VOLUME 73 | ISSUE 2 | APRIL - JUNE 2022

REVIEW

The role of JAK1/2 kinases in the development of neurogenic heterotopic ossification following spinal cord injury

Kali E[1], Benetos IS[2], Pneumaticos S[3], Vlamis J[4]

[1]*BSc: Physiotherapist, Physical and Rehabilitation Medicine Department, Hygeia Hospital, Athens, Greece*

[2]*MD, PhD: Orthopaedic Surgeon, 3RD Department of Orthopaedic Surgery, KAT Hospital, University of Athens, Greece*

[3]*MD, PhD: Orthopaedic Surgeon, Professor and Head, 3RD Department of Orthopaedic Surgery, KAT Hospital, University of Athens, Greece*

[4]*MD. PhD: Orthopaedic Surgeon, Assistant Professor, 3RD Department of Orthopaedic Surgery, KAT Hospital, University of Athens, Greece*

ABSTRACT

Neurogenic heterotopic ossification is a complex disease that is characterized by the formation of heterotopic bone in soft tissues following central nervous system injuries and various neurological disorders. The exact mechanism of the disease and the factors that play a role in its development are still unknown and they comprise a promising research field. However, understanding the pathophysiology of the disease can lead us to diagnosis and help us find more effective ways of treatment.

The JAK-STAT signaling pathway is a mechanism of cellular signaling that is involved in many processes of the organism, such as the development of the skeletal system and the regulation of the neuroinflammatory response that follows spinal cord injuries. The purpose of this study is the research of the bibliography concerning the role of these JAK1 and JAK2 tyrosine-kinases in the development of neurogenic heterotopic ossification following spinal cord injuries.

KEY WORDS: Neurogenic heterotopic ossification, spinal cord injuries, JAK-STAT, JAK1, JAK2

Introduction

Heterotopic ossification, or *de novo* bone formation, represents pathologic condition during which benign, mature, lamellar bone is formed in tissues which do not belong in the skeletal system. Heterotopic bone formation is observed in tissues with high concentration of connective tissue cells (i.e. periosteum, peritenon, perimysium). Ectopic bone

CORRESPONDING AUTHOR, GUARANTOR

Kali Evangelia,
Physiotherapist, Athens, Greece
Email address: evangelia.kali@gmail.com

VOLUME 73 | ISSUE 2 | APRIL - JUNE 2022

has also been observed in the walls of blood vessels, ligaments and even in tissues of the abdomen [1-9].

In general, heterotopic ossification can be divided into three categories, depending on the etiology behind its formation: (a) traumatic, (b) neurogenic and (c) genetic [1,2,4-6,9,10]

Neurogenic heterotopic ossification is a type of *de novo* bone formation which best describes the cases of heterotopic bone formation following Central Nervous System (CNS) trauma, such as traumatic brain injuries (TBI) or spinal cord injuries (SCI). There is an ongoing debate as to the mechanisms and factors that play a role in the development of the disease. Nowadays, the role of JAK-STAT signaling pathway in the pathogenesis of the disease has been investigated due to its involvement in the neuroinflammatory process that follows spinal cord injury. This review will primarily focus on neurogenic heterotopic ossification following spinal cord injury and the role of JAK1/2 kinases.

A thorough literature search was performed using the 'PRISMA' systematic review guidelines and in the PubMed database using the key words "heterotopic ossification", "neurogenic heterotopic ossification", "JAK-STAT & spinal cord injury" and "JAK-STAT & heterotopic ossification". We limited our search to articles written in the English language and published from 1990 to 2020. The search yielded 7201 results in total. Removal of duplicate articles, resultied in 7071 articles of which 6965 were excluded since they weren't relevant to this review. The full text articles of the remaining 106 records were then investigated for eligibility and 50 of those were excluded for various reasons, leaving 56 articles for the synthesis of this review. There is abundant literature that highlights the role of JAK1and JAK2 proteins in various physiological processes but there is only one article that highlights the role of those tyrosine kinases in the formation of neurogenic heterotopic ossification following spinal cord injury (Table 1).

Discussion

The pathophysiology of Neurogenic Heterotopic Ossification

Neurogenic heterotopic ossification occurs in patients that have sustained traumatic brain and spinal cord injuries. Rarely, it may also occur in patients with Guillain-Barré syndrome, as well as in cases with tumors or hemorrhage of the central nervous system. Additionally, patients suffering from meningitis, myelitis, multiple sclerosis or are comatose for a long period of time also risk developing neurogenic heterotopic ossification [11-19]

The pathophysiology of heterotopic ossification is not clearly understood, even today, due to the complexity of the disease. In recent years, thanks to various research studies on Fibrodysplasia Ossificans Progressiva, progress has been made in understanding the mechanisms behind its appearance and we now know that various systemic and local factors play a role in its pathogenesis. In various studies, the local induction properties of Bone Morphogenetic Proteins –BMPs has been noted, as well as the systemic effect of prostaglandin E2 -PGE2. According to the bibliography, despite the existence of some common cellular mechanisms between Fibrodysplasia Ossificans Progressiva and the acquired types of heterotopic ossification, a unifying mechanism has not been found yet. It is important to note that the pathophysiology of the disease bears some similarities to the normal fracture healing process, making heterotopic ossification an obstacle when it comes to the treatment of orthopaedic patient [1,3,6,7,20–22]

Normal bone formation requires inductive signaling pathways that consist of chemokines, cytokines, bone morphogenetic proteins, growth factors, prostaglandins, interleukins, inductive osteoprogenitor cells and a permissive osteoinductive environment. Moreover, a proper quantity of osteoclasts which are responsible for the metabolic changes observed in normal bone, so that the skeletal system can adapt to various changes such as development and growth due to physical activities. During the normal bone development process, bone is formed after multipotent osteoprogenitor cells migrate, proliferate and differentiate. This process seems to bear some similarities to the pathological bone formation process that has been observed in cases of heterotopic ossification. It is also important to note that the heterotopic bone is similar to normal bone in

VOLUME 73 | ISSUE 2 | APRIL - JUNE 2022

some aspects but it does not have a periosteum and a high concentration of osteoblasts and osteoclasts. This type of bone is characterized by high biological activity and increased formation rates [1,3,6,7,20-22]

Recently, experimental studies have revealed certain mechanisms and molecules involved in the bone formation process of neurogenic heterotopic ossification and have led to the development of theories for the pathophysiology of the disease. The main theory comes from the observation that the serum of patients that sustained central nervous system injuries, when in contact with osteoprogenitor muscle cells, inducts mitosis and shows osteogenic properties. Debaud *et al,* developed an animal model where mice with thoracic spinal cord were injected with the snake poison cardiotoxin, to promote an inflammatory reaction. They reported that inflammation of the nervous system, due to peripheral trauma, leads to an increase of the heterotopic bone that is formed thus proving a correlation between neurogenic heterotopic ossification and abnormal molecular signaling. This theory supports that central nervous system injuries lead to a release of signaling molecules that interact with local and circulating progenitor cells, leading to their proliferation and differentiation to osteoblasts and finally to the formation of heterotopic bone. Three factors have been proven to be important in the pathophysiology of the disease and seem to mimic the stages of the normal fracture healing process: osteogenesis, osteoinduction, and osteoconduction) [1,3,6,7,11,13,15-18,20-28].

Inflammation and spinal cord injury

Central nervous system injuries are the first step in the process that leads to heterotopic bone formation, as they induce an inflammatory response. Three different stages can be distinguished: (i) the acute phase, (ii) the secondary and (iii) the chronic phase. The acute phase refers to the immediate tissue damage and usually lasts a few days. Mechanical injury leads to lesions of the nervous system and ischemia due to limited blood supply. The secondary phase refers to the inflammation process that follows the abnormal release of neurotransmitters and the subsequent damage to the tissues. In this stage, oxidative stress is often observed due to the established ischemia and the release of molecules related to inflammation. The chronic phase includes the Wallerian degeneration and the formation of scar tissue since healing is often incomplete, and the continuation of the cell apoptosis. The inflammatory response involves cells of the nervous system as well as cells recruited from the periphery [29-34].

Central nervous system has its own system of immunological response due to the presence of the blood brain barrier and the blood spinal cord barrier. Several studies support that there are lymphatic vessels in the blood spinal cord barrier that connect the central nervous system and the peripheral nervous system and during inflammation a migration of cells from the latter to the first one is observed. Important cells of the inflammatory process of the central nervous system are the microglial cells. Activation and proliferation of these cells results in the production of cytokines and recruitment of WBCs from the periphery to induce phagocytosis of injured tissues. Astrocytes are another type of central nervous system cells that participate in the inflammation process and are responsible for the recruitment of microglial cells. Astrocytes produce cytokines, such as CCL2, CXCL1, CXCL2, CXCL10, GM-CSF and IL-6, and recruit cells from the periphery. They are also responsible for the regulation of homeostasis. Other central nervous system cells are the oligodendrocytes that are responsible for the axonal myelin. They are part of the inflammatory response and they are sensitive to damage, leading to pathological conditions since inflammation induces autoimmunity against myelin. During inflammation oligodendrocytes precursor cells migrate to the point of damage [29,30,33-35]

Neutrophils are an example of cells that are recruited from the periphery during the inflammatory response, reaching maximum concentration after 1-3 days. These are phagocytic cells and secrete cytokines that help activate other immune cells. Macrophages are another type of cells that are recruited from the periphery. These cells exhibit different phenotypes, determined by their environment, adapt to the different stimuli and participate in the wound healing process. Macrophages exhibit two pheno-

VOLUME 73 | ISSUE 2 | APRIL - JUNE 2022

types during inflammation, M1 and M2, and their transcription is a complicated process with different factors regulating each type. M1 macrophages' transcription is regulated by STAT1, IRF-5 while M2 is regulated by STAT6, IRF-4 and PPARs. PPARs are transcription factors expressed in microglial cells, astrocytes, neurons and oligodendrocytes [29,30,33-35]. M1 macrophages, combined with endogenous microglial cells, express cytokines, such as TNF-α, IL-6, IL-12, and IL-1β, which induce CD4+ T cells that are responsible for both the phagocytosis of damaged cells and the recruitment of new neutrophils. At this stage, M2 macrophages express arginase-1 and Ym1 and there is an increase in markers such as IL-4, CD206 and Fizz-1, which are characteristic M2 activation markers. Macrophages and neutrophils also produce reactive oxygen species (ROS) and inducible nitric oxide synthase (i-NOS). These molecules are usually observed under normal conditions but after spinal cord injury or other pathological conditions they induce oxidative stress and neuronal death. Ahn *et al,* showed that apoptosis following spinal cord injury seems to depend on the caspase-3 pathway, activated through PPARs, inhibiting DNA repair processes. Therefore we can conclude that the complex macrophage population that is observed has a dual role as on one hand M1 promote the inflammatory response and phagocytosis and on the other hand M2 have anti-inflammatory properties and promote healing [29,30,33-35]

During inflammation, proliferation and differentiation of oligodendrocyte precursor cells are also observed, as well as astrocyte-driven scar tissue formation and Wallerian degeneration. At this stage M2 macrophages gradually decrease in numbers. Differentiation of oligodendrocyte precursor cells continues but full remyelination of axons isn't observed. It is easy to conclude that normal macrophages' function, during the inflammatory process does not promote full healing of the lesion and that abnormal expression of macrophages can lead to chronic inflammatory conditions and incomplete injury healing [29,30,33-35]

The role of JAK-STAT pathway

The JAK-STAT signaling pathway is a type of conserved cellular signaling which is involved in the processes of cell proliferation, differentiation, migration and the inflammatory response. It was first discovered due to interleukin IL-6 and IFNγ signaling. IL-6, together with IL-11, oncostatin M (OSM), leukemia inhibitory factor (LIF), CT-1 and neurotrophin-1, belong to the gp130 superfamily. Binding of these cytokines to the receptor leads to the homodimerization of gp130 and the activation of the appropriate JAK proteins [36-41].

The JAK protein family consists of four tyrosine kinases JAK1-3 and TYK2, while the STAT family consists of seven proteins STAT1-4, STAT5A/B and STAT6. STAT proteins have 6 regions with conserved structure and function. These six regions include a DNA binding region, an SH2 region and a region for transcription activation (TAD). JAKs have 7 domains with JAK homology (JH1-JH7). JH2 is a pseudokinase region and it is next to JH1. JAK-STAT proteins are expressed in all cells and their specificity is ensured by the variety of cytokines and growth factors that bind to them [36-40].

The JAK-STAT pathway is responsible for signaling processes of the nervous and skeletal system. The interaction between JAK-STAT signaling and the inflammatory process following spinal cord injury is a promising research field. It has been shown that cytokines of the gp130 family are responsible for the induction of inflammation and the activation of the JAK-STAT signaling pathway through JAK1 and JAK2 kinases and STAT1 and STAT3.[29,33,38-40,42-45].

JAK1 and JAK2 are involved in the process of bone formation. Oncostatin M, which belongs to the gp130 family, has the ability to bind to two receptors. Type I receptor consists of gp130 and LIF while the type II receptor consists of the complex gp130 and oncostatin M receptor (OSMR). Oncostatin M is secreted from hematopoietic cells, such as macrophages, neutrophils and dendritic cells and from osteocytes, osteoblasts and microglial cells. It has been shown that oncostatin M is responsible for the induction of mesenchymal spinal cord cells' differentiation to osteogenic cells and the *in vitro* differentiation of osteoblasts. Oncostatin M induces the phosphorylation of JAK1 and JAK2 in osteoblasts

VOLUME 73 | ISSUE 2 | APRIL - JUNE 2022

in animal models, while JAK1 and JAK2 knockout mice die early, either due to weight loss or anemia, prior to bone formation.

Skeletal growth also seems to be affected by STAT1 and STAT3. STAT1 related genes affect the inflammatory process while STAT3 seems to have an anti-inflammatory and pro-proliferation effect. STAT1 deficient mice exhibit higher bone mass and faster fracture healing since STAT1 naturally inhibits the transcription of Runx2 of osteoblasts. On the other hand, STAT3 deletion in mice osteoblasts leads to lower bone mass and inhibition of endochondral ossification, suggesting that STAT3 plays a role in embryonic development and in the processes of cell growth, inhibition of apoptosis and bone homeostasis through regulation of the B-xL, Bcl-2, Fas, Cyclin D1, Survivin and C-Myc genes. STAT3 mutations are associated with abnormal bone growth and increased bone decomposition, which supports the hypothesis that STAT3 promotes osteoblasts' function. There are various studies that suggest that STAT3 is important for chondrocytes' and osteoclasts' differentiation and therefore for the *in vivo* bone formation.[28,31,32,34,36,42,46-50].

The JAK-STAT pathway also seems to be involved in the central nervous system through its role in neurogenesis and scar tissue formation. It has been shown that JAK1,STAT1 and STAT3 are activated in cortical precursor embryonic cells following CNTF receptor activation, inducing the differentiation of neural stem cells and neural progenitor cells into astrocytes, through the gp130 signaling pathway. In general it has been shown that binding of IL-6 leads to the dimerization of gp130 and the subsequent activation of JAK-STAT and phosphorylation of STAT3. Moreover, prolactin induces both differentiation of astrocytes through phosphorylation of JAK2, STAT1, STAT5a and STAT5b [31,33,35,39,42,51,52]

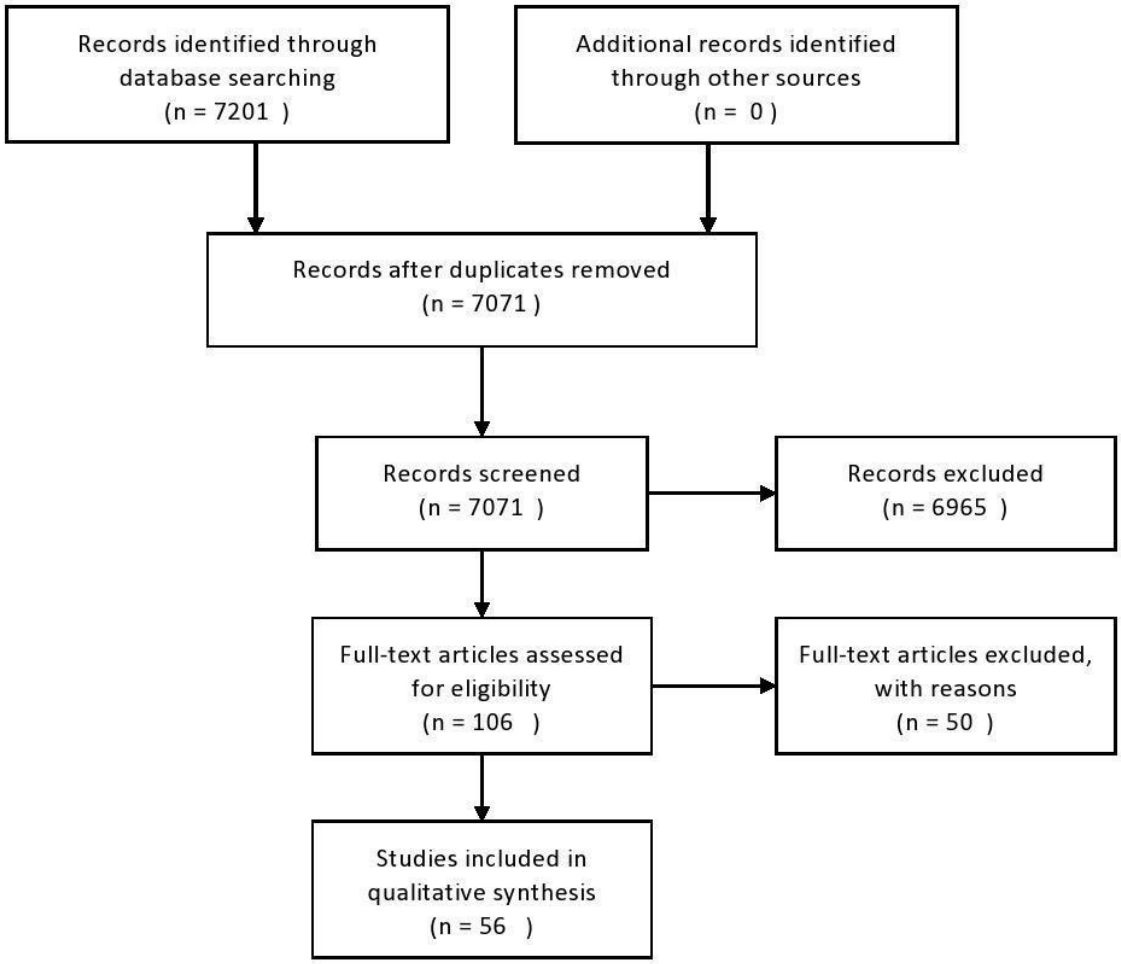
This pathway is also involved in the inflammation process following spinal cord injury. Inhibition of IL-6 receptor inhibits astrocytes' differentiation through JAK-STAT and astrogliosis leading to unhealed axons. It has also been shown that STAT3 inhibition leads to a decrease of active astrocytes, thus affecting barriers of the central nervous system. Leung *et al* showed that STAT3 expression in astrocytes lasted longer after deletion of SOCS3 and wound healing was improved. Astrocytes demonstrate neuroprotective abilities through the release of various cytokines. Among those are IL-6, transforming growth factor β1 and various neurotrophic factors such as NGF, CNTF and b-FGF. Research data supports that, following spinal cord injury, CNTF is produced by astrocytes and leads to phosphorylation of JAK1, JAK2, STAT1 and STAT3. Yamauchi *et al* studied the concentration of JAK1 and STAT3 in accordance to time and they proved that the maximum IL-6 concentration occurs simultaneously with the time point where JAK1 and STAT3 expression was at its maximum. They also showed that JAK1 expression starts immediately after the injury and when it reaches its peak, it gradually decreases, same as IL-6 expression.. Other studies have shown that administration of the inhibitor for miR-21, leads to inhibition of IL-6R/JAK-STAT pathway and better wound healing. Dai *et al,* through the usage of spinal cord injury mice models, noted that there appears to be a correlation between time and the expression of JAK2 and STAT3, which reached a peak after the 10th day post injury. SOCS3, which is a negative regulator of JAK-STAT and is induced by IL-6 cytokines through activation of STAT3, inhibits cellular apoptosis. Oncostatin M induces the activation of STAT3 and the subsequent activation of SOCS3. Experimental studies showed that GM-CSF factors induced activation of JAK2/STAT5 through IL-6, thus affecting cell proliferation and differentiation. On the other hand, use of IFNγ activates JAK2/STAT1 and JAK2/STAT3. All of the above suggest that SOCS3 is an important regulator of JAK-STAT signaling that reduces the risk of inflammation-related complications [16,18,23,26,28,30-32,34,35,38,42,46,47,53-56.]

Various studies that support the role of JAK1 and JAK2 proteins in the function of oncostatin M. Levy *et al* proved that OSM induces phosphorylation of JAK1 and JAK2 kinases and the subsequent activation of STAT1 and STAT3 in osteoblasts, in vitro. Moreover, macrophages activated after spinal cord injury, release oncostatin M among other osteogenic factors. Torrosian *et al* proved that in patients with neurogenic heterotopic ossifica-

VOLUME 73 | ISSUE 2 | APRIL - JUNE 2022

TABLE 1.

Flow diagram showcasing the results of a search in the PubMed database and the selection process for the articles used in this review



tion, activated macrophages induce inflammation through production of high OSM levels and that high plasma OSM concentration can be found in these patients. They also showed that deletion of the OSMR (receptor of OSM) gene in mice leadS to heterotopic ossification inhibition. Alexander *et al.* injected spinal cord injured mice with cardiotoxin and noted that spinal cord injury allows the entrance of monocytes/macrophages in the injured tissue and the production of OSM. Binding of OSM to the receptor promotes phosphorylation of JAK1, JAK2 and activation of STAT3 [16,18,23,26,28,30-32,34,35,38,42,46,47,53-56].

Conclusion

Neurogenic heterotopic ossification is a complex disease which still today is poorly understood. It is characterized by heterotopic bone formation following central nervous system trauma and after certain conditions such as Guillain-Barré syndrome or tumors. Spinal cord injuries are responsible for the formation of neurogenic heterotopic ossification which is observed in 20-30% of cases with such trauma. Heterotopic bone is often formed in the hip region but other areas are also susceptible, such as knees, elbows, shoulders, arms and spine. Spinal cord injury of the thoracic and cervical spine, leads to het-

VOLUME 73 | ISSUE 2 | APRIL - JUNE 2022

erotopic bone formation and ankylosis.

This disease exhibits similarities to the normal bone formation process; however its pathophysiology is not completely understood. Recent data highlight the importance of the inflammatory process that follows spinal cord injury. Up to this moment there is no conclusive data about the cell types involved in the formation of heterotopic bone, although literature data suggest that all of them bear similarities to osteoprogenitor cells. Chemokines, cytokines, macrophages and macrophage-derived factors such as oncostatin M and BMP-2 have been shown to have osteoinductive abilities, suggesting a possible correlation with heterotopic bone formation. A permissive environment is also of the utmost importance.

The JAK-STAT signaling pathway is a signaling pathway involved in many biological functions. There is abundant literature that highlights the role of JAK1 and JAK2 tyrosine kinases in normal skeletal development and neural cell differentiation and proliferation. In recent years, related research has being conducted on their role in spinal cord injuries and the inflammatory process that they trigger. Besides the research of Alexander *et al.,* there is no other research that directly implicates JAK1 and JAK2 proteins in the formation of heterotopic bone in neurogenic heterotopic ossification. The role of JAK1 and JAK2 following spinal cord injuries suggests that these proteins may also be involved in neurogenic heterotopic ossification and can be potential therapeutic targets.

REFERENCES

1. D. Shehab, A. H. Elgazzar, and B. D. Collier, "Heterotopic ossification.," *J. Nucl. Med.* 2002;43:345-53, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/11884494.
2. Subbarao JV, Garrison SJ. "Heterotopic ossification: Diagnosis and management, current concepts and controversies," *J. Spinal Cord Med* .1999;22:273–283
3. Nauth A *et al.*, "Heterotopic ossification in orthopaedic trauma.," *J. Orthop. Trauma 2012;26:684-8*, doi: 10.1097/BOT.0b013e3182724624.
4. Reichel LM, Salisbury E, Moustoukas MJ, DavisAR, Olmsted-Davis E, "Molecular Mechanisms of Heterotopic Ossification," *J. Hand Surg. Am.*,2014;39:563-566, doi: 10.1016/j.jhsa.2013.09.029.
5. Pape CH, MarshS, Morley JR, Krettek C, Giannoudis PV, "Current concepts in the development of heterotopic ossification," *J. Bone Joint Surg. Br.*, 2004;86-B:783–787, doi: 10.1302/0301-620X.86B6.15356.
6. Vanden Bossche L, Vanderstraeten G. "Heterotopic ossification: A review," *J. Rehabil. Med.* 2005;37:129–136, doi: 10.1080/16501970510027628.
7. Zychowicz ME. "Pathophysiology of heterotopic ossification.," *Orthop. Nurs.*, 2013;32:173–7, doi: 10.1097/NOR.0b013e3182920d85.
8. McCarthy EF, Sundaram M. "Heterotopic ossification: a review," *Skeletal Radiol.* 2005;34:609–619, doi: 10.1007/s00256-005-0958-z.
9. Meyers C*et al.* "Heterotopic Ossification: A Comprehensive Review," *JBMR Plus*, 2019;3:e10172, doi: 10.1002/jbm4.10172.
10. Hinck S., "Heterotopic Ossification: A Review of Symptoms and Treatment," *Rehabil. Nurs.* 1994;19:169–173, doi: 10.1002/j.2048-7940.1994.tb01578.x.
11. da Paz AC, Carod Artal FJ, Kalil RK. "The function of proprioceptors in bone organization: A possible explanation for neurogenic heterotopic ossification in patients with neurological damage," *Med. Hypotheses* 2007;68:67–73, doi: 10.1016/j.mehy.2006.06.035.
12. Seipel R, Langner S, Platz T, Lippa M, Kuehn JP, Hosten N. "Neurogenic heterotopic ossi-

fication: epidemiology and morphology on conventional radiographs in an early neurological rehabilitation population," *Skeletal Radiol.* 2012;41:61–66, doi: 10.1007/s00256-011-1115-5.

13. Sakellariou VI, Grigoriou E, Mavrogenis AF, Soucacos PN, Papagelopoulos PJ. "Heterotopic ossification following traumatic brain injury and spinal cord injury: insight into the etiology and pathophysiology" *J. Musculoskelet. Neuronal Interact.*, 2012;12:230–40, Available: http://www.ncbi.nlm.nih.gov/pubmed/23196266.
14. Mavrogenis AF, Guerra G, Staals EL, Bianchi G, Ruggieri P. "A classification method for neurogenic heterotopic ossification of the hip," *J. Orthop. Traumatol.*, 2012;13:69–78, doi: 10.1007/s10195-012-0193-z.
15. Van Kuijk AA. Geurts ACH, Van Kuppevelt HJM. "Neurogenic heterotopic ossification in spinal cord injury," *Spinal Cord.* 2002;40:313–326, doi: 10.1038/sj.sc.3101309.
16. Sullivan MP, Torres SJ, Mehta S,. Ahn J. "Heterotopic ossification after central nervous system trauma," *Bone Joint Res.* 2013;2:51–57, doi: 10.1302/2046-3758.23.2000152.
17. Brady RD, Shultz SR, McDonald SJ, O'Brien TJ. "Neurological heterotopic ossification: Current understanding and future directions" *Bone* 2018;109:35–42, doi: 10.1016/j.bone.2017.05.015.
18. Genêt F *et al.*, "Neurological heterotopic ossification following spinal cord injury is triggered by macrophage-mediated inflammation in muscle," *J. Pathol.* 2015;236:229–240, doi: 10.1002/path.4519.
19. Reznik JE *et al.*, "Prevalence and risk-factors of neurogenic heterotopic ossification in traumatic spinal cord and traumatic brain injured patients admitted to specialised units in Australia.," *J. Musculoskelet. Neuronal Interact.*,2014;14:19–28, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/24583537.
20. Edwards DS, Clasper JC. "Heterotopic ossification: a systematic review," *J. R. Army Med. Corps*, 2015;161:315–321, doi: 10.1136/jramc-2014-000277.
21. Zeckey C, Hildebrand F, Frink M, Krettek C. "Heterotopic ossifications following implant surgery--epidemiology, therapeutical approaches and current concepts.," *Semin. Immunopathol.*, 2011;33:273–86, doi: 10.1007/s00281-011-0240-5.
22. Ranganathan K *et al.*, "Heterotopic Ossification," *J. Bone Jt. Surg.* 2015;97:1101–1111, doi: 10.2106/JBJS.N.01056.
23. Debaud C *et al.*, "Peripheral denervation participates in heterotopic ossification in a spinal cord injury model," *PLoS One*, 2017;12:e0182454, doi: 10.1371/journal.pone.0182454.
24. Zhao Y *et al.* "Development process of traumatic heterotopic ossification of the temporomandibular joint in mice," *J. Cranio-Maxillofacial Surg.* 2019;47:1155–1161, doi: 10.1016/j.jcms.2018.11.026.
25. Tuzmen C, Verdelis K, Weiss L, Campbell P. "Crosstalk between substance P and calcitonin gene-related peptide during heterotopic ossification in murine Achilles tendon," *J. Orthop. Res.*, 2018;36:1444–1455, doi: 10.1002/jor.23833.
26. Davis EL,. Davis AR, Gugala Z Olmsted-Davis EA. "Is heterotopic ossification getting nervous?: The role of the peripheral nervous system in heterotopic ossification," *Bone*, 2018;109:22–27, doi: 10.1016/j.bone.2017.07.016.
27. Ramirez DM, Ramirez RM,. Reginato AM, Medici D. "Molecular and cellular mechanisms of heterotopic ossification.," *Histol. Histopathol.*, 2014;29:1281–5, doi: 10.14670/HH-29.1281.
28. Łęgosz P, Drela K, Pulik L, Sarzyńsk S, Małdyk P, "Challenges of heterotopic ossification—Molecular background and current treatment strategies," *Clin. Exp.*

Pharmacol. Physiol., 2018;45:1229–1235, doi: 10.1111/1440-1681.13025.

29. Xiao Q, Du Y, Wu W, Yip HK, "Bone morphogenetic proteins mediate cellular response and, together with Noggin, regulate astrocyte differentiation after spinal cord injury," *Exp. Neurol.*, 2010;221:353–366, doi: 10.1016/j.expneurol.2009.12.003.
30. Gensel JC, Zhang CB. "Macrophage activation and its role in repair and pathology after spinal cord injury," *Brain Res.* 2015;1619:1–11, doi: 10.1016/j.brainres.2014.12.045.
31. Dai J *et al.* "MicroRNA-125b promotes the regeneration and repair of spinal cord injury through regulation of JAK/STAT pathway," *Eur. Rev. Med. Pharmacol. Sci* 2018;22:582–589, doi: 10.26355/eurrev_201802_14271.
32. Dai J *et al.*, "MicroRNA-210 promotes spinal cord injury recovery by inhibiting inflammation via the JAK-STAT pathway," *Eur. Rev. Med. Pharmacol. Sci.*,2018;22:6609–6615, doi: 10.26355/eurrev_201810_16135.
33. Ahn YH, Bae Yeon Y, Lee G, Kang Mee K, Kang SK. "Molecular insights of the injured lesions of rat spinal cords: Inflammation, apoptosis, and cell survival," *Biochem. Biophys. Res. Commun.*, 2006; 348:560–570, doi: 10.1016/j.bbrc.2006.07.105.
34. Yan Z, Gibson SA, Buckley JA, Qin H, Benveniste NE, "Role of the JAK/STAT signaling pathway in regulation of innate immunity in neuroinflammatory diseases.," *Clin. Immunol.*, 2018;189:4–13, doi: 10.1016/j.clim.2016.09.014.
35. Yamauchi K *et al.*, "Activation of JAK/STAT signalling in neurons following spinal cord injury in mice," *J. Neurochem.*, 2006;96:1060–1070, doi: 10.1111/j.1471-4159.2005.03559.x.
36. Moresi V, Adamo S, Berghella L. "The JAK/STAT Pathway in Skeletal Muscle Pathophysiology," *Front. Physiol.*, 2019;10:1–10, doi: 10.3389/fphys.2019.00500.
37. Roskoski R, "Janus kinase (JAK) inhibitors in the treatment of inflammatory and neoplastic diseases," *Pharmacol. Res.*, 2016;111:784–803, doi: 10.1016/j.phrs.2016.07.038.
38. Levy JB, "Activation of the JAK-STAT signal transduction pathway by oncostatin-M cultured human and mouse osteoblastic cells," *Endocrinology*, 1996;137:1159–1165, doi: 10.1210/en.137.4.1159.
39. Wang T *et al.*, "The role of the JAK-STAT pathway in neural stem cells, neural progenitor cells and reactive astrocytes after spinal cord injury," *Biomed. Reports*, 2015;3:141–146, doi: 10.3892/br.2014.401.
40. Li J. JAK-STAT and bone metabolism. *JAK-STAT*, 2013;2:e23930, doi: 10.4161/jkst.23930.
41. Gotthardt D, Trifinopoulos J, Sexl V, Putz EM, "JAK/STAT Cytokine Signaling at the Crossroad of NK Cell Development and Maturation," *Front. Immunol.*, 2019;10:1–16, doi: 10.3389/fimmu.2019.02590.
42. Park KW, Lin CY, Benveniste EN, Lee YS. "Mitochondrial STAT3 is negatively regulated by SOCS3 and upregulated after spinal cord injury.," *Exp. Neurol.*, 2016;284:98–105, doi: 10.1016/j.expneurol.2016.08.002.
43. Tapia VS, Herrera-Rojas M, Larrain J. "JAK-STAT pathway activation in response to spinal cord injury in regenerative and non-regenerative stages of Xenopus laevis," *Regeneration*, 2017;4:21–35, doi: 10.1002/reg2.74.
44. Hudson SJ, Brett SJ. "Heterotopic ossification--a long-term consequence of prolonged immobility.," *Crit. Care*, 2006;10:174, doi: 10.1186/cc5091.
45. Zhang X *et al.*, "SOCS3 Attenuates GM-CSF/IFN-γ-Mediated Inflammation During Spontaneous Spinal Cord Regeneration," *Neurosci. Bull.*,2020;36:778–792, doi: 10.1007/s12264-020-00493-8.
46. Ning SL, Zhu H, Shao J, Liu YC, Lan J, Mia J., "MiR-21 inhibitor improves locomotor function recovery by inhibiting IL-6R/JAK-STAT pathway-mediated inflammation after spinal cord injury in model of rat," *Eur.*

VOLUME 73 | ISSUE 2 | APRIL - JUNE 2022

Rev. Med. Pharmacol. Sci.,2019;23:433–440, doi: 10.26355/eurrev_201901_16852.

47. Alexander KA *et al.*, "Inhibition of JAK1/2 Tyrosine Kinases Reduces Neurogenic Heterotopic Ossification After Spinal Cord Injury.," *Front. Immunol.*, 2019;10:377, doi: 10.3389/fimmu.2019.00377.
48. H. Tseng *et al.*, "Neurogenic heterotopic ossifications develop independently of granulocyte-colony stimulating factor and neutrophils," *J. Bone Miner. Res.*, p. jbmr.4118, Jun. 2020, doi: 10.1002/jbmr.4118.
49. Li JW, Kuang Y, Chen YL, Wang JF. "LncRNA ZNF667-AS1 inhibits inflammatory response and promotes recovery of spinal cord injury via suppressing JAK-STAT pathway," *Eur. Rev. Med. Pharmacol. Sci.*, 2018;22:7614–7620, doi: 10.26355/eurrev-201811-16375.
50. El Jammal T, Gerfaud-Valentin M, Sève P, Jamilloux Y. "Inhibition of JAK/STAT signaling in rheumatologic disorders: The expanding spectrum," *Jt. Bone Spine*, 2020;87:119–129, doi: 10.1016/j.jbspin.2019.09.005.
51. Cui M, Ma X, Sun J, He J, Shen L, Li F. "Effects of STAT3 inhibitors on neural functional recovery after spinal cord injury in rats," *Biosci. Trends*,2016;10:460–466, doi: 10.5582/bst.2016.01160.
52. Salisbury E, Sonnet C, Heggeness M, Davis AR, and E. Olmsted-Davis E. "Heterotopic ossification has some nerve.," *Crit. Rev. Eukaryot. Gene Expr.*,2010;20:313–24, doi: 10.1615/critreveukargeneexpr.v20.i4.30.
53. Dong L, Dong G, Cao J, Zhang J. "Association of α2-HS Glycoprotein with Neurogenic Heterotopic Ossification in Patients with Spinal Cord Injury," *Med. Sci. Monit.*, 2017;23:5382-5388, doi: 10.12659/MSM.904626.
54. Torossian F *et al.*, "Macrophage-derived oncostatin M contributes to human and mouse neurogenic heterotopic ossifications," *JCI Insight*, 2017;2:21,Cdoi: 10.1172/jci.insight.96034.
55. Tirone M *et al.*, "Severe Heterotopic Ossification in the Skeletal Muscle and Endothelial Cells Recruitment to Chondrogenesis Are Enhanced by Monocyte/Macrophage Depletion," *Front. Immunol.*, 2019;10:1640,, doi: 10.3389/fimmu.2019.01640.
56. Zhang X *et al.*, "Oncostatin M receptor β deficiency attenuates atherogenesis by inhibiting JAK2/STAT3 signaling in macrophages," *J. Lipid Res.*,2017;58:895–906, doi: 10.1194/jlr.M074112.

READY - MADE CITATION

Kali E, Benetos IS, Pneumaticos S, Vlamis J. The role of jak1/2 kinases in the development of neurogenic heterotopic ossification following spinal cord injury. *Acta Orthop Trauma Hell* 2022; 73(2): 145-154.