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

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Chapter 8

Conclusion and discussion

"Blessed are the hearts that can bend; they shall never be broken." (Albert Camus)

This study has investigated, how macrophage infiltration of the herniated disc influences the clinical and radiological outcomes in sciatica. More specifically, the association between alterations in macrophage differentiation and inflammatory protein expression and radiological outcomes was investigated. For this purpose, herniated lumbar disc samples harvested during discectomy surgery were examined. This allowed us to study the grade of inflammation using immunohistochemistry and proteomics and relate these findings to clinical and radiological outcomes, both at baseline and at follow-up. Through evaluation by immunohistochemistry we learned that the extent of macrophage infiltration is related to the type of disc herniation, with extruded disc material containing more cells than bulging disc material. Furthermore, we found that a higher number of macrophages present in the lesioned disc material associates with less disc herniation on MRI one year post surgery, which we interpreted as the macrophages contributing to the resorption process. In terms of clinical outcomes, a remarkable discrepancy in the effect of patients with Modic Changes (MC) and those without MC was demonstrated. In patients with MC, more infiltrated macrophages resulted in a less satisfactory recovery. On the other hand, in patients without MC, more infiltrated macrophages resulted in a more satisfactory recovery.

Furthermore, in an additional immunohistochemistry study, we found that this discrepancy in effect of macrophage infiltration between patients with and without MC seems to be attributed to a difference in macrophage differentiation: patients with MC have a considerably lower fraction of CD163+ positive macrophages, which is a marker for tissue remodeling. In addition, in the proteomic study, examination of protein expression in nucleus pulposus samples revealed that patients with MC showed a decrease in detoxification of reactive oxygen species (ROS) compared to patients without MC. ROS is a marker for inflammation and tissue damage, and a decrease in the detoxification of ROS illustrates that there exists dysfunction of the immune response. This is in line with the immunohistochemistry results that showed a lower percentage of CD163+M2 macrophages in MC+ patients, and with our earlier findings that show a slower recovery of MC patients with inflammatory cells. At last, we found that a large % of the identified macrophages did not stain positively for any of the M1(iNOS/CD40) or M2 (CD163/Arg1) markers. Whether this is due to an absence of differentiation or because the limited number of included markers could not cover all macrophage differentiations, remains a question for further study.

Since only surgical samples were examined to answer the research question, the abovementioned conclusions may be limited in their generalizability. Surgery is only indicated for severe sciatica cases, hence the distribution of inflammation and the importance of its effect on clinical outcomes described in this study may be different from that in a population with mild complaints. These limitations emphasize the importance of careful evaluation of the results. Hence, in the paragraphs below, we will not only discuss the study findings but also advantages and disadvantages of the methodologies used in this study. In addition, the implications and future perspectives of the results of this study will also be discussed.

Macrophage differentiation

M1 and M2 macrophages, an effective dichotomization?

Macrophage differentiation or polarization has recently emerged as a topic of major interest in multiple fields [1]. This complex phenomenon is influenced by numerous factors, and results in a wide variety of macrophage phenotypes with according behaviors and expression profiles including: antimicrobial activity, immune cell activation, tumor resistance, allergy, immune modulation and tissue remodeling [2]. It seems an oversimplification to polarize these phenotypes as M1 (pro-inflammatory/anti-microbial) and M2 (anti-inflammatory / tissue remodeling), yet it is still broadly used in the literature. For this study, the M1/M2 dichotomization was also used with the following justification. Sciatic pain is a complex phenomenon that is influenced by many variables amongst which: the type of disc herniation, the extent and type of macrophage infiltration, the inflammatory status of the endplate, and the severity of the nerve compression.

When it comes to understanding such complex clinical phenomena, this M1/M2 macrophage oversimplification may help to understand the bigger picture of the interactions between these many variables. Once research has provided a more solid basis for the theories on the role of macrophages that we have presented, the nuances in activation profiles become more important. For now, we feel confident to use the M1/M2 dichotomization, as long as we clearly define which expression factors/markers were used to identify M1 and M2 macrophages, in accordance with current expert guidelines [1].

Implications on disc herniations

A disc herniation is a traumatic event, in which other immune stimulators should not play a big role. Hence, at the time of surgery, the expected macrophage differentiation response would be towards M2, and is aimed at phagocytizing the herniated material without further immune recruitment and excessive damage. This effect was demonstrated in chapter 3, in which evidence was provided for disc resorption due to macrophages, accompanied by the observation that macrophage infiltration resulted in earlier recovery (chapter 2 and 4), and by the finding that the majority of macrophages was CD163 positive (M2 marker; chapter 6). In addition, evidence from the literature presented in chapter 2 showed that the cytokines (IL-4 and IL-10), which are expressed by M2 macrophages, also associate with a quicker recovery and relieve of pain symptoms. Collectively, this suggests that for these cases, macrophage infiltration is a beneficial event, and should not be inhibited, but rather stimulated with therapeutic strategies.

Unfortunately, macrophage infiltration is not beneficial in all cases. In chapter 4, it is shown that macrophage infiltration is not beneficial for patients that demonstrate MC in the adjacent vertebra. This is likely caused by an alteration in macrophage differentiation due to an alternative pro inflammatory environment, in which a treatment focusing on inhibition of inflammation may be more beneficial. Taken together, insight in the pathological process in herniated discs could impact the choice of treatment.

Therapeutic implications

Our current treatment approach for sciatica patients is conservative care for at least 8-12 weeks, during which the majority of patients recovers spontaneously [3]. However, in the subgroup of patients with persisting symptoms, surgery can be considered. Identifying this subgroup in an early disease stage, could advance the decision to operate, which would save them from an ineffective prolonged conservative approach. Based on our findings, assessing the inflammation status may provide such knowledge in an early stage. Because a hernia is likely to resolve spontaneously in the case of M2 infiltration, knowing whether M2 macrophages are present in the first weeks after the onset of symptoms could lead to a decision to treat conservatively for a longer period of time. Furthermore, if an absence of M2 macrophage infiltration can be assessed in the first weeks, spontaneous resorption is less likely, thereby making early surgery the preferential option. Saving patients from an ineffective conservative approach would not only reduce the duration of the disease, but also the cost for society since these patients can return to work quickly. When measuring macrophage differentiation in an early phase, it is vital to wait at least 5 days before M2 infiltration is assessed [4], as the inflammation profile on the first day of a normal wound healing response is mainly dominated by M1 macrophages [5]. In a normal wound healing process, M1 macrophages slowly differentiate into M2 during the following days [6]. Whether macrophage differentiation also occurs this quickly in the poorly vascularized intervertebral disc still has to be unraveled.

By contrast, if a M1 dominant infiltration is seen after the first week, neither decompression surgery nor conservative care may be the best option. For these patients, it is important to know if bacteria are present. If so, an antibiotic treatment approach can be considered. By contrast, in those with a M1 dominant infiltrate without a bacterial infection, the problem is more likely to be of degenerative nature, and arthrodesis or perhaps future regenerative disc therapies may be the preferable approach.

Alternatively, it could be that M1 and M2 differentiation is only a part of the picture, and that we should look at a more basic level and therefore consider vascular supply. With a poor vascular system, macrophages, other cells and essential nutrients may be unable to reach the herniated disc, which prevents a healthy wound healing response and thus disc resorption. For example, if a patient with poor blood supply due to hypercholesteremia, diabetes and/or extensive smoking has a small wound, the wound may take months to heal. In the worst case scenario, the wound may never fully heal. For a patient with a healthy vascular supply, such a wound can heal within a couple of weeks. Since resorption of a herniated disc can be seen as a wound in an area with little vascular supply, it is reasonable that in this location, a patients vascular status is even more important for recovery. Following this line of reasoning, recent studies showed that high LDL levels were a risk factor for disc herniations [7, 8], but whether it also contributes to the duration of symptoms still has to be elucidated. Nevertheless, the important role of macrophages and the likely role of the vascular system in the resorption of disc herniations indicate that we should look at a herniated disc as a wound, and focus our treatment strategies on optimization of the wound healing process. However, before we start developing new treatments, we first need to develop new biomarkers that help to identify which patients have altered macrophage differentiation and/or a poor vascular supply in the disc.

MRI features as biomarkers

Modic Changes as a biomarker

In this thesis, the predictive value of observed MC on MRI was investigated in multiple ways: In chapter 3 we did not find the presence of MC to be of influence on the association between the rate of disc resorption and macrophage infiltration. However, it should be noted that the original study population used in chapter 3 was insufficiently powered for this sub analysis. Hence, we recommend repeating this study with a larger sample size before a final conclusion can be drawn.

Regarding the proposed alternative macrophage differentiation in MC patients: in chapter 6 we illustrated that in MC patients, macrophages expressed CD163 (M2 marker) less frequently. Since M2 macrophages play an important role in down-regulation of the inflammation response, lower levels of CD163 are proposed to correspond to higher levels of pro-inflammatory cytokines in the local environment such as TNF-α [9], which in turn result in an increase in pain symptoms and a slower recovery rate as illustrated in chapter 2. In line with the decrease in M2 macrophage marker CD163, an increase in M1 markers was to be expected in MC patients. However, we did not find an increase in M1 markers CD40 or iNOS. Both M1 markers were only expressed in a small fraction of the total macrophage population (medians <3%), whereas CD163 was expressed in a moderate fraction (median 21%). With these markers, a large percentage of macrophages is neither marked as M1 nor as M2, which insinuates that additional markers are required to make a more complete categorization of macrophage differentiation. Hence additional M1 markers such as CD63, CD80 or CD86 could still reveal higher M1 differentiation status in MC patients [10, 11].

The underlying cause for the alternative macrophage differentiation found in patients with MC remains unknown. Literature suggests that this alternative differentiation may be caused by the presence of anaerobic bacteria in the disc in patients with MC in the adjacent vertebra, mostly Propionibacterium Acnes [12]. Alternatively, a degenerated disc or endplate with high levels of apoptosis and necrosis could also lead to alternative macrophage differentiation, without intervention of bacteria. Processes of apoptosis and necrosis, with or without anaerobic bacteria, are associated with an increase in oxidative stress, and high levels of reactive oxygen species (ROS) [13], which induce cell damage and result in an upregulation of pro-inflammatory gene expression and macrophage differentiation towards M1 [13]. In a normal inflammation response, high levels of ROS are eventually cleared by a process called 'detoxification', in which M2 macrophages play a pivotal role through expression of antioxidant enzymes such as catalase [14, 15]. In MC patients however, we found a decrease in the detoxification of ROS (chapter 7), which is likely leading to higher levels of ROS. This is in accordance with the low levels of M2 (CD163) macrophages demonstrated in patients with MC (chapter 6). Whether a lower number of M2 macrophages is the result of an increase in ROS or vice versa remains to be elucidated. Nevertheless, the two findings combined could very well explain the slow recovery of MC patients with inflammation that was demonstrated in chapter 3.

Taken together, MC may thus function as an indicator for a pro-inflammatory reaction aimed at either a bacterial infection or at a degenerated environment. However, not all patients with MC have a bacterial infection nor do they all have macrophage infiltration, and if they do, the distribution of M1 and M2 still varies from patient to patient. Hence, we must conclude that MC on its own is not a viable biomarker to predict a pro-inflammatory reaction/bacterial infection that could explain the persisting sciatica symptoms.

A possible reason why MC by itself are not so specific for a proinflammatory response, is the existence of multiple types of MC, of which type 1 and type 2 are the most prevalent. Even though MC type 1 and MC type 2 show similarities when their molecular signature is examined, they still differ in gene and protein expression profile [16]. MC type 1 is considered the active/acute inflammation type, and can be recognized by marrow-edema, whereas MC type 2 is more regarded as a chronic inflammation with pro-osteoclastic and fatty changes [16-18]. Hence, MC type 1 seems to be more relevant for identifying a pro inflammatory state in patients with a disc herniation. Unfortunately, solely focusing on MC type 1 is not always a viable option as MC type 1 and 2 are often mischaracterized.

Reliability of reporting the type of MC

The presence of MC can be reliably identified on MRI, as was illustrated by the substantial inter-observer agreement in chapter 3-5. Nevertheless, the distribution of MC type 1 and MC type 2 varies greatly amongst studies. In our study, < 10% of the patients with MC were characterized as MC type 1, whereas others have reported percentages as high as 78% [19]. Since MC type 1 is believed to be an acute process and MC type 2 a chronic inflammatory process, differences in distribution of MC type 1 vs MC type 2 could potentially be attributed to the timing of the MRI. However, the average duration of symptoms till baseline in our study was +/- 9 weeks, whereas the average duration of symptoms till baseline in the study from Matsuyama et al. who found 78% MC type 1 was +/-19 weeks [19]. Of course, when evaluating duration of symptom data, the influence of recall bias should not be underestimated. This makes the quality of the data less reliable and thus the conclusions drawn from it. Nevertheless, it seems that the duration of symptoms is not the only factor of influence on the distribution of MC type 1 and 2.

MC type 1 is identified by marrow edema, which is hyperintense on T2 and hypointense on T1, whereas MC type 2 is identified by fatty marrow changes, which is hyperintense on T1 and T2. The sensitivity of identifying hypo and hyperintensities on T1 depends on different MRI settings. For instance, when a low field strength is used (<1.0T), marrow inhomogeneities are less pronounced, which makes MC type 1 easier and MC type 2 harder to identify compared to 1.5T magnets. When comparing a 0.3T to a 1.5T scanner, the 1.5T resulted in twice as much identification of MC type 2 and reduced the identification of MC type 1 to 25-33% [20]. Moreover, an MRI with 3.0T or higher have superior conventional fat suppression, which increases the identification of MC type 1 as it reduces the chance that marrow edema is overlooked. In addition, another technique to increase the detection rate of MC type 1 is the usage of a fat suppression sequence. To what extent this increases the detection rate of MC type 1 is not properly studied [21]. Nevertheless, in order to prevent this overestimation of MC type 2 and underestimation of MC type 1, we recommend using a 3.0T scanner and or fat suppression techniques for future studies.

Mischaracterizing the MC type makes it challenging to distinguish the reported differences and associations regarding MC type 1 and MC type 2. since we used a 1.5T scanner and no fat suppression, our prevalence of MC type 1 is likely to be underestimated, whereas the prevalence of MC type 2 is probably overestimated. Thus, our conclusions regarding the altered macrophage behavior in patients with MC type 2 may actually be for MC type 2 and MC type 1.

With regards to future projects, it could be that accurately distinguishing MC type 1 from MC type 2, would result in MC type 1 being a useful predictor for an inflammatory environment due to a bacterial infection, and may consequently influence therapeutic strategies. Alternatively, as MC type 1 is characterized by marrow edema, it could simply only be a predictor for a poor vascular status with vascular malformation, which in turn could be associated with a higher occurrence of anaerobic bacterial infections and alternative differentiation of the infiltrated macrophages. In that case, MC type 1 would not be a sensitive predictor and may thus not be useful on its own.

By contrast, MC type 2 could also be the type to focus on, with the rationale that the inflammation response in MC type 2 is chronic, which could signify that the body was unable to clear a bacterial infection and that the infiltrated macrophages have remained in an ineffective M1 state and cause further damage. Such a process is unlikely to resolve spontaneously, which the acute MC type 1 response might.

In conclusion, this study showed that MC have a predictive value in the characterization of the inflammatory profile of herniated discs. However, its sensitivity and specificity seem to be insufficient to use in clinical practice on its own. Until the problem of mischaracterization of the MC types is solved, MC should only be used as an additional predicter combined with other parameters such as disc inflammation. Further, caution should be taken with comparing different types of MC and sticking to absence vs presence of MC may be the preferred approach if MRI parameters are not optimal and mischaracterization is likely.

Disc herniation features as a biomarker

Alternatively, considering that the presence of MC (both type 1 and 2) are associated with endplate avulsion, the limited predictive value could indicate that MC is a confounder for endplate avulsion. An avulsed endplate would provide a proper way of entry for both bacteria as well as macrophages to enter and could perhaps be a more accurate predictor. In chapter 7 we examined the effect of both endplate pathologies on the proteomic signature and showed that endplate avulsion was associated with an increase in the detoxification of ROS and an increase in immune activation and coagulation. Together, this illustrates a healthy traumatic immune response, making it less likely that a bacterial infection is present. Because, in contrast with endplate avulsion, MC showed a decrease in detoxification of ROS and immune system, it is unlikely that MC is a confounder for endplate avulsion. Instead, these two parameters should be treated independently. Nonetheless, still little is known regarding the effect of endplate avulsion on disc inflammation and recovery, and more research is needed in order to assess whether and how this parameter should be used.

A different feature to focus on is the extent of the disc herniation. In chapter 3, we found that extrusion is a predictor for macrophage infiltration and disc resorption after one year. However, these associations with disc extrusion were only moderate, which indicates that extrusion cannot be used as a reliable biomarker for disc resorption as their sensitivity and specificity would be too low to base clinical decisions on. Nonetheless, it could prove to be of adequate predictive value when combined with other predictive parameters.

Alternative Imaging techniques

A different promising technique that could be of use is Magnetic resonance spectroscopy (MRS), which is an imaging technique that can characterize metabolic features of the disc in vivo. For example, increases in Alanine and Lactate have been associated with increased pain symptoms and is regarded as a marker for inflammation [22]. However, whether MRS can also distinguish bacterial infection from macrophage infiltration and M1 from M2 differentiation, still has to be elucidated.

Studying inflammation in the disc

Immunohistochemistry: a qualitative, quantitative and illustrative technique
Of course, finding predictors for a pro-inflammatory environment such as MC is only useful if such an environment can be identified reliably. A suitable way to get information regarding the inflammatory status of the herniated disc is immunohistochemistry of the disc material. Immunohistochemical staining revealed that macrophages were seen abundantly in NP tissue but not in the cartilage endplate tissue, which was frequently present in the sample. Further, it was demonstrated that macrophages cluster around the edges of the collected tissue samples, and were barely seen in the central parts, which suggests that macrophages work their way inwards. When this last observation is combined with the finding that they were more prominent in extruded compared to bulging discs, it suggests that the observed macrophages were not resident macrophages, but that their presence is rather a consequence of the herniated part of the disc being exposed to the systemic circulation.

Immunohistochemistry: Distinguishing NP cells from macrophages
When compared to a purely quantitative technique such as ELISA or Western blotting, which provide a value for the protein expression of interest, immunohistochemistry can provide additional information that would otherwise have been overlooked. Besides, for many markers, there are multiple cells that express them: in chapter 7 it is shown that many resident cells of the disc (NP cells and chondrocytes) also express macrophage markers CD68, CD40, iNOS and Arg1, but that macrophages can be identified based on their morphology. Hence by using immunohistochemistry, quantification of protein expression could be restricted to macrophages, whereas with a purely quantitative technique, protein expression of all cell types would have been included and may have provided inadequate conclusions.

Interestingly, the expression intensity and expression by different cell types may vary greatly upon the specific brand of the antibody, buffer and pretreatment of the material. During the study, multiple antibodies for CD40 and CD163 were tested with various buffers and pretreatments and discarded if the results were unsatisfactory. This eventually resulted in the antibody panel that was used in chapter 7. This also shows, that if previous reports mention that a certain marker is specific for macrophages, this claim first has to be verified when not the exact same material and lab settings are used. In chapters 3 to 5, only CD68 was used because this marker is abundant in macrophages, and the samples used for this study were old, which may have compromised their quality and hence the identifiable protein expression that can be stained positively. For CD68, this resulted in reduced but still considerable amount of positively stained macrophages, thereby making CD68 useable as a parameter. Despite, the exact cell counts were likely to be less accurate, thus in order to maintain the validity of the data, macrophage infiltration was analyzed as a categorical variable with categories on a logarithmic scale. A positive consequence of the reduced staining intensity of CD68, was that NP cells and chondrocytes were not stained positively. Unfortunately, we were unable to include more advanced analysis with M1 or M2 distributions on this old tissue, as their corresponding antibodies were barely positive.

Immunohistochemistry: Cell counting

The downside of immunohistochemistry is that after finishing the lab procedure, there is no quantitative number that can be used directly. In order to quantify protein expression, a positive surface count or positive cell count can be performed. This is a labor intensive task that leaves room for subjectivity as it can be challenging to distinguish positive from negative cells when they are faintly stained. Hence it is advisable to use 2 raters in order to improve the quality of the data. Alternatively, in order to reduce the labor required and improve the objectivity and reproducibility of the cell counts, an algorithm can be developed that will count all tissue above a certain intensity threshold. The possible downside of the algorithm is that it does not distinguish between a positive macrophage and a positive chondrocyte, and that it tends to count double folded tissue as positive. Although some additional measures can be taken to combat these downsides, it does not work for all antibodies and tissues. In such cases, or if one is interested in the expression of a large number of proteins, other techniques such as proteomics can be a more attractive option.

Proteomics, a technique for biomarker screening

A different laboratory method that can be used to quantify protein expression is Proteomics, using Liquid Chromatography – Mass Spectrometry (LC-MS). Proteomics is a relatively new technique that quantifies all expressed proteins in the selected tissue. In short, it does so through first dissecting all proteins of the tissue into peptides, which are then possessed in the Mass Spectrometry machine, where all peptides are sorted based on their mass to charge ratio. Each mass to charge ratio corresponds to a peptide which is subsequently translated to its corresponding protein based on a database [23]. The interesting advantage of this exploratory technique is that it quantifies a large number of proteins at once, hence it is very suitable to select interesting targets for further research in an exploratory phase, and it is easy to retrieve information regarding the correlation between sets of proteins.

With regard to macrophage differentiation in disc herniations, proteomic seems remarkably beneficious when it comes to the identification of new biomarkers. At this point, we know that macrophage infiltration and their differentiation profile play a role in the etiology of disc herniations. However, how exactly the differentiation profile functions and what proper targets could be for future therapy still has to be elucidated. For example, we hypothesize that patients with MC tend to have more bacteria present, which in turn will likely alter macrophage differentiation from M2 towards M1. In chapter 6, we found that not all M1 markers (CD40 and iNOS) are suitable to study this question. This is exactly where proteomics proves its benefit. Instead of trying new markers one by one until one suffices, proteomics will reveal the full spectrum of proteins from which a proper candidate can be chosen. Besides, this spectrum of proteins is not limited to macrophage markers, so by using this approach, other important areas of interest can also be brought into view. This way, we can shed light on candidate targets and markers, instead of staying in the dark.

As of today, our knowledge on how MC relate to the inflammatory profile of the disc is still extremely limited, and even less is known for endplate avulsion, as this concept was only recently discovered by Rajasekaran et al. in 2013 [24]. Hence an exploratory technique seemed the most suitable for investigating these phenomena. Providing an overview of all abundantly expressed proteins in the disc could aid in selecting specific targets for future studies. Also, by focusing on the differences in expression profiles instead of individual markers, we could verify the immunohistochemistry results that illustrated a detrimental effect of inflammation in patients with MC.

As this methodology provides a tremendous amount of data, it can be very overwhelming, which is why we used a bioinformatic approach to translate protein expression into pathways. By identifying alterations in pathways, which were a downregulation of detoxification of reactive oxygen species (ROS), complement system and immune system for MC, and an increase in coagulation and detoxification of ROS for EPA, we have provided a general overview of relevant pathways and identified new specific fields of interest for future research. For example, future research can focus on important players that modify these pathways. And since, LC-MS is unable to detect proteins in very low quantity, alterations of other relevant proteins in these pathways can be unravelled with different, more sensitive techniques.

Before the suggested personalized treatment strategies could be implemented in clinical practice, highly sensitive and specific biomarkers would have to be developed. In chapter 5, we evaluated gadolinium enhancement as a tool to assess macrophage infiltration, but found it to be inadequate. Hence, new non-invasive biomarkers are required that identify bacterial infection, macrophage infiltration and differentiation in the herniated disc.

Serology

Considering the infiltrated macrophages in the disc originate from the systemic circulation, analyzing patients' serum can provide information on the inflammatory status of the disc. In chapter two, this study discussed the serum levels IFN-γ, TNF-α, IL-6, IL-8, IL-1β, IL-10, and IL-4, some of which correlated with an increase in pain symptoms (TNF-α/IL-8/IL-6), while others resulted in decrease of pain symptoms (IL-4/IL-10). This indicates that even though the site of inflammation is small and relatively poorly vascularized, serology can still be used to search for biomarkers. Hence, we suggest future studies should focus on finding serological markers that correlate to bacterial infection and M1/M2 macrophage infiltration. This way, patients could receive personalized treatment strategies at minimal diagnostic cost.

Drug treatment strategies

Acquiring knowledge on the presence and type of macrophage infiltration will not only influence the choice to treat conservatively or surgically, but may also lead to new personalized drug treatment strategies. Knowing that a macrophage response will accelerate the resorption process, a possible new treatment approach is to stimulate macrophage proliferation and differentiation, locally, in the herniated disc. A possible example would be an epidural injection with Macrophage Colony Stimulating Factor (CSF-1), which can stimulate local proliferation, increases the cell number and skews macrophages towards M2 [25]. Besides, administration of CSF-1 has shown to accelerate wound healing in vitro [26]. Short term oral treatment with CSF-1 has been successfully administrated to patients with immune cell depletion due to chemotherapy, but side effects were not reported [27]. whether CSF-1 is also tolerated in non-immune-depleted radiculopathy patients remains unknown. If systemic therapy is not well tolerated due to systemic side effects, epidural injections can be considered.

Stimulating macrophage infiltration is likely to be beneficial in traumatic herniations, but in a disc with a M1 dominated response due to an infection or degenerative changes, increasing the number of macrophages may not have the desired effect, as these macrophages are more likely to differentiate towards M1. In these patients, an alternative strategy would be to skew macrophage differentiation towards M2, preferably combined with antibiotics in case of an infection. Possible ways of doing so are with steroid injections (methylprednisolone), which have shown to decrease M1 and increase M2 differentiation in a mice model with lung injury [28]. Furthermore, since M1 activation resulted in an increased expression of COX-2, but a decreased expression of COX-1, whereas M2 activation resulted in an increased expression of COX-1 [29]. Moreover, inhibition of COX-2 in an in vitro mice model resulted in an inhibition of macrophage differentiation towards M1 and promoted polarization towards M2 instead [30]. Hence, a different approach would be to use selective COX-2 inhibitors, such as Etoricoxib or Celecoxib.

A big advantage of these widely prescribed drugs is that they can safely be taken orally. However, the question remains whether oral administration will result in a sufficient concentration in the herniated disc, as the efficacy of these drugs for sciatic symptoms are still up for debate. Thus, we recommend studying different ways of administration and their corresponding efficacy, and targeting sciatica patients with a M1 dominant infiltrate in the herniated disc.

For those patients in which bacteria are present in the intervertebral disc, an antibiotic treatment strategy should be developed depending on the pathogen. With a pooled prevalence of 56.4%, the most common bacteria is Propionibacterium acnes [12], other reported bacteria are Staphylococci epidermidis, Corynebacterium propinquum, Pepto streptococci, Staphylococci aureus, Bacillus Cereus and Citrobacter braaki/freundi [31]. All these bacteria do well in an anaerobic environment [32-34], which makes a degenerated disc or endplate an ideal place to grow. Specific antibiotic strategies would have to be tailored to the resistance and susceptibility profile of each bacteria, although most (P. Acnes, S. epidermidis, S. Aureus and Pepto streptococci) will likely react to amoxicillin–clavulanate [35]. The efficacy of antibiotic treatment was demonstrated by Albert et al (2013) [36], who performed a RCT and randomized patients between conventional care and administering amoxicillin/clavulanate in a dose of 500/125 mg three times a day, at 8h intervals, for 100 days to long term radiculopathy patients with MC type 1. She and her group demonstrated a significant clinical improvement in the antibiotic treated group compared to control patients. Up till now this experiment was not repeated and the rationale for the outcome remains to be unraveled.

The other 3 bacteria are often resistant to Amoxicillin-clavulanate, but have reported susceptibility to Ceftriaxone (Corynebacterium) [37], imipenem/meropenem (Citrobacter) [32] and ciprofloxacin/ vancomycin (Bacillus) [38]. Nevertheless, the susceptibility of these bacteria should first be assessed using bacteria cultured from intervertebral discs, as these could be slightly different strains and their resistance profile could be altered. At last, duration and dosages of the antibiotics would have to be increased since the disc has a very limited vascular supply and is more dependent on diffusion. The extent of this increase depends on the pharmacokinetics of the respective antibiotics. Alternatively, the possibilities for local administration during surgery, or through epidural injection could also be explored.

To our knowledge, this study is the first to focus on macrophage differentiation and to integrate it with clinical outcomes and other inflammation parameters of the disc. This study has provided some insight on how macrophages play a crucial role in the course of disc resorption and its clinical implications, but has also created many new questions. These questions fuel the inspiration for new interesting studies that shall greatly improve our understanding of the pathology and will likely enlighten innovative ways to treat sciatica. For example, future research should focus on specifying the role of macrophages in relation to a bacterial infection and the inflammatory environment of MC, not to forget how these interactions may affect the clinical outcomes and whether they require different treatment strategies. On that account, a new study protocol was developed in chapter 9, which aims to answer these questions and search for biomarkers to integrate this material in clinical practice: the EIMICOR study (Effect of Infection, Modic and Inflammation on Clinical Outcomes in Radiculopathy). With the results of this new study protocol, a better understanding of the role of inflammation in sciatica will be created through evaluation of the disc material and blood samples. Bacterial status, M1/M2 differentiation and proteomic signature of the herniated disc and MC status of the adjacent endplate will all be associated with clinical outcomes. Subsequently, biomarkers that can predict the inflammation status of the disc will explored in blood. This way, differences in pathology between herniated discs can be determined and patient subgroups can be characterized with the help of non-invasive biomarkers. By doing so, we can work towards personalized treatment strategies and improve the quality of life in patients suffering with sciatica.

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