

Junjie Lin

Papel da Osteopontina na Patologia da Coluna - uma revisão sistemática

Role of Osteopontin in Spinal Pathologies - a systematic review

Janeiro, 2021

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Role of Osteopontin in Spinal Pathologies: a systematic review

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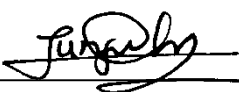
Janeiro, 2021

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Faculdade de Medicina da Universidade do Porto, 08/02/2021

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DESIGNAÇÃO DA ÁREA DO PROJECTO

Neurocirurgia – Patologia Raquidiana

TÍTULO DISSERTAÇÃO

Role of Osteopontin in Spinal Pathologies

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Prof. Doutor Paulo Pereira

COORIENTADOR (se aplicável)

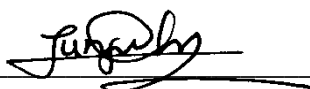
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DEDICATÓRIA

À minha família, o meu maior e sincero obrigado pelo apoio absoluto, pela fé inabalável em mim, pelas oportunidades, pelas experiências, pela paciência, pelos ensinamentos da vida, pelos bons e maus momentos, pelos valores que me norteiam hoje e no futuro, pelo encorajamento e por fim, porque lhes devo muito daquilo que sou.

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Valete, Fratres!

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Role of Osteopontin in Spine Pathologies: a systematic review

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LIST OF ABBREVIATIONS

$\alpha\text{v}\beta\text{3}$ - integrin binding motif

AGEs - advanced glycation end products

AIS - adolescent idiopathic scoliosis

ALP - alkaline phosphatase

CD44 - cluster of differentiation 44

CSM - cervical spondylotic myelopathy

CSNs - neonatal corticospinal neurons

DRG - dorsal root ganglion

ECM - extracellular matrix

EF - epidural fibrosis

G - guanine

HIV - human immunodeficiency virus

IGF-1 - insulin-like growth factor 1

IVD - intervertebral disc

IVDD - intervertebral disc degeneration

LFC - ligamentum flavum cells

MTB - *Mycobacterium tuberculosis*

OLF - ossification of ligamentum flavum

OPLL - ossification of posterior longitudinal ligament

OPN - osteopontin

OPN-SV - osteopontin splice variants

PLL - posterior longitudinal ligament

PRISMA - preferred reporting items for systematic reviews and meta-analysis statement

PTMs - post-translational modifications

SCI - spinal cord injuries

SNP - single nucleotide polymorphisms

STB - spinal tuberculosis

T - thymine

ttw - twy mice

ABSTRACT

Objective: Worldwide, spinal diseases are an increasingly prevalent health problem. Deeper knowledge about the pathophysiology of this complex group of diseases could improve current prevention strategies and treatment options. Osteopontin (OPN) is a multifunctional protein with great interest among the scientific community in these diseases. The aim of this revision is to appraise the role of osteopontin and its expression in different spinal pathologies.

Methods: A systematic literature review search was conducted on PubMed and Scopus databases for published studies until 6th October 2020, concerning the role of osteopontin in spinal pathologies.

Results: Among the 674 studies retrieved, 79 were selected for further analysis. A correlation between the expression of OPN and the presence or clinical severity of 8 different spinal pathologies of diverse etiologies was found and analyzed in this review.

Conclusion: OPN appears to have a broad spectrum of functions in spinal pathologies, mainly as an inflammatory cytokine or as a bone metabolism regulator. In the future, OPN might be a potential target for development of novel therapies or work as a prognostic tool in the onset and progression of spinal diseases.

Key-words: osteopontin, spine, intervertebral disc degeneration, spinal diseases, spinal cord injuries;

INTRODUCTION

Spinal diseases represent a wide and heterogeneous spectrum of conditions, with increasing prevalence and consequent upscaling economic and social burden worldwide^{1,2}.

Deeper knowledge about the pathophysiology of these diseases could potentially improve current prevention strategies and treatment possibilities. Osteopontin is a pleiotropic protein, already recognized for its significant roles in several systemic functions and disorders. In the last decade, at least 500 articles per year related to OPN were indexed in PubMed, revealing this molecule as a target for research, with promising results and new insights about its function.

Osteopontin and its Functions

OPN is an extracellular matrix protein of bone which is highly phosphorylated and negatively charged acidic protein, due to the presence of aspartic and glutamic acid, and serine in its composition³. Besides this polyaspartic acid motif, where the hydroxyapatite and calcium binding occurs, it also has 2 heparin binding zones (for CD44) and an integrin receptor binding domain, which interacts with different ubiquitously expressed cell surface receptors (**figure 1**).

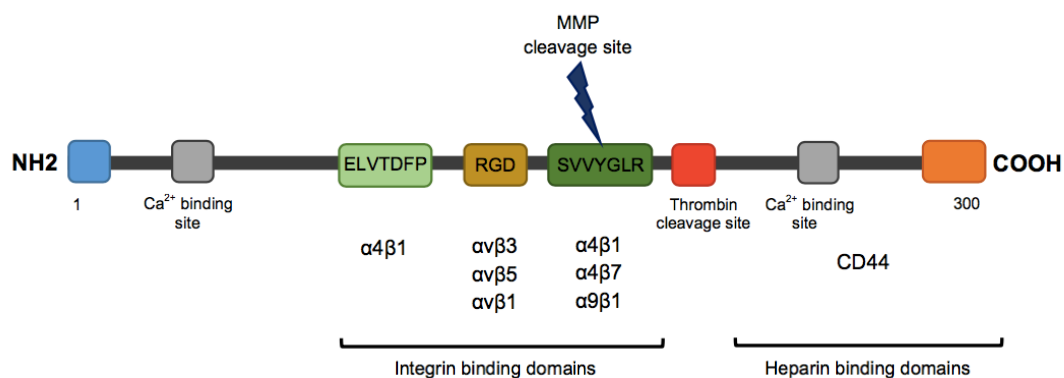


Figure 1. Structural form of OPN and interactions. Adapted from Boggio E, et al. Journal of Immunology Research, 2016⁴

Apart from being abundantly expressed in a variety of tissues (kidney, salivary glands, vascular tissues, pancreas, bone, lung, brain cells, immune cells, urinary and reproductive tracts, among others)⁵, OPN has also been recognized as a multifunctional protein, with an important biological role in several systems (**figure 2**). Its (over)expression influences physiological and pathological processes.

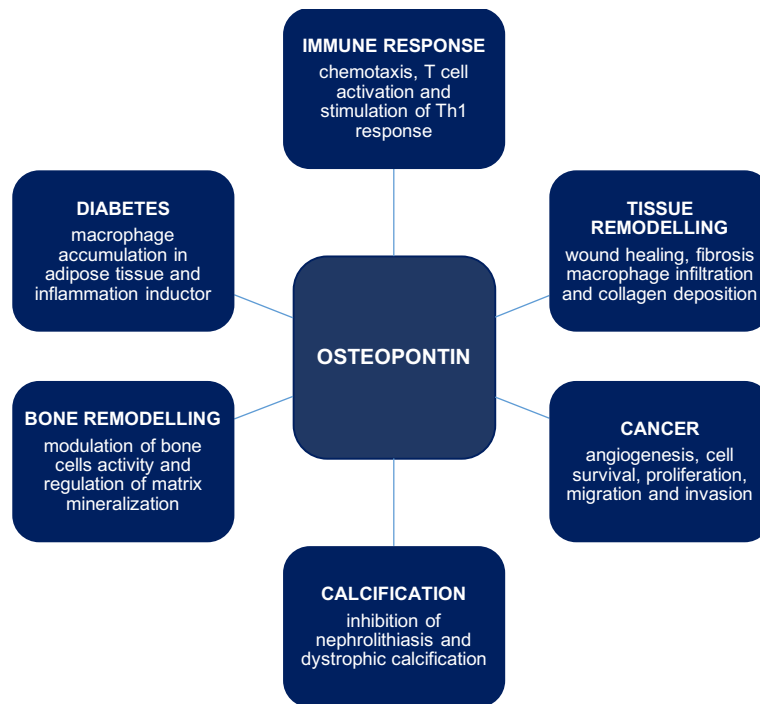


Figure 2. Major functions of OPN

Epigenetic processes also seem to influence OPN activity. For instance, OPN splice variants (OPN-SV) have currently attracted attention after the detection of different combinations of these forms in malignant carcinomas^{6,7}. Additionally, OPN polymorphisms have been related to increased susceptibility and severity of some diseases, such as spinal tuberculosis or cervical spondylotic myelopathy^{8,9}.

The extension of the post-translational modifications (PTMs)^{10,11} can also modulate OPN activity and tissue specificity. Within these, phosphorylation stands as the most reviewed one, as it influences the adhesion and bone resorption by osteoclasts, migration of malignant cells, and regulation of mineralization and crystallization^{12,13}.

Previous researches have emphasized OPN's role in biomineralization. In mineralized tissues, OPN is synthesized and released by osteoblasts and osteoclasts¹⁴. This protein has a particular distribution in bones, more specifically on the cement lines in remodelling bone, and at *laminae limitantes* at bone surfaces. In these microenvironments, OPN mediates osteoblast cell adhesion and guides early calcification events. The binding of osteoclasts onto the bone surfaces may also be promoted by OPN, due to higher abundance in regions of the bone surface where osteoclasts are anchored, by increasing mineralized collagen matrix¹⁵.

Osteopontin in spinal pathologies

Being a pleiotropic and multifunctional protein, new insights about OPN's function in several physiologic and pathologic processes are published continually, including in spinal diseases. Multiple studies describe the relation of OPN with conditions of different etiologies, reporting that its expression influences both the disease course and outcome^{8,9,16-33}.

OPN has been implicated in several spinal diseases, such as intervertebral disc degeneration, cervical spondylotic myelopathy and scoliosis^{8,16-22,28-32}. Its role in the immune response might be crucial in some infectious diseases, in particular spinal tuberculosis^{9,33}. In damaged tissues, wound healing promoted by OPN may be employed in epidural fibrosis or spinal cord injury²³⁻²⁷.

In this comprehensive review, we summarize the present knowledge about the functions of OPN in spine pathologies.

MATERIAL AND METHODS

Search strategy

This study followed the protocols specified in the 2009 Preferred Reporting Items for Systematic Reviews and Meta-analysis statement (PRISMA). A comprehensive literature search of PubMed and Scopus was conducted to identify all relevant articles published until October 6, 2020.

The following query was used on both databases: ("Osteopontin" OR "OPN") AND ("Spine" OR "Spinal" OR "Vertebr*" OR "Column"). Further manual search and analysis were also conducted, using the identified articles reference lists, in order to find additional significant studies.

Inclusion criteria

All studies focusing on current knowledge of the role of OPN in spinal pathologies, mainly its biological functions and potential clinical application use were included in this review. Both human and nonhuman studies were considered for evaluation. No language restrictions were applied.

Exclusion criteria

Studies addressing the role of OPN in areas other than spinal related diseases were excluded from this review.

Data extraction and quality assessment of studies

The databases were searched independently by two researchers, who later evaluated the eligibility and quality of the identified studies, based on the title and

abstract. If there was a disagreement regarding the inclusion of the articles, a consensus between the authors was reached through meticulous review and discussion.

Methodological quality and risk of bias of each study was performed by the reviewers according to the ROBINS-I risk of bias tool for observational studies, SYRCLE's risk of bias tool for animal studies and NIH quality assessment tool for cross-sectional studies.

RESULTS

A total of 674 articles were identified, 263 from PubMed and 411 from Scopus databases. Afterwards, the reference lists of these articles provided 15 additional studies. After the removal of duplicates (217), 472 studies were subjected to an initial analysis, based on the titles and abstracts and 117 were selected for full-text analysis. Finally, 79 studies were included in the systematic review for qualitative synthesis, following the exclusion of 38 as the inclusion criteria were not met. A PRISMA flow diagram is presented in **figure 3**.

Study characteristics

From each study, the specific pathology in which the research focused on, the authors, the experimental model, the objectives, the OPN role, the main results and the interpretation of the outcomes were collected.

The features of the studies which have directly discussed OPN involvement in spinal pathologies, are summarized in **table 1**, including the quality analysis. Most scientific researches included human or animal models. By far, the most commonly used animals were muroid rodents, mainly C57BL/6J mice and Wistar rats.

The majority of studies found a correlation between OPN expression and the presence or severity of disease, demonstrating that OPN might be a predictor of disease. Within the spinal diseases, intervertebral disc degeneration, cervical spondylotic myelopathy and epidural fibrosis had incomparably the greatest amount of information available in the literature. OPN in spinal cord injury, adolescent idiopathic scoliosis and spinal tuberculosis were also examined in our work, with interesting results. The main functions described by the studies were in the areas of immunology, bone remodeling, fibrosis and wound healing. It is important to highlight that most of the investigations in this field are from recent years.

DISCUSSION

Intervertebral Disc Degeneration

Low back pain is recognized as a major cause of disability worldwide³⁴. A main contributor to this prevalent disorder is Intervertebral Disc Degeneration (IVDD), a complex multifactorial process, far from being clearly understood, where environmental and genetic factors play a role^{34,35}. The degeneration process includes a decrease in nutrient supply to the disc, which impairs its ability to preserve the extracellular matrix (ECM). Hence, modifications in the contents of ECM ensue, and finally changes in cell metabolism, loss of tissue biomechanical properties, and reduced capability of the intervertebral disc (IVD) to resist load³⁵. Presently, there are no effective treatments to stop disc degeneration or lead to disc regeneration.

OPN has been considered as a marker of calcification, as it is invariably associated with normal and pathological mineral deposits. Although it has been demonstrated to be overexpressed in hyperostotic spinal lesions and other ectopic calcifications, the real role of this molecule is yet to be clarified³⁶⁻³⁸. It seems to act as a regulator of calcification^{12,39}. One hypothesis is that OPN might play a role in IVD degeneration, since calcification of the vertebral endplates reduces the nutrition and blood supply, leading to disc degeneration^{35,36}.

A study in ovariectomized mice (OVX), from Xiao *et al.* examined the role of postmenopausal osteoporosis in lumbar IVDD¹⁶. The ovariectomy led to increased ossification and hypertrophy of the endplate, and narrowing of the intervertebral disc space, processes linked to disc degeneration. A main finding was the significant increase

in expression of OPN both in the endplate and annulus fibrosus in OVX mice, signalling abnormal ossification¹⁶.

Another study focused on the presence of ectopic calcifications in degenerated IVDs, hypothesizing that advanced glycation end products (AGEs) accumulation in IVDs is related with ectopic calcifications, inducing hypertrophy and osteogenic differentiation of IVD cells, consequently promoting degeneration¹⁷. Immunohistochemical analyses demonstrated that OPN co-localizes with methylglyoxal-hydroimidazolone-1, an AGE precursor and marker of ectopic calcification, in both nucleus pulposus and cartilage endplate of degenerated IVDs, suggesting a focal endochondral ossification role of OPN in IVDs¹⁷.

Tomaszewski *et al.*, also found a positive correlation between endplate calcification and both endplate and IVD degeneration in the cervical spine, favoring the hypothesis that endplate calcification is related to IVD degeneration¹⁸. However, they were unable to demonstrate a correlation between OPN expression and endplate calcification or IVD degeneration, since their immunohistochemical studies showed low expression of OPN in all IVD regions, regardless of the degree of degeneration¹⁸. The results of this study stand in opposition to both the aforementioned experiment and previous studies regarding OPN in ectopic calcification in other tissues^{36,38,40}.

The reason for these distinct results from similar studies remains unclear. This disparity might be related to differences in immunohistochemical techniques, or the role of OPN in these processes may be more indirect, with the intervention of other molecules, such as AGEs. Also, the avascular nature of the IVD can be a reason for conflicting results with calcification in other tissues.

OPN has been recognized as an active player not only in biomineralization and tissue remodeling, but also in inflammation⁴¹. Marfia et al. investigated the role of OPN as a pro-inflammatory molecule in IVD degeneration¹⁹. In fact, inflammation has been associated with degenerative disc disease and mainly considered a deleterious process leading to disease progression⁴². Being an extracellular matrix protein, OPN is believed to act as a proinflammatory cytokine promoting the recruitment of macrophages and mediating cytokine secretion⁴¹.

In order to elucidate the relation between OPN, inflammation and degeneration of IVD, Marfia and colleagues studied the expression of OPN and inflammatory cytokines in cells from IVDs¹⁹. A marked increase of OPN gene expression in degenerated IVDs was detected, and it correlated with the severity of disc degeneration. The specific processes of how OPN acts in these conditions are not clarified. Being an avascular structure, the removal of cytokines produced by disc cells is impaired, leading to a proinflammatory cytokine-rich microenvironment which might induce OPN gene expression and protein synthesis. Consequently, the higher concentration of OPN could promote IVD degeneration¹⁹. Whether those changes could promote mineralization, linking the processes of inflammation and ectopic IVD calcification was not investigated.

In light of the emerging evidence, degenerative disc disease presents as an ever more complex process. OPN may be related to degeneration, as a regulator of IVD calcification. Nonetheless, the role of OPN in ectopic calcification of the endplate and IVD remains unclarified and might be multifactorial. Further in-depth research in this field is needed.

Myelopathy

Cervical Spondylotic Myelopathy

IVD degeneration, mentioned in the previous topic, can also lead to cervical spondylotic myelopathy (CSM), which is a common condition resulting from compression of the spinal cord due to degeneration of the spinal elements. It is characterized by IVD degeneration and facet joint osteoarthritis, along with pathologic changes in the spinal ligaments, including the posterior longitudinal ligament (PLL), most frequently hypertrophy and ossification^{8,43,44}. The etiology of this disease remains unclear, as well as risk factors and mechanism of progression⁸.

In fact, some of the current investigations in CSM have been focusing on the influence of genetic factors in the development and prognosis of the disease, specifically on the role of single nucleotide polymorphisms (SNP) and abnormal gene expression⁴⁵. Among them, one prospective study investigated the relation between OPN expression and its gene polymorphisms and CSM⁸.

Wu *et al.* studied three SNP of the OPN gene and demonstrated that the -66GG genotype carriers had a higher susceptibility to CSM⁸. Additionally, -66GG genotype carriers had higher OPN expression in the intervertebral disk, along with increased expression of other inflammatory cytokines⁸. It was postulated that the correlation of the -66T>G SNP genotype and inflammatory response could be related to the development of CSM. Interestingly, -66T>G SNP also revealed to be associated with clinical outcome after surgical treatment⁸.

Ossification of the Posterior Longitudinal Ligament

As previously mentioned, cervical myelopathy can also be related to OPLL^{46,47}.

The role of OPN in ectopic calcification of this spinal ligament was reported in an study using twy mice, a mutant (gene symbol: *ttw*) characterized by spinal hyperostosis and considered as a model for OPLL. This study demonstrated enhanced expression of

OPN in spinal hyperostotic lesions, particularly in cells of the posterior longitudinal ligament and chondrogenic cells in the outer layer of the annulus fibrosus⁴⁸. A recent study involving patients with cervical OPLL revealed increased OPN levels in males, which correlated with the extension of this disease. Male patients with OPLL over three spine levels had a higher expression of this protein²⁰. However, they were unable to demonstrate similar results in females²⁰.

Additional evidence indicating that OPN may be involved in OPLL was the demonstration of a positive linkage between this condition and OPN, on a Japanese large-scale screening for candidate genes of OPLL²¹.

Ossification of Ligamentum Flavum

Similar to OPLL, ossification of ligamentum flavum (OLF) can cause spinal cord injury as a result of severe compression. Although rare, it may be responsible for thoracic myelopathy, given its higher incidence in the low thoracic spine and it may occur isolated or in combination with OPLL, exacerbating the spinal stenosis⁴⁹.

A recent study suggested that OPN is implicated in the OLF. Xu *et al.* demonstrated not only that OPN expression is increased in ossified human ligamentum, but it also can promote proliferation of ligamentum flavum cells (LFC), in a dose- and time- dependent effect. Another interesting finding was that OPN could promote progression of OLF by increasing the expression of alkaline phosphatase (ALP), a glycoprotein crucial for bone formation. However, OPN treatment was not able to promote the ossification²².

Overall, OPN seems to function as a pro-inflammatory molecule inducing changes that promote bone remodelling. Multiple studies revealed increased expression of this

protein in different processes that ultimately lead to myelopathy, with higher levels correlating to more extensive and severe forms. Available data suggest that OPN can be used as a marker of disease severity and prognosis^{8,20}. Despite current evidence regarding the role of OPN in these conditions, the information available is still insufficient for translation to clinical practice and additional investigation is necessary.

Epidural Fibrosis

Extensive epidural fibrosis (EF) following a spinal surgery may be an underlying factor for the so-called failed-back surgery syndrome⁵⁰. Adhesion of nonphysiologic scar tissues to the nerve roots may cause dural tethering and/or compression of the nerve roots^{51,52}, which can lead to low back and radicular pain after surgery^{50,52}. Nevertheless, the correlation between these two entities is still debated and the pathophysiological mechanism of EF remains unclear²⁴. After a surgical insult, the formation of EF is attributed to collagen synthesis by fibroblasts that infiltrate the postsurgical hematoma.

The involvement of OPN in fibrosis has been investigated by numerous studies. It is known to participate in the composition of collagen fibrils in the course of tissue remodelling⁵³. In fact, animal studies on OPN-null mutant mice demonstrated changes in the wounds, with reduced collagen fibrils diameter and matrix disorganization⁵⁴. Besides, OPN acts as a pro-inflammatory cytokine, stimulating migration of macrophages and neutrophils, and modulating angiogenesis and fibrosis formation⁵³. Another study showed that OPN mediates macrophage accumulation with subsequent fibrosis in renal tissue. A major finding was that both types I and IV collagen deposition were reduced in OPN null mutant mice, which led the authors to conclude that the overall effect of OPN in this model appears to be proinflammatory and profibrotic⁵⁵.

Despite all accumulated knowledge in the field, only a few studies focused on the contribution of OPN to postsurgical epidural scar formation^{23,24,56-58}.

Brzezicki *et al.* investigated the involvement of OPN in the development of EF, scar vascularization and neural response to injury in rats submitted to laminectomy. Increased OPN immunoreactivity was detected in arachnoid adhesions, dura and tissue surrounding the dorsal root ganglion (DRG). A correlation was found between the expression of OPN and increased thickness of the dura, due to inflammatory and fibrotic processes. A significant increase in percentage of OPN-positive neurons in DRG in the surgical group was identified, along with a reduction of somatosensory evoked potential amplitudes, favoring the hypothesis of DRG involvement in peridural scar-related recurrent radiculopathy. Another finding was the correlation between the number of OPN-positive neurons, and both the extent of EF and the intensity of OPN expression in it and in the fibrous tissue surrounding the DRG, supporting the theory of nerve root tethering as a source of recurrent pain. These results point out OPN as a leading contributor to EF formation and as a probable player in postoperative recurrent pain through modulation of DRG responses²³.

Pereira *et al.* investigated the role of OPN in the development of EF and the correlation with persistent or recurrent symptoms, specifically after lumbar spine surgery²⁴. Samples of EF were collected from patients who underwent epiduroscopy and adhesiolysis. The presence of OPN was detected at the periphery of collagen fibers both in the posterior epidural space and in the posterior peri-radicular space. Nonetheless, OPN was absent in neighboring tissues, including the annulus fibrosus and the ligamentum flavum, suggesting that OPN does not have a ubiquitous distribution in these tissues, therefore its presence could be directly related to wound healing and scar formation. Thus,

OPN presence can potentially play a role in postoperative pain through its contribution to tethering of the nerve roots²⁴. Therefore, the authors raised the hypothesis that inhibition of OPN at the surgical site could potentially decrease EF formation and the prevalence of recurrent symptoms.

A series of studies tested the hypothesis that activation of fibrinogenesis could be triggered by an inflammatory process initiated through chemotactic factors released by erythrocytes and thrombocytes in the epidural space. Accordingly, the authors examined the effects of bevacizumab, cetuximab and the sorafenib on formation of EF. The results showed the potential of these agents in preventing the development of EF, however, OPN levels did not correlate with histopathological EF evaluation. An explanation to the discrepancy between these findings and previous reports was not provided by the authors. Nevertheless, their conclusions support the role of inflammation as a determinant factor in the development of EF, leading the way for further investigations on pro-inflammatory molecules, such as OPN, as potential therapeutic targets⁵⁶⁻⁵⁸.

Further research is necessary to consolidate the knowledge about the role of OPN in EF formation and progression, with the ultimate objective of using it as a therapeutic target.

Spinal Cord Injuries

Spinal cord injury (SCI) is a debilitating neurological condition causing permanent or long-lasting physical disability⁵⁹. Given the absence of an efficient treatment to reverse the damage to the spinal cord, research in this area for new therapeutic strategies has been intense, with promising results, some of which involving OPN⁶⁰.

Following a deleterious stimulus, microglia and astrocytes are premature responders initiating the process of clearance of toxic metabolic remnants, not only due to their phagocytic capacity, but also through the production inflammatory mediators and recruitment of leucocytes that contribute to debris clearance^{61,62}. Nonetheless, the aforementioned process must be well-adjusted, as excessive inflammatory response can increase tissue damage⁶³.

As a pro-inflammatory cytokine and as an anchor for phagocytosis, OPN assists the migration of inflammatory and glial cells into the damage cavity after injury²⁵. As a major player in this chemotactic process, specifically in microglia and macrophages response, OPN is more active during early response²⁵. In fact, Moon *et al.* found an elevation of OPN levels in the early days (1 to 7) after a severe SCI²⁵. In this study, expression of OPN was low in normal spinal cord and gradually increased in damaged tissues in a variety of cells: activated microglia/macrophages, astrocytes and neurons²⁵. Thus, OPN seems to play an important role in mediating the inflammatory process in early stages of SCI, with cell proliferation and migration intimately associated with OPN expression.

Similar results were obtained by Hashimoto *et al.*, as an increased expression of OPN by microglia cells in the injury site was registered, soon after the injury (early and subacute stages)²⁶. The authors argument that OPN may suppress scar formation and promote axonal regeneration and astrocyte migration²⁶. In a posterior study, OPN-deficient mice exhibited a reduced local expression of inflammatory cytokines, appart from greater neuronal loss and less white matter sparing, associated with worse locomotor recovery²⁷. These results support the hypothesis that OPN might exert a regulatory role in the inflammatory response, with neuroprotective functions after SCI. Furthermore, its

upregulation is fundamental to enhance neurological recovery and its expression is correlated with the extension of injury²⁷.

Further investigations suggested that OPN increases axon regeneration when conjugated with growth factors, such as Insulin-like Growth Factor 1 (IGF-1) or Brain-Derived Neurotrophic Factor⁶⁴. IGF-1 promotes the growth of neonatal corticospinal neurons (CSNs), but has limited effects in reactivating the growth capacity in adults. However, OPN is able to sensitize CSNs' signaling responses to IGF1, by increasing IGF1 receptors, promoting regenerative growth in adult neurons and relevant functional recovery after spinal cord injury, as demonstrated by Liu *et al.*⁶⁵ This theory was supported by Anderson *et al.*, who described a reactivation of mature descending propriospinal neurons by OPN, IGF1 and ciliary neurotrophic factor⁶⁶.

Taken together, these results suggest that OPN can play an important role in body's response to spinal cord injury, not only minimizing tissue damage, but also promoting axon regeneration and neurological recovery.

Idiopathic Scoliosis

Adolescent idiopathic scoliosis (AIS) is a common spinal deformity, usually diagnosed in late childhood or adolescence⁶⁷. It is currently accepted that AIS develops under the influence of a myriad of factors, including genetic deficiencies, melatonin signaling malfunctions, autonomic nervous system abnormalities and platelet-calmodulin dysfunction^{30,67,68}.

A few reports focused on the role of OPN in AIS, specifically as a serum molecule, with systemic actions. Moreau *et al.* reported not only a significant increase in OPN plasma levels of AIS patients, but also an association with curve severity²⁸. More

importantly, they demonstrated that genetic depletion of OPN prevented spinal deformation²⁸. Additionally, Yadav *et al.*, showed that overexpression of OPN is linked not only to scoliosis but also other skeletal abnormalities²⁹.

These findings are in line with the results of experiments performed in a model of bipedal mice. Xie *et al.* demonstrated a significant higher incidence and severity of scoliosis in the group treated with OPN³⁰. Another analogous experiment found consistent results³¹. The incidence of scoliosis was significantly higher in the high-concentration osteopontin female and male mice groups, but curve severity was considerably higher in the female group³¹. In conjunction, these results suggest the involvement of OPN in the etiopathogenesis of scoliosis, both in its onset and disease progression²⁸⁻³⁰.

Sun *et al.* noticed that high serum osteopontin levels are associated with low cortical bone mineral density in girls with AIS. They postulate that high osteopontin levels in plasma may reflect underlying abnormalities of bone mineralization in AIS subjects³².

Several studies investigated the association between OPN and melatonin, possibly through the participation in the same effector pathway. Melatonin is a circadian hormone, previously established as a key player in AIS development^{69,70}. In animal models, bipedal mice with melatonin deficiency had a higher development rate of scoliosis, whereas protection against the disease was conferred by restoring melatonin levels⁷¹. Deficiency of OPN also protected transgenic melatonin-deficient mice from scoliosis, with high OPN blood levels being related to scoliosis development²⁸. Whether OPN exerts an individual influence in the etiopathogenesis of AIS or act has a downstream effector regulated by melatonin, needs further investigations^{28,30}.

Considering the abovementioned results, it follows that OPN might be a target for the ongoing investigation on serum markers predictors for the onset and progression of AIS. The possible influence of OPN genetic polymorphisms in AIS, as already demonstrated in other diseases such as CSM, needs further research⁷²⁻⁷⁴.

Spinal Tuberculosis

In addition to other pro-inflammatory molecules, OPN involvement has been identified in the systemic response to several infectious conditions. These investigations demonstrated an increased plasma level of OPN in sepsis, systemic inflammatory response syndrome, and septic shock^{75,76}. The results also correlated a crescent OPN levels with increasing mortality rates in patients presenting with sepsis^{75,77}.

Of particular importance in spinal pathologies is spinal tuberculosis (STB), a devastating disorder with growing incidence particularly in patients with HIV infection⁷⁸. As for other infectious conditions, the role of OPN was investigated in *Mycobacterium tuberculosis* (MTB) infections.

Up-regulation of OPN is implicated in the formation of granulomas in tuberculosis, sarcoidosis and silicosis, all of which involve cell-mediated immune reactions⁷⁹. In the absence of OPN expression, the formation of granulation tissue is dramatically reduced⁸⁰. Nau *et al.* found that OPN expression was inversely correlated with disseminated infection⁸¹.

Shiratori *et al.* reported that OPN activates cellular immune response and it may constitute a biomarker for the diagnosis and monitoring the course of the disease³³. In line with this results, another study established a correlation between serum levels of OPN

and the presence of MTB infection, particularly in patients presenting with a lung cavity^{33,82}.

As in other spinal conditions, the influence of OPN genetic polymorphisms in spinal tuberculosis (STB) was also investigated. Wang *et al.* detected a higher frequency of the -66GG genotypic carriers of -66T>G polymorphisms in STB patients, with the G allele being considered as a risk factor for the occurrence of STB. Furthermore, the OPN mRNA expression and cleaved protein were higher in the GG genotype, correlating with increased severity of STB. With all these findings considered, OPN genetic polymorphisms may be a potential useful biomarker for the susceptibility and a predictor of clinical severity of STB⁹.

The current scientific knowledge draws the attention to OPN, not only as a crucial determinant for the susceptibility and severity of *Mycobacterium tuberculosis* spinal infections, but also fostered new investigations in other spinal infectious diseases⁹.

LIMITATIONS

Some important limitations are present in our systematic review. Firstly, the retrieved studies were heterogeneous regarding the characteristics of the study and sample size, with some studies presenting small numbers. Occasionally, the number of mice used was not revealed or a control group was not available, which may prevent the findings from being extrapolated. Secondly, considering the limited number of studies regarding the role of OPN in spinal pathologies, the accuracy and validity of the relationship between the expression of this protein and the presence or severity of the

condition may also be questionable. Lastly, there were some conflicting results, mostly regarding the role of OPN in EF.

It is clear that some studies are in their preliminary stages and additional research, with a larger sample size and a more extensive research, is essential for further comprehension of the direct role of OPN in the etiopathogenesis. Only then, its importance in the diagnosis and treatment of patients with spinal pathologies can be determined.

CONCLUSION

Despite being initially described more than 20 years ago, in recent years OPN has regained the interest of the scientific community, as it is proving to be a promising and impressively multifunctional molecule. Although further research is needed to explicit the full role of OPN in spinal diseases, the results of existing studies are very motivating. In the future, OPN might be a potential target for development of novel therapies or play a role as a prognostic element in the onset and progression of different spinal diseases.

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Conflict of interest

The authors report no conflict of interest.

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Table 1. Main results of published studies of OPN role in Spinal Pathologies

Pathology	Study	Experimental Model	Objective	OPN role	Main OPN Results	Interpretation	Quality Analysis
Intervertebral Disc Degeneration	Xiao, Z., <i>et al.</i> , 2018 [16]	30 female C57BL/6 J mice;	To examine the roles of postmenopausal osteoporosis in IVDD and its underlying mechanism, including the expression of OPN;	Osteochondral remodelling;	Ovariectomy induced intervertebral disc alterations; Upregulation of OPN in the endplate and annulus fibrosus of OVX mice;	Ovariectomy induces IVDD; OPN is involved in abnormal ossification of intervertebral disc, stimulating disc degeneration.	Low risk of bias - SYRCLE's RoB tool
	Tomaszewski, K., <i>et al.</i> , 2014 [18]	60 human cadaveric cervical IVDs	To analyse the expression and role of proteins commonly associated with IVD;	Calcification and degeneration in cervical vertebral endplate;	Poor expression of OPN, both in the vertebral endplate and the IVD;	No correlation between OPN and the degree of degeneration and endplate calcification.	Low risk of bias - ROBINS I
	Illien, S., <i>et al.</i> , 2016 [17]	5 human cadaveric lumbar IVDs 4 bovine tail IVDs (for bovine cell culture)	To evaluate the relation between advanced glycation end products (AGEs) in IVDs with ectopic calcifications; AGE role in the inducement of hypertrophy and osteogenic differentiation of IVD cells;	Osteogenic differentiation;	AGE stimulation of bovine NP and CEP cells in culture induced ectopic calcifications; OPN co-localizes with a precursor marker (AGE) of ectopic calcification in both locations;	AGE induces hypertrophy and osteogenic differentiation in IVD cells, inducing endochondral ossification; OPN with focal endochondral ossification role in IVDs.	Serious risk of bias - ROBINS I
	Marfia, G., <i>et al.</i> , 2015 [19]	21 patient lumbar IVDs	To identify genes implicated in IVD pathology, through analysis of gene profile in mesenchymal stem cells (MSCs) from human herniated IVD (H-IVD) and degenerated IVD (D-IVD);	Tissue mineralization inductor; Proinflammatory cytokine;	CCL25, IL37, CCR1 and CCR9 upregulation; OPN markedly increased (>400x) in D-IVD-MSCs compared to H-IVD-MSCs; OPN protein expression was detectable only in D-IVD-MSCs, and its levels is directly related to the IVD Thompson grade;	Abnormal expression of OPN plays a pivotal role in the pathophysiological process of human disc degeneration; OPN might favour degenerative changes in D-IVD-MSCs; OPN may indicate directly D-IVD severity.	Moderate risk of bias - ROBINS I

Cervical Spondylotic Myelopathy	Wu, J., et al., 2014 [8]	187 patients with CSM 233 controls	To evaluate the association of OPN Polymorphism with the Susceptibility to CSM and its Outcome;	Pro-inflammatory cytokine; Immune response regulator;	Lower OPN expression in the -66GG carriers; No association between -443 C>T and -156G>GG and levels of OPN; -66T>G polymorphism influences the local OPN expression; -66GG carriers with higher expressions of key cytokines; No correlation between the OPN polymorphism and the severity of CSM (mJOA score);	OPN -66T>G polymorphism increases the susceptibility to CSM; Involvement of -66 G>T polymorphism in the development of CSM via increased production of cytokines and inflammatory activation; OPN genetic polymorphism can be used as a disease severity, prognosis marker and indicative of the outcome of ACF surgery.	Low risk of bias - ROBINS I
	Cai, G., et al., 2017 [20]	121 patients with cOPLL 60 age-matched healthy subjects	To investigate potential pathogenic mechanisms, mainly serum hormones and cytokines in cOPLL patients;	Ossification;	Osteopontin and fibroblast growth factor-23 (FGF-23) were significantly increased in male cOPLL patients; OPN with no statistical differences in cOPLL females; FGF-23 and OPN increased in the E-type cOPLL group;	OPN is involved in cOPLL and it is associated with the extension.	Moderate risk of bias - ROBINS I
	Furushima, L., et al., 2002 [21]	126 affected sib-pairs 250 unrelated OPLL cases, and 200 non-OPLL controls (for the allelic association study)	To screen for candidate genes of ossification of the posterior longitudinal ligament (OPLL);	Ossification;	BMP4 reached criteria of suggestive evidence of linkage; OPN (NPL 1.15), and PTHR1 (NPL 1.00) showed positive evidence of linkage;	BMP4 plays an important role in the pathogenesis of OPLL; OPN may be involved in Ossification of OPLL.	NI

Cervical Spondylotic Myelopathy	Xu, Z., <i>et al.</i>, 2016 [22]	8 patient ossified ligamentum flavum 8 patient normal ligamentum flavum	To understand the role and mechanism of OPN expression in ligamentum flavum cell (LFC) isolated from patient with normal ligamentum flavum (NLF) and patient with ossification of ligamentum flavum (OLF);	Bone remodelling; Proliferation promotor;	Increased expression of OPN and its receptors in ossification tissue; OLF group: OPN staining with 1 negative case, 1 positive case, and 6 strong positive case; NLF group: OPN staining with 7 negative cases, 1 positive case, and 0 strong positive case; OPN increases the expression of ALP in OLF-LFCs, but not in NLF-LFCs; OPN treatment promoted LFC proliferation (dose- and time-dependent increase); Increased expression of OPN and CD44 in OLF group;	OPN may promote the progression of OLF by increasing the expression of ALP; OPN is increased in ossifying area, thus OPN may have an important role in bone remodelling; OPN induces osteogenic differentiation of LFCs; OPN may cause hypertrophy of ligamentum flavum by promoting cell proliferation.	Moderate risk of bias - ROBINS I
Epidural Fibrosis	Brzezicki, G., <i>et al.</i>, 2011 [23]	25 male Wistar rats divided in 2 groups: 15 in L5 laminectomy group and 10 in sham procedure group	To evaluate the postlaminectomy osteopontin expression in peridural scar tissue and structures within the vertebral canal; To correlate the findings with peridural scar extent, scar vascularity, and neurophysiological findings;	Wound healing; Organ fibrosis; Neural response to injury;	Laminectomy resulted in increased OPN expression in bony margins of the laminectomy defect, scar tissue, arachnoid adhesions (with nerve root adhesions) and thickened dura; Postlaminectomy increases percentage of OPN positivity in dorsal root ganglions neuron; Decrease in the amplitude of the somatosensory-evoked potentials in postlaminectomy animals; Number of OPN positive neurons was affected by extension and OPN staining intensity of peridural fibrosis;	Laminectomy and peridural scar formation can elicit a response in DRG, which may lead to recurrent radicular symptoms; OPN as a major player in wound healing and in epidural fibrosis formation; OPN as a marker of DRG response to peridural scar formation; Positive OPN staining of adhesions may be a useful tool in distinguishing “true” adhesions from artifacts.	High risk of bias - SYRCLE's RoB tool

Epidural Fibrosis	Pereira, P., <i>et al.</i> , 2014 [24]	24 patients with persistent or recurrent low back and/or leg pain after lumbar spine surgery epidural scar tissue (no control group)	To investigate the presence of nerve fibers and the expression of OPN in epidural fibrous tissue after lumbar surgery in humans;	Tissue remodelling; Scar formation; Wound healing	Presence of OPN at the periphery of collagen fibers in scar tissues, irrespective of the location; OPN not detected in neighbouring tissues, including the annulus fibrosus of the intervertebral disc and the ligamentum flavum;	OPN seems to play a role in the formation of epidural fibrosis; OPN presence can be directly related to the pathophysiology of wound healing and scar formation.	Fair - NIH quality assessment tool for cross-sectional studies
	Tanriverdi, O., <i>et al.</i> , 2018 [58]	16 Wistar albino rats divided in 2 groups: control (L1-2 laminectomy only) and sorafenib treatment (L1-2 level laminectomy + topical sorafenib)	To determine if Sorafenib can prevent the development of spinal epidural fibrosis (EF);	Fibrosis; Anti-inflammatory;	Sorafenib decreased neovascularization in damaged tissue; Scores for inflammatory cell density and fibrosis score were lowest in sorafenib-treated group; No significant difference in microvessel density scores between groups after osteopontin staining; OPN levels did not correlate with histopathological EF evaluation or CD105 levels;	Sorafenib reduced the development of EF, and the degree of EF; OPN cannot be considered a reliable marker for the immunohistochemical detection of EF.	High risk of bias - SYRCLE's RoB tool
	Tanriverdi, O., <i>et al.</i> , 2019 [56]	16 Wistar albino rats divided in 2 groups: control (L1-2 laminectomy alone) and treatment	To assess EGFR inhibition with Cetuximab, for the postoperative treatment of fibrosis;	Fibrosis; Anti-inflammatory;	Degree of EF was lower with local cetuximab administration; Cetuximab decreases neovascularization and inhibits fibroblasts in the damaged tissue; CD105 staining was higher in treatment group; Levels of OPN did not agree with	Cetuximab inhibits the development of EF; CD105 is a reliable marker for grading EF; OPN is not considered to be a reliable marker for identifying EF.	High risk of bias - SYRCLE's RoB tool

Epidural Fibrosis		group (L1-2 level laminectomy + topical cetuximab)			histopathological EF assessment methods and CD105 levels;		
	Yilmaz, I., <i>et al.</i> ; 2018 [57]	16 Wistar albino rats divided in 2 groups: control (L1-2 laminectomy alone) and treatment group (L1-2 laminectomy + topical bevacizumab)	To evaluate Bevacizumab for epidural fibrosis (EF) treatment using histopathology and immunohistochemical staining for CD105 and OPN;	Fibrosis; Anti-inflammatory;	The extent of EF was lower in animals that received local bevacizumab; Grading of EF based on microvessel density as assessed by CD105 immunostaining was consistent with fibroblast count and fibrosis density; Bevacizumab reduced neovascularization in damaged tissue; No significant difference in OPN expression between the two groups;	Local application of bevacizumab after laminectomy significantly reduces EF; CD105 is a reliable marker for EF evaluation, consistent with grading; Grading using OPN immunostaining is not reliable.	Low risk of bias - SYRCLE's RoB tool
Spinal Cord Injuries	Moon, C., <i>et al.</i> , 2004 [25]	18 Sprague-Dawley rats subjected to laminectomy at T9/T10	To study the expression of OPN in the spinal cords of rats with clip compression injury;	Inflammatory (macrophage activation and migration) regulator; Neural response to injury;	Expression of OPN was low in normal spinal cord and gradually increased on post-injury days in the majority of cells; OPN expression is related to the expression of Proliferating Cell Nuclear Antigen; Majority of proliferating cells in damaged tissues of early SCI express OPN;	OPN plays an important role in mediating the inflammatory process in early stages of SCI; OPN facilitates cell migration of macrophages and astrocytes after SCI; Cell proliferation is intimately associated with OPN expression; Remodelling of damaged spinal cord is influenced by OPN expression.	High risk of bias - SYRCLE's RoB tool
	Hashimoto, M, <i>et al.</i> , 2003 [26]	Male Wistar rats submitted to extradural compression	To assess the upregulation of OPN expression in rat spinal cord microglia after traumatic injury, by analysing temporal and	Inflammatory cytokine; Chemotactic activity;	Activated microglia and the level of OPN expression increased gradually, reaching a peak level at 3 days;	OPN expression is synchronized with the activation of microglia and macrophages; OPN secreted from activated microglia may stimulate astrocyte	High risk of bias - SYRCLE's RoB tool

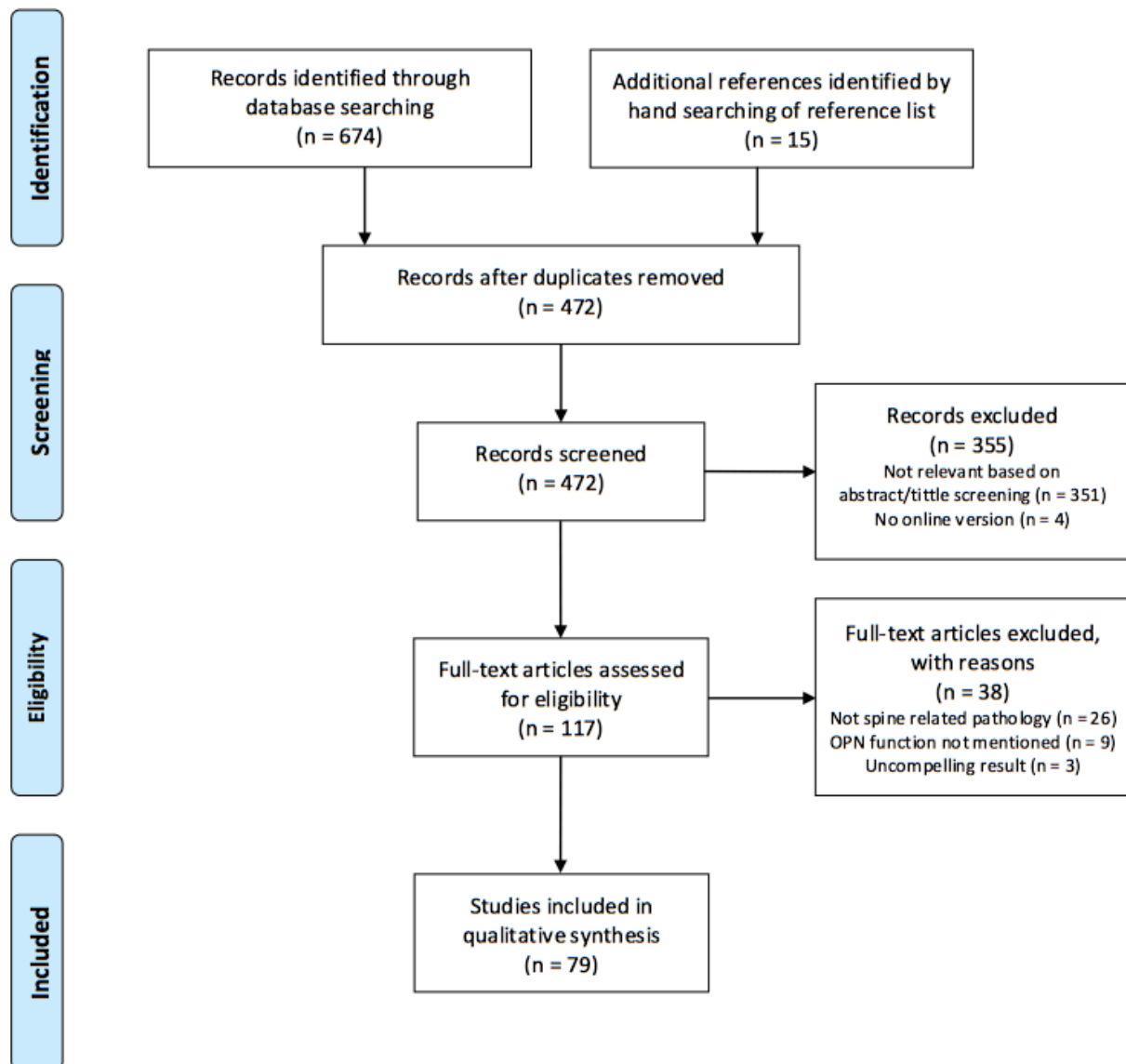
Spinal Cord Injuries		(unknown number)	spatial changes of OPN mRNA and protein;	Adhesion molecule in microglial phagocytosis;	Activated microglia expressed OPN and were, mainly distributed in the white matter of the spinal cord; Correlation between OPN expression and demyelination; OPN mRNA and protein reached maximum levels earlier in SCI than brain ischemia;	migration; Level of expression OPN correlates with the extent of inflammation.	
	Hashimoto, M, <i>et al.</i> , 2004 [27]	45 Male C57BL/6 mice subjected to laminectomy at T7/8 level, in which the study group received additional extradural compression ;	To profile gene expression of cathepsin D, metallothioneins-1 and -2, OPN, and tenascin-C in a mouse spinal cord injury (SCI) model by cDNA microarray analysis;	Chemotactic activity; Opsonin for phagocytosis; Wound healing;	SCI led to the up-regulation of OPN and cathepsin D expression in activated macrophages/microglia at 3 and 7 days; Only OPN expression was up-regulated at all time; Gene expression profiling was shown to exhibit temporal and spatial changes of expression after SCI;	One role of OPN expression may be chemotaxis of macrophages/microglia to recruit them into the injured area; OPN may facilitate cleaning of debris in damaged tissues and promote wound healing.	High risk of bias - SYRCLE's RoB tool
Adolescent Idiopathic Scoliosis	Moreau, A., <i>et al.</i> , 2009 [28]	320 patients with IS, compared with 120 healthy control subjects and 82 asymptomatic offspring;	To investigate the involvement of OPN and its soluble CD44 receptor (sCD44), in Idiopathic Scoliosis pathomechanism;	Multifunctional cytokine;	Mean plasma OPN levels were significantly increased in IS patients and correlated with disease severity; Elevated plasma OPN levels were also found in the asymptomatic at-risk group; Mean plasma sCD44 levels were significantly lower only in IS patients with Cobb angle ≥ 45 degree;	OPN is essential to induce scoliosis formation and curve progression through interactions with CD44 receptors; Elevation of OPN levels precede idiopathic scoliosis onset; Plasma OPN and sCD44 values could be useful markers for diagnosis of IS and prognosis of curve progression.	Moderate risk of bias - ROBINS I

Adolescent Idiopathic Scoliosis					All transgenic C57B1/6j mice devoid of OPN or CD44 receptor were protected against scoliosis;		
	Yadav, M., <i>et al.</i> , 2014 [29]	Phospho1 ^{-/-} mice, with C57BL/6 background; (unknown number) Wild-type littermate control mice (unknown number)	To study the effects of ablation and accumulation of OPN in the skeletal phenotype of Phospho1 ^{-/-} Mice;	Inhibitor of mineralization;	OPN expression is upregulated in the skeleton, particularly in the vertebrae; Increased proportion of phosphorylated OPN (p-OPN) peptides in Phospho1 ^{-/-} mice; Rescue of the scoliosis phenotype and improvement at other skeletal sites in the Phospho1 ^{-/-} mice by the deletion of Spp1;	Accumulation of plasma OPN causes the skeletal abnormalities in Phospho1 ^{-/-} mice; Inhibiting the expression of OPN can prevent the occurrence of adolescent idiopathic scoliosis and leads to improvements in the skeletal phenotype.	High risk of bias - SYRCLE's RoB tool
	Xie, N., <i>et al.</i> , 2015 [30]	80 male C3H/HeJ mice with amputated forelimbs divided in 2 groups: A - 40 mice treated with OPN, and B - 40 mice treated with saline; 40 quadruped control mice in Group C;	To determine the role of OPN in the pathomechanism of scoliosis;	Downstream effector; Bone metabolism regulator;	Osteopontin level was significantly higher in Group A compared with other groups; Higher incidence of spinal deformity in group A, followed by group B and then C; The average Cobb angle (measure of the curvature of the spine) was 29.8° in Group A, 20.9° in Group B, and 17.5° in Group C;	Elevated OPN level might play an important role in the etiopathogenesis of scoliosis; OPN increases the risk for scoliosis in bipedal mice and contributes to curve progression; Serum OPN level might be a potential risk predictor for the onset and progression of AIS.	Low risk of bias - SYRCLE's RoB tool

Adolescent Idiopathic Scoliosis	Tang, F., <i>et al.</i> , 2016 [31]	20 female and 20 male C3H/HeJ mice in high-concentration OPN treatment (hc-OPN) group, with equivalent control groups;	To observe the occurrence of scoliosis of the bipedal female mice induced by high concentration of OPN, and to provide evidence for establishing ideal drug models of AIS;	Bone metabolism regulator;	Higher incidence of scoliosis in the hc-OPN female and male mice groups than in the control groups; No significant difference in the incidence of scoliosis between hc-OPN female and male mice groups Curve magnitude was severer in the hc-OPN female mice group than in the hc-OPN male mice group; Higher and worse scoliosis incidence in hc-OPN-induced bipedal mice;	Increased levels of OPN may lead to the development of adolescent idiopathic scoliosis; The risk and severity of AIS are correlated with OPN expression.	Low risk of bias - SYRCLE's RoB tool
	Sun, G., <i>et al.</i> , 2012 [32]	45 AIS and 20 normal age-matched girls;	To document the OPN levels in AIS girls and its associations with BMD vs. normal control girls;	Bone metabolism regulator;	AIS girls was found to have longer arm span and lower body weight than the healthy controls; OPN level of AIS girls is significantly higher than the controls; OPN predicted cortical BMD in AIS group but not in normal controls;	Low cortical BMD in AIS is significantly correlated with high OPN level; High OPN level in plasma may reflect the underlying abnormalities in bone mineralization in AIS.	Moderate risk of bias - ROBINS I
Spinal Tuberculosis	Shiratori, B., <i>et al.</i> , 2014 [33]	37 HIV-negative patients with Tuberculosis (TB) infection and no prior history of TB treatment; Controls	To evaluate the reliability of Loop-mediated isothermal amplification (LAMP) for detecting Mycobacterium tuberculosis (MTB) infection; To identify the most prevalent MTB genotype in Metro Manila, including analysis of biomarkers of MTB infection;	Immune response regulator (T _H 1 response inductor);	MTB-LAMP with 100% sensitivity in culture positive samples and 94,52% specificity in culture negative samples; OPN and gal-9, and found statistically higher levels of both proteins in TB patients compared to HC individuals; OPN and gal-9 correlated with IL-8 and IP-10; Among the different biomarkers,	OPN and gal-9 activate the expression of chemokines and cytokines in macrophages, helping to recruit immune cells to the site of MTB infection; Increased IP-10, OPN, and neutrophils levels reflect the acute stage of TB infection; Determining TB severity and prognosis may be possible through measurement of IP-10,	Low risk of bias - ROBINS I

Spinal Tuberculosis		were healthy volunteers lacking signs of TB			OPN had the highest discriminatory property in differentiating healthy control from infection;	OPN, and neutrophils levels.	
	Wang, G., et al., 2017 [9]	150 patients with spinal tuberculosis (STB), with 149 healthy controls	To investigate the clinical significances of bone morphogenetic protein-4 (<i>BMP-4</i>), osteopontin (<i>OPN</i>), and vitamin D receptor (<i>VDR</i>) gene polymorphism, mRNA and protein expression in spinal TB patients;	<i>Mycobacterium tuberculosis</i> infection marker; Immune response regulator;	Higher frequency of GG genotype and G allele at -66T>G polymorphism in OPN gene; -66GG genotype correlated with a high OPN mRNA and protein expression; -66T>G polymorphism in OPN gene, was significantly associated with the clinical severity; Low BMP-4 mRNA and protein, high OPN mRNA and cleaved protein, and low VDR mRNA and protein expression significantly correlated with more severe clinical symptoms;	-66T>G polymorphism of OPN can be used as a biomarker for the susceptibility and a predictor of clinical severity of spinal TB; High <i>OPN</i> mRNA and protein expression are indicators of clinical severity in spinal TB patients.	Moderate risk of bias - ROBINS I

Figure 3. Flow diagram of the systematic review search process



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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ANEXOS



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Section/topic	#	Checklist item	Reported on page and paragraph/ table #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both. - MANDATÓRIO	Page 6: Role of Osteopontin in Spine Pathologies: a systematic review
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. – SEGUIR RECOMENDAÇÕES DA REVISTA	Page 9: “Objective: Worldwide, spinal diseases are an increasingly prevalent health problem. Deeper knowledge about the pathophysiology of this complex group of diseases could improve current prevention strategies and treatment options. Osteopontin (OPN) is a multifunctional



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			protein with great interest among the scientific community in these diseases. The aim of this revision is to appraise the role of osteopontin and its expression in different spinal pathologies. Methods: A systematic literature review search was conducted on PubMed and Scopus databases for published studies until 6th October 2020, concerning the role of osteopontin in spinal pathologies. Results: Among the 674 studies retrieved, 79 were selected for further analysis. A correlation
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			between the expression of OPN and the presence or clinical severity of 8 different spinal pathologies of diverse etiologies was found and analized in this review. Conclusion: OPN appears to have a broad spectrum of functions in spinal pathologies, mainly as an inflammatory cytokine or as a bone metabolism regulator. In the future, OPN might be a potential target for development of novel therapies or work as a prognostic tool in the onset and progression of spinal diseases.”
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INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. – MANDATÓRIO <i>O rationale corresponde à justificação da importância da revisão sistemática</i>	Page 10: “Osteopontin is a pleiotropic protein, already recognized for its significant roles in several systemic functions and disorders. In the last decade, at least 500 articles per year related to OPN were indexed in PubMed, revealing this molecule as a target for research, with promising results and new insights about its function.”



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Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). - MANDATÓRIO	Page 9: “The aim of this revision is to appraise the role of osteopontin and its expression in different spinal pathologies.”
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. – FACULTATIVO	A protocol was not prepared and the review was not registered.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. – MANDATÓRIO <i>É altamente recomendado, de acordo com as boas práticas da Cochrane, que não sejam aplicados critérios de exclusão baseados na língua e/ou data de publicação dos estudos.</i>	Page 13: “All studies focusing on current knowledge of the role of OPN in spinal pathologies, mainly its biological functions and potential clinical application use were included in this review. Both human and nonhuman studies were considered for evaluation. No



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			language restrictions were applied.”
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. – MANDATÓRIO <i>Em consonância com as boas práticas da Cochrane, é mandatório que se verifique pesquisa em pelo menos duas bases de pesquisa bibliográfica (idealmente, deverão ser pesquisadas duas bases generalistas e uma específica da área). No caso de revisões sistemáticas de estudos experimentais/ensaio clínico aleatorizados, é altamente recomendado que uma das bases pesquisadas corresponda à CENTRAL ou a bases de ensaios clínicos como a ClinicalTrials.gov.</i> <i>Estudos de revisão da literatura em que a pesquisa decorra numa única base de dados não serão classificados como revisões sistemáticas.</i>	Page 9: “A systematic literature review search was conducted on PubMed and Scopus databases for published studies until 6th October 2020”
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. – MANDATÓRIO <i>A query de pesquisa deve ser obrigatoriamente disponibilizada. A utilização de filtros de pesquisa da InterTASC é altamente recomendada (https://sites.google.com/a/york.ac.uk/issg-search-filters-resource/home)</i>	Page 13: A comprehensive literature search of PubMed and Scopus was conducted to identify all relevant articles published until October 6, 2020. The following query was used on both databases: ("Osteopontin" OR "OPN") AND ("Spine" OR "Spinal" OR



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			"Vertebr*" OR "Column"). Further manual search and analysis were also conducted, using the identified articles reference lists, in order to find additional significant studies.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). – MANDATÓRIO <i>As fases de selecção dos estudos primários devem ser descritas. Em consonância com as boas práticas da Cochrane, é mandatório que o processo de selecção envolva duas fases (fase de rastreio, em que os registos são seleccionados por título e abstract, e fase de inclusão, na qual se procede à leitura integral dos full texts). Em cada uma destas fases, o processo de selecção deve mandatoriamente envolver dois investigadores actuando de forma independente.</i>	Page 14: A total of 674 articles were identified, 263 from PubMed and 411 from Scopus databases. Afterwards, the reference lists of these articles provided 15 additional studies. After the removal of duplicates



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			(217), 472 studies were subjected to an initial analysis, based on the titles and abstracts and 117 were selected for full-text analysis. Finally, 79 studies were included in the systematic review for qualitative synthesis, following the exclusion of 38 as the inclusion criteria were not met.
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. – MANDATÓRIO <i>Trata-se de descrever de que forma se procedeu à extracção de dados dos estudos primários. Em consonância com as boas práticas da Cochrane, tal processo deverá envolver dois investigadores de forma independente.</i>	Page 13 and 14: “The databases were searched independently by two researchers, who later



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			evaluated the eligibility and quality of the identified studies, based on the title and abstract. If there was a disagreement regarding the inclusion of the articles, a consensus between the authors was reached through meticulous review and discussion.”
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. – MANDATÓRIO <i>Trata-se de descrever as variáveis para as quais foi obtida informação.</i>	Table 3
Risk of bias in individual studies / Risk of bias across studies	12/ 15	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. – MANDATÓRIO <i>Em todas as revisões sistemáticas, deverá existir um processo de avaliação da qualidade dos estudos primários. No caso de revisões sistemáticas de estudos experimentais/ensaios clínicos aleatorizados, a aplicação dos critérios de risco de viés (Risk of Bias) da Cochrane é altamente recomendada. No caso de revisões sistemáticas de estudos observacionais, poderão ser seguidos os critérios ROBINS ou os critérios dos National Institutes of Health (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools).</i>	Page 14: Methodological quality and risk of bias of each study was performed by the reviewers according to the ROBINS-I risk of bias tool for observational studies, SYRCLE’s risk of bias tool for animal studies



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			and NIH quality assessment tool for cross-sectional studies.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). – FACULTATIVO. APENAS NECESSÁRIO SE FOR FEITA META-ANÁLISE	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise.
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis. – FACULTATIVO. APENAS NECESSÁRIO SE FOR FEITA META-ANÁLISE	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise.
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. – FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise.
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. – MANDATÓRIO	Figure 3
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. – MANDATÓRIO	The features of the studies which have directly discussed OPN involvement in spinal pathologies, are summarized in



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			Table 1
Risk of bias within and across studies	19/22	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). – MANDATÓRIO	Page 28
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. – FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency. – FACULTATIVO. MANDATÓRIO APENAS SE FOR FEITA META-ANÁLISE	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise.
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). – FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise.
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). – MANDATÓRIO	Page 18-28: “Considering the abovementioned results, it follows that OPN might be a target for the ongoing



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			investigation on serum markers predictors for the onset and progression of AIS. The possible influence of OPN genetic polymorphisms in AIS, as already demonstrated in other diseases such as CSM, needs further research ⁷²⁻⁷⁴ .”
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). – MANDATÓRIO	Page 28 and 29: “Lastly, there were some conflicting results, mostly regarding the role of OPN in EF.”
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research. – MANDATÓRIO	Page 29: “In the future, OPN might be a potential target for development of novel therapies or play a role as a prognostic element in the onset and progression of



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			different spinal diseases.”
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. – SEGUIR RECOMENDAÇÕES DA REVISTA	Page 29: “This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.”

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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Instructions for Contributors

Revised: July 10, 2018

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Neurospine provides spine clinicians and researchers with peer-reviewed articles on basic and clinical investigation of spine and spinal cord to enhance patient management, education, clinical or experimental research, and professionalism. The journal will consider submissions in areas on craniocervical to lumbosacral spine including the followings; neuroscience and pain research, bone mineral research, disc and joint research, bio and industrial technology, pathophysiology, risk factors, symptomatology, imaging, treatment, rehabilitation of spine, and spinal cord/ peripheral nerve diseases. Specifically, basic and technology researches include the most influential research papers from all fields of science and technology, revolutionizing what physicians and researchers practicing the art of spinal neurosurgery worldwide know. Thus, we welcome valuable basic and translational technology research articles to introduce cutting-edge research of fundamental sciences and technology in clinical spinal neurosurgery. Clinical or Basic Research Articles, Review Articles, Case Reports, Technical Notes, and Letters to the Editor written in English will be accepted.

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1. Title Page

- 1) The title pages must be composed of external and internal title pages.
- 2) The external title page must contain the article title, and full names of all authors with their institutional affiliations both. The type of manuscript (original articles, review articles, case reports, technical notes, letters to the editor, brief communications) should also be addressed. When the work includes multiple authors with different affiliations, the institution where the research was mainly conducted should be spelled out first, and then be followed by foot notes in superscript Arabic numerals

beside the authors' names to describe their affiliation in a consecutive order of the numbers. Running head must be included consisting of no more than 65 characters/spaces.

The external title page must also contain the address, telephone and facsimile numbers, and e-mail address of the corresponding author at the bottom of the page, as well as information on the previous presentation of the manuscript in conferences and funding resources, if necessary.

- 3) The internal title page should only contain the article title. The internal title page must not contain any information on the names and affiliations of the authors.

2. Manuscript Format

- 1) The manuscript should be composed of no more than 5,000 English words for original and review articles, 3,000 English words for technical reports and case reports except for references, tables, and figures. It should be composed of no more than 600 English words for letters to the editor.
- 2) The article should be organized in the order of title, abstract, introduction, materials and methods, results, discussion, conclusion, references, tables, and figures or illustrations.
- 3) There should be no more than 40 references in original articles. In case reports, materials and methods and results can be replaced with cases. The number of references should be 20 or less and the figure number 5 or less.
- 4) Manuscript format may vary in review articles. There should be no more than 100 references in review articles.
- 5) Text should be written in 11 point fonts with double line spacing.

3. Abstract

- 1) Objective, Methods, Results, and Conclusion sections should be included in abstract of clinical or laboratory research, but are not necessary in other types of studies.
- 2) The abstract should include brief descriptions on the objective, methods, results, and conclusion as well as a detailed description of the data. An abstract containing 250 words or less is required for original articles and 200 words or less for case reports and review articles.
- 3) Abstract can be revised by the decision of editorial board, and some sentences can be modified as a result of revision.
- 4) A list of key words, with a minimum of two items and maximum of six items, should be included at the end of the abstract.
- 5) The selection of Key Words should be based on Medical Subject Heading (MeSH) of Index Medicus and the web site (<http://www.nlm.nih.gov/mesh/MBrowser.html>).

4. Introduction

The introduction should address the purpose of the article concisely,

and include background reports mainly relevant to the purpose of the paper. Detailed review of the literature should be addressed in the discussion section.

5. Materials and Methods

- 1) The article should record research plans, objective, and methods in order, as well as the data analysis strategies and control of bias in the study. Enough details should be furnished for the reader to understand the method(s) without reference to another work in the study described.
- 2) When reporting experiments with human subjects, the authors must document the approval received from the local Institutional Review Board. When reporting experiments with animal subjects, the authors should indicate whether the handling of the animals was supervised by the research board of the affiliated institution or such. Approved number of IRB must be noted.
- 3) Photographs disclosing patients must be accompanied by a signed release form from the patient or family permitting publication.
- 4) Ensure correct use of the terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors), and, unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex and gender. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity and justify their relevance.

6. Results

- 1) The authors should logically describe their results of observations and analyses performed using methodology given in the previous section and provide actual data.
- 2) For biometric measurements in which considerable amount of stochastic variation exists, a statistical evaluation is mandatory. The results must be solely from the findings of the current study and not refer to any previous reports.
- 3) While an effort should be made to avoid overlapping descriptions by Tables and by main text, important trends and points in the Table should be described in the text.

7. Discussion

Discussions about the findings of the research and interpretations in relation to other studies are made. It is necessary to emphasize the new and critical findings of the study, not to repeat the results of the study presented in the previous sections. The meaning and limitation of observed facts should be described, and the conclusion should be related to the objective of the study only when it is supported by the results of the research.

8. Conclusion

The conclusion section should include a concise statement of the major findings of the study in accordance with the study purpose.

9. References

The authors are responsible for the accuracy of the references. Key the references (double-spaced) at the end of the manuscript. End-Note users can access a direct download of the updated *Neurospine* Publications style at <https://www.e-neurospine.org>. References should be numbered consecutively in the order in which they are first mentioned in the text. All references cited in the text must be both listed and cited by the reference number (footnotes are not accepted). Use superscript numerals outside periods and commas, inside colons and semicolons. When more than 2 references are cited at a given place in the manuscript, use hyphens to join the first and last numbers of a closed series; use commas without space to separate other parts of a multiple citation (e.g., As reported previously,^{1,3-8,19}...The derived data were as follows^{3,4,12}.)

Do not link the references to the text. Cite unpublished data, such as papers submitted but not yet accepted for publication or personal communications, in parentheses in the text. If there are more than three authors, name only the first three authors and then use et al. Refer to the List of Journals Indexed in Index Medicus for abbreviations of journal names, or access the list at <https://www.nlm.nih.gov/archive/20130415/tsd/serials/lji.html>. Sample references are given below:

• Journal article

1. Sakai K, Okawa A, Takahashi M, et al. Five-year follow-up evaluation of surgical treatment for cervical myelopathy caused by ossification of the posterior longitudinal ligament. *Spine (Phila Pa 1976)* 2012;37:367-76.

• Book chapter

2. Sweitzer S, Arruda J, DeLeo J. The cytokine challenge: Methods for the detection of central cytokines in rodent models of persistent pain. In: Kruger L, editor. *Methods in pain research*. Boca Raton, FL: CRC Press; 2001:109-32.

• Entire book

3. Atlas SW. *Magnetic resonance imaging of the brain and spine*. Philadelphia: Lippincott Williams & Wilkins; 2001.

• Software

4. Epi Info [computer program]. Version 6. Atlanta: Centers for Disease Control and Prevention; 1994.

• Online journals

5. Friedman SA. Preeclampsia: A review of the role of prostaglan-

dins. *Obstet Gynecol* [serial online]. January 1988;71:22-37. Available at: BRS Information Technologies; McLean, VA. Accessed December 15, 1990.

• Database

6. CANCERNET-PDQ [database online]. Bethesda (MD): National Cancer Institute; 1996. Updated March 29, 1996.

• World Wide Web

7. Gostin LO. Drug use and HIV/AIDS [Internet]. June 1, 1996. Available at: <http://www.ama-assn.org/special/hiv/ethics>. Accessed June 26, 1997.

10. Tables

- 1) Tables should be created using the table formatting and editing feature of Microsoft Word. The title of the table must be noted. Tables cannot be submitted in a picture format.
- 2) Tables should be prepared in detail, in order to understand the contents of the manuscript without further reference.
- 3) Tables should be submitted separately from manuscript. Do not include vertical lines in table, and refer to the table formats in formal papers in *Neurospine*.

11. Figures and Illustrations

- 1) Figures should have resolution of 300 dpi or above and should be submitted individually (Namely, if Figure 1 is divided into A, B, C, and D, do not combine them into one, but submit each of them separately). Allowable file format for figures are JPG or TIF (TIFF) only.
- 2) Figures should be named according to figure name (example: Fig-1A.tif). If the quality of the photographs is considered as inappropriate for printing, resubmission of them can be requested by the journal.
- 3) Authors should submit figures in black and white if they want them to be printed in black and white. Authors are responsible for any additional costs of producing color figures (Additional cost for color printing is determined by the editorial board).
- 4) Line art should have resolution of 1,200 dpi or more in JPG or TIF format.

12. Author Check List

- 1) Before submitting the manuscript, authors should double-check all requirements noted in the agreement form regarding the registration and copyrights of their manuscript. A manuscript that does not fit the author instructions of the journal regarding format and references will be returned to the authors for further correction.
- 2) The page numbers in the manuscript should be counted from the page with the abstract, and the name and affiliation of the

authors should not appear thereafter.

- 3) Author check list should be prepared, signed by corresponding author, submitted with manuscripts, and then registered online. Relevant forms can be downloaded at manuscript submission site.

IV. Peer Review Process

All manuscripts are considered confidential. They are peer-reviewed by at least 2 anonymous reviewers selected by the Editor. The corresponding author is notified as soon as possible of the Editor's decision to accept, reject, or ask for revisions. The average time interval for an initial review process that involves both editorial and peer reviews is approximately 1 month; occasionally, there are unavoidable delays, usually because a manuscript needs multiple reviews or several revisions. When manuscripts are returned for revision, a cover letter from the Editor provides directions that should be followed carefully. When submitting the revised manuscript, authors should include a Response Letter, which describes how the manuscript has been revised. A point-by-point response to the Editor should be included with the revised manuscript. Authors who plan to resubmit but cannot meet this deadline should contact the Editorial Office. Manuscripts held for revision will be retained for a maximum of 90 days. The revised manuscript and the author's comments will be reviewed again. If a manuscript is completely acceptable, according to the criteria set forth in these instructions, it is scheduled for publication in the next available issue.

We neither guarantee the acceptance without review nor very short peer review times for unsolicited manuscripts. Commissioned manuscripts also are reviewed before publication.

We adopt double-blind peer review in which case, not only authors but also reviewers do not know each other.

V. Publication and Charges

- 1) Once a manuscript is accepted for publication by the journal, it will be sent to the press, and page proofs will be sent to authors. Authors must respond to the page proofs as soon as possible after making necessary corrections of misspellings, and the location of the photographs, figures or tables. Authors can make corrections for only typing errors, and are not allowed to make any author alteration or substantive changes of the text. Proofs must be returned to the press within 48 hours of receipt. No response from the authors within this time frame will lead the publication of the proof read without corrections, and the editorial board will not be responsible for any mistakes or errors occurring in this process.
- 2) There is no article processing charge (APC), also known as a publication fee including submission fee, for accepted articles.

VI. Ethical Guidelines

1. Research Ethics

- 1) All of the manuscripts should be prepared in strict observation of research and publication ethics guidelines recommended by the Council of Science Editors (CSE), International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), and the Korean Association of Medical Journal Editors (KAMJE).
- 2) Any study including human subjects or human data must be reviewed and approved by a responsible institutional review board (IRB). Please refer to the principles embodied in the Declaration of Helsinki (<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>) for all investigations involving human materials.
- 3) Animal experiments also should be reviewed by an appropriate committee (Institutional Animal Care and Use Committee, IACUC) for the care and use of animals. Also studies with pathogens requiring a high degree of biosafety should pass review of a relevant committee (Institutional Biosafety Committee, IBC). The editor of *Neurospine* always request submission of copies of informed consents from human subjects in clinical studies or IRB approval documents.

2. Conflict of Interest

- 1) The corresponding author of an article is asked to inform the Editor of the authors' potential conflicts of interest possibly influencing their interpretation of data. A potential conflict of interest should be disclosed in the cover letter even when the authors are confident that their judgments have not been influenced in preparing the manuscript. Such conflicts may be financial support or private connections to pharmaceutical companies, political pressure from interest groups, or academic problems. Disclosure form shall be same with ICMJE Uniform Disclosure Form for Potential Conflicts of Interest (http://www.icmje.org/coi_disclosure.pdf).
- 2) The Editor will decide whether the information on the conflict should be included in the published paper. Before publishing such information, the Editor will consult with the corresponding author. In particular, all sources of funding for a study should be explicitly stated. The *Neurospine* asks referees to let its Editor know of any conflict of interest before reviewing a particular manuscript.

3. Journal Policies on Authorship and Contributorship

- 1) Authors are required to make clear of their contribution to their manuscript in cover letter. To be listed as an author one should have contributed substantially to all three categories established

by the International Committee of Medical Journal Editors (IC-MJE): (1) conception and design, or acquisition, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published; and (4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

- 2) When a large, multicenter group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. When submitting a manuscript authored by a group, the corresponding author should clearly indicate the preferred citation and identify all individual authors as well as the group name. Journals generally list other members of the group in the Acknowledgments. Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship. Authors are responsible for replying to all questions asked by reviewers or editors that relate to the accuracy or integrity of any part of the work. All persons who have made a substantial contribution, but who are not eligible as authors, should be named in the acknowledgments. Authors are expected to consider carefully the way authors should be listed and ordered before submitting their manuscripts, and to provide a definitive list of authors with their original submission. Any addition, deletion, or rearrangement of author names in the authorship list should be made before the manuscript has been accepted—and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for requesting a change in the list of authors; and (b) written confirmation (by e-mail or letter) from all authors to say that they agree with the addition, removal, or rearrangement.

4. Redundant Publication and Plagiarism

- 1) Redundant publication is defined as “reporting (publishing or attempting to publish) substantially the same work more than once, without attribution of the original source(s)”. Characteristics of reports that are substantially similar include the following: (a) “at least one of the authors must be common to all reports (if there are no common authors, it is more likely plagiarism than redundant publication);” (b) “the subject or study populations are often the same or similar;” (c) “the methodology is typically identical or nearly so,” and (d) “the results and their interpretation generally vary little, if at all.”
- 2) When submitting a manuscript, authors should include a letter informing the editor of any potential overlap with other already published material or material being evaluated for publication and should also state how the manuscript submitted to *Neurospine* differs substantially from this other material. If all or

part of your patient population was previously reported, this should be mentioned in the Materials and Methods, with citation of the appropriate reference(s).

- 3) The editorial committee checks the similarity by using the iThenticate (<http://www.ithenticate.com/>) program for all submitted articles to prevent plagiarism. The editorial committee rejects the article suspected of plagiarism and asks the author to check whether it is plagiarized and make a resubmission.

5. Readership

It is primarily for clinicians and researchers who care patients with spine and spinal cord diseases. They are able to obtain tailored information to adopt for their research and practice. Its readership can be expanded to other positions: • Researchers can get the recent topics of clinical research in spine and spinal cord field and detailed research methods; • Clinicians in the field can get the new information and recent development for care of patients; • Medical teacher can access and adopt a variety of data in medical education; • Allied health professionals including nurses are able to get the recent information for care of patients with spine and spinal cord diseases; • Medical health students can understand the recent trends of the field and interesting cases for their work; • Policy makers are able to reflect the results of the articles to the nation-wide health care policies for patients with spine and spinal cord diseases; • The public, especially family of patients with spine and spinal cord diseases are able to read the advancement in their family's diseases so that they have a better knowledge on the diseases and a confidence in the clinicians' devotion to their family.

6. Obligation to Register Clinical Trial

- 1) Clinical trial defined as “any research project that prospectively assigns human subjects to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome” should be registered to the primary registry to be prior publication.
- 2) *Neurospine* accepts the registration in any of the primary registries that participate in the WHO International Clinical Trials Portal (<http://www.who.int/ictrp/about/details/en/index.html>) as well as <https://www.anzctr.org.au/>, www.clinicaltrials.gov, www.umin.ac.jp/ctr/index/htm and www.trialregister.nl. The clinical trial registration number shall be published at the end of the abstract.

7. Process for Identification of and Dealing With Allegations of Research Misconduct

When the Journal faces suspected cases of research and publication misconduct such as a redundant (duplicate) publication, plagiarism, fabricated data, changes in authorship, undisclosed conflicts of interest, an ethical problem discovered with the submitted manuscript, a

reviewer who has appropriated an author's idea or data, complaints against editors, and other issues, the resolving process will follow the flowchart provided by the Committee on Publication Ethics (<http://publicationethics.org/resources/flowcharts>). The Editorial Board will discuss the suspected cases and reach a decision. We will not hesitate to publish errata, corrigenda, clarifications, retractions, and apologies when needed.

Neurospine adheres to the research and publication ethics policies outlined in International Standards for Editors and Authors (<http://publicationethics.org>) and the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (<http://icmje.org>). Any studies involving human subject must comply with the principles of the World Medical Association Declaration of Helsinki. Clinical research should be approved by the Institutional Review Board, as well through patient consent. A patient's personal information cannot be published in any form. However, if it is absolutely necessary to use a patient's personal information, the consent of the patient or his/her guardian will be needed before publishing. Animal studies should be performed in compliance with all relevant guidelines, observing the standards described in the NIH Guide for the Care and Use of Laboratory Animals.

Cases that require editorial expressions of concern or retraction shall follow the COPE flowcharts available from: <http://publicationethics.org/resources/flowcharts>. If correction is needed, it will follow the ICMJE Recommendation for Corrections, Retractions, Republishings and Version Control available from: <http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/corrections-and-version-control.html> as follows:

Honest errors are a part of science and publishing and require publication of a correction when they are detected. Corrections are needed for errors of fact. Minimum standards are as follows: First, it shall publish a correction notice as soon as possible, detailing changes from and citing the original publication on both an electronic and numbered print page that is included in an electronic or a print Table of Contents to ensure proper indexing; Second, it shall post a new article version with details of the changes from the original version and the date(s) on which the changes were made through CrossMark; Third, it shall archive all prior versions of the article. This archive can be either directly accessible to readers; and Fourth, previous electronic versions shall prominently note that there are more recent versions of the article via CrossMark.

8. Handling Complaints and Appeals

The policy of the journal is primarily aimed at protecting the authors, reviewers, editors, and the publisher of the journal. If not described below, the process of handling complaints and appeals follows the guidelines of the Committee of Publication Ethics available from:

<https://publicationethics.org/appeals>

Who complains or makes an appeal?

Submitters, authors, reviewers, and readers may register complaints and appeals in a variety of cases as follows: falsification, fabrication,

plagiarism, duplicate publication, authorship dispute, conflict of interest, ethical treatment of animals, informed consent, bias or unfair/inappropriate competitive acts, copyright, stolen data, defamation, and legal problem. If any individuals or institutions want to inform the cases, they can send a letter to editor through <https://www.e-neurospine.org/about/contact.php>. For the complaints or appeals, concrete data with answers to all factual questions (who, when, where, what, how, why) should be provided.

Who is responsible to resolve and handle complaints and appeals?

The Editor, Editorial Board, or Editorial Office is responsible for them.

What may be the consequence of remedy?

It depends on the type or degree of misconduct. The consequence of resolution will follow the guidelines of the Committee of Publication Ethics (COPE).

9. Postpublication Discussions and Corrections

The postpublication discussion is available through letter to the editor. If any readers have a concern on any articles published, they can submit letter to the editor on the articles. If there founds any errors or mistakes in the article, it can be corrected through errata, corrigenda, or retraction.

10. Policies on data sharing and reproducibility

Until 2020, authors will be encouraged to share their data openly, but starting in 2021, they will be mandated to do so. The related regulation follows the open data sharing policy outlined below.

1) Open data sharing policy

For clarification on result accuracy and reproducibility of the results, raw data or analysis data will be deposited to a public repository, for example, Harvard Dataverse (<https://dataverse.harvard.edu/>) after acceptance of the manuscript. Therefore, submission of the raw data or analysis data is mandatory. If the data is already a public one, its URL site or sources should be disclosed. If data cannot be publicized, it can be negotiated with the editor. If there are any inquiries on depositing data, authors should contact the editorial office.

2) Clinical data sharing policy

This journal follows the data sharing policy described in "Data Sharing Statements for Clinical Trials: A Requirement of the International Committee of Medical Journal Editors" (<https://doi.org/10.3346/jkms.2017.32.7.1051>). As of July 1, 2018 manuscripts submitted to ICMJE journals that report the results of interventional clinical trials must contain a data sharing statement as described below. Clinical trials that begin enrolling participants on or after January 1, 2019 must include a data sharing plan in the trial's registration. The ICMJE's policy regarding trial registration is explained at <http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html>. If the data sharing plan changes

Table. Examples of Data Sharing Statements That Fulfill These ICMJE Requirements*

	Example 1	Example 2	Example 3	Example 4
Will individual participant data be available (including data dictionaries)?	Yes	Yes	Yes	No
What data in particular will be shared?	All of the individual participant data collected during the trial, after deidentification.	Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices).	Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices).	Not available
What other documents will be available?	Study Protocol, Statistical Analysis Plan, Informed Consent Form, Clinical Study Report, Analytic Code	Study Protocol, Statistical Analysis Plan, Analytic Code	Study Protocol	Not available
When will data be available (start and end dates)?	Immediately following publication. No end date.	Beginning 3 months and ending 5 years following article publication.	Beginning 9 months and ending 36 months following article publication.	Not applicable
With whom?	Anyone who wishes to access the data.	Researchers who provide a methodologically sound proposal.	Investigators whose proposed use of the data has been approved by an independent review committee (learned intermediary) identified for this purpose.	Not applicable
For what types of analyses?	Any purpose.	To achieve aims in the approved proposal.	For individual participant data meta-analysis.	Not applicable
By what mechanism will data be made available?	Data are available indefinitely at (<i>Link to be included</i>).	Proposals should be directed to xxx@yyy. To gain access, data requestors will need to sign a data access agreement. Data are available for 5 years at a third party website (<i>Link to be included</i>).	Proposals may be submitted up to 36 months following article publication. After 36 months the data will be available in our University's data warehouse but without investigator support other than deposited metadata. Information regarding submitting proposals and accessing data may be found at (<i>Link to be provided</i>).	Not applicable

* These examples are meant to illustrate a range of, but not all, data sharing options.

after registration this should be reflected in the statement submitted and published with the manuscript, and updated in the registry record. All of the authors of research articles that deal with interventional clinical trials must submit data sharing plan of example 1 to 4 in Table 1. Based on the degree of sharing plan, authors should deposit their data after deidentification and report the DOI of the data and the registered site.

For the policies on the research and publication ethics not stated in this instructions, International standards for editors and authors (<https://publicationethics.org/resources/resources-and-further-reading/international-standards-editors-and-authors>) can be applied.

All correspondences, business communications and manuscripts should be mailed to:

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