Serum concentration	12-month FU pain (VAS)	Pos
Wang et al. [19]	138	6/10	Serum concentration	Pre-op pain (VAS) Pre-op ODI	No No
IL-1β					
Ahn et al. [17]	23	5/10	mRNA expression NP	Pre-op pain (VAS) Pre-op SLR	No No
Andrade et al. [24]	15	8/10	mRNA expression NP/AF protein expression NP/AF	Pre-op pain (VAS) 6-week FU pain (VAS) 12-month FU pain (VAS) Pre-op pain (VAS) 6-week FU pain (VAS) 12-month FU pain (VAS)	No/no No/no No/no No/no No/no No/no
Brisby et al. [26]	39	3/10	CSF and serum concentration	Pre-op pain (VAS) Pre-op SLR	No No
IL-1α					
Ahn et al. [17]	23	5/10	mRNA expression NP	Pre-op pain (VAS) Pre-op SLR	No No
IFN-γ					
Brisby et al. [26]	39	3/10	CSF and serum concentration	Pre-op pain (VAS) Pre-op SLR	No No
Cuellar et al. [21]	24	5/10	Protein expression NP	Pre-op VAS	Pos
M2 expression profile					
IL-10					
Ahn et al. [17]	23	5/10	mRNA expression NP	Pre-op pain (VAS) Pre-op SLR	No No
Wang et al. [19]	138	6/10	Serum concentration	Pre-op pain (VAS) ODI	Neg Neg
IL-4					
Wang et al. [19]	138	6/10	Serum concentration	Pre-op pain (VAS) ODI	No No
Zu et al. [20]	262	9/10	Serum concentration at baseline Serum concentration at 1-month FU Serum concentration at 12-month FU	12-month FU pain (VAS) 12-month FU pain (VAS) 12-month FU pain (VAS) 12-month FU ODI	Neg Neg Neg No

Study	Cohort size (n)	Risk of bias	Specification	Clinical parameter	Association
TGF-β					
Ahn et al. [17]	23	5/10	mRNA expression NP	Pre-op pain (VAS) Pre-op SLR	No No

‘No’ infers that no association was established. If an association is indicated as ‘positive’, the inflammatory factor positively association to the clinical outcome parameter (as an example: ‘Andrade 2011’: if the TNFR1 expression was higher, patients experienced more postoperative pain). ‘Negative’ infers a negative association between the inflammatory and clinical outcome parameter in question. As an example: in Andrade 2011, high TNFR2 expression associated with less postoperative pain. The negative associations indicate a protective effect of an inflammatory reaction on pain or disability. Of these fourteen studies, three used a correlation test instead of an association test: Brisby (2002), Cuellar (2010), Wang (2016). NP = nucleus pulposus, AF = annulus fibrosis, FU = follow-up, Pre-op = pre-operative, Pos = positive association, Neg = negative association