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INTEGRATED STUDY MASTER'S THESIS

Treatment of Heterotopic Ossification

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Declaration

I hereby declare that I have written this dissertation without the unauthorized help of third parties and without the use of other than the stated aids; the thoughts taken directly or indirectly from outside sources are identified and marked as such.

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1 ABBREVIATIONS

AF – Avulsion fractures

ALK2 – Activin receptor-like kinase 2

ALP – Alkaline phosphatase

BMP – Bone morphogenetic protein

CPDD – Calcium Pyrophosphate Deposition Disease

CT – Computed tomography

DC – Dystrophic calcification

Dkk1 – Dickkopf-1

EndMT – Endothelial-to-mesenchymal transition

GS – Glycine-serine

Hh – Hedgehog

HIF-1 – Hypoxia-inducible factor 1

HO – Heterotopic Ossification

ISS – Injury severity score

MO – Myositis Ossificans

MRI – Magnetic resonance imaging

mTOR – Mammalian target of rapamycin

OS - Osteosacroma

RAR – Retinoic acid receptors

SCI – Spinal cord injury

SOST – Sclerostin

TBI – Traumatic brain injury

TC – Tumoral calcinosis

THA – Total hip arthroplasty

TKA - Total knee arthroplasty

Trf-2 – Transferrin receptor-2

US – Ultrasonography

2 SUMMARY

The treatment of heterotopic ossification (HO), a pathological disease in which ectopic bone forms in soft tissues, is examined in this thesis. HO can be acquired, for example after burns, trauma, surgery, or brain injury, or it can be caused by genetic disorders such as progressive osseous heteroplasia (POH) or fibrodysplasia ossificans progressiva (FOP). Examining new and existing HO therapy modalities is the goal of this thesis. It focuses on pharmacological, physiotherapeutic, surgical, and experimental techniques. Identifying clinical difficulties, assessing current treatment outcomes, and demonstrating future advancements based on molecular pathway blockage. A comprehensive literature review was conducted to collect and examine information on the epidemiology, pathophysiology, diagnosis, and management of acquired and genetic HO. This involved a critical assessment of current advancements in targeted molecular therapy, case reports, and clinical research. Since these are important mechanisms for ectopic bone formation, special attention was paid to signaling pathways like BMP/Smad, RAR, Hedgehog, Wnt/β-catenin, EndMT, and mTOR/HIF-1α. The results demonstrate that although surgical excision is still the most effective treatment for mature HO, there are risks involved, including infection, bleeding, and even recurrence. Physiotherapy has a helpful but discussed role. Aggressive movement may exacerbate the ossification, whereas mild, painless mobilization is helpful. In high-risk patients, pharmacologic prophylaxis with NSAIDs, bisphosphonates, or radiation has been shown to be successful in lowering the risk of HO. Furthermore, promising new treatments specifically target molecular pathways involved in inflammation and bone formation, have shown to be helpful as well. In conclusion, treating HO requires a customized, multidisciplinary strategy. Although conservative and surgical approaches are currently the norm, customized therapies that act at the molecular level to stop or reverse ectopic bone growth are the way of the future.

3 KEYWORDS

Heterotopic Ossification, Acquired HO ,Fibrodysplasia Ossificans Progressiva (FOP), Diagnostic tools, Treatment Strategies, Surgical Excision, Physical Therapy, Pharmacological Prophylaxis, Innovative Therapies

4 INTRODUCTION

Heterotopic ossification (HO) is a pathological condition characterized by the abnormal formation of bone in soft tissues such as muscles, tendons, or ligaments. Joint pain, stiffness, and in extreme situations, ankylosis or complete immobility, can result from it. In general, there are two types of HO, acquired and genetic. The most prevalent causes of acquired HO include burns, trauma, surgery, and neurologic traumas, such as traumatic brain injuries or spinal cord injuries. On the other hand, certain gene mutations that result in progressive and spontaneous ossification generate hereditary forms such as FOP. Despite decades of research, HO is difficult to diagnose and treat due to its complex mechanism and wide range of clinical manifestations. There are few treatment options available, and they often rely on the location of the ectopic bone, the degree of symptom intensity, and the maturity of the ossification. Despite the risks of recurrence and consequences like bleeding or infection, surgical excision is still the most common treatment for mature HO. Additionally, non-surgical therapies like radiotherapy, NSAIDs, and physical therapy are employed, either as preventative measures or in combination with surgery. New treatment strategies that target certain signaling pathways involved in HO formation, such as BMP, RAR, Hedgehog, and Wnt/β-catenin, have shown promising results. These techniques give hope for future therapies that are more tailored and successful. This thesis offers a thorough overview of the many approaches being used to treat HO, ranging from new targeted treatment to traditional surgical and pharmaceutical approaches. Additionally, it looks at the distinctions between acquired and genetic HO and talks about how these differences affect therapeutic management.

5 LITERATURE REVIEW

5.1 DEFINITION

„Heterotopic ossification (HO) is the formation of ectopic osseous lesions within soft tissue or joints”(1). It can be acquired or genetic HO. Acquired HO is often caused by trauma, e.g. fractures, severe soft tissue damage, burns, amputations, spinal cord injury or traumatic brain injury. It can also be due to an iatrogenic trauma after surgery. These osseous lesions can cause nerve compression, resulting in intense discomfort, open or non-healing sores, and a limited range of motion due to physical obstruction inside joints. Genetic HO is due to a genetic mutation in bone

morphogenetic protein (BMP) receptors. Acquired HO represents a far greater number of HO patients than genetic HO.(1)

5.2 EPIDEMIOLOGY

Nongenetic HO is often seen in young adults, most following a traumatic event. In up to 75% it develops after trauma. The risks of developing HO are positively correlated with the injury severity score (ISS) in trauma patients. (1) Additionally, sex-related variables influence the tendency for HO. Males are more likely to be impacted than females at a 3:2 ratio , possibly due to the differences in muscle mass, different mechanisms of injury, and different hormonal signaling pathways that influence osteogenesis.(2) (3) The risk for HO is also influenced by the mechanism of injury, length of immobility, percentage of total body surface area burnt, and degree of spasticity. (2) A considerably increased risk of developing HO is linked to several disorders. These include bone fractures and dislocations, which can account for up to 30% of cases, and orthopedic procedures, such as total hip arthroplasty (THA), and put patients at risk; research has shown that up to 58% of THA patients acquire HO. High-energy limb trauma, traumatic brain injury, spinal cord injury (with HO occurring in as many as 50% of cases), and severe burns, which impact up to 20% of patients with third-degree burns, are other high-risk situations. Notably, the incidence of HO may surpass 90% in cases of severe traumatic amputations.(3)

5.3 PATHOPHYSIOLOGY

The exact mechanism of HO is unknown. But studies suggest that after the traumatic injury or neurological event the biological response on tissue level starts. Bone morphogenic proteins (BMPs) are expressed in the injured area. These proteins encourage the migration of mesenchymal stem cells, sometimes referred to as satellite cells, to the location, where they develop into fibroblasts and then osteoblasts, which are cells that form bone. Alkaline phosphatase and other enzymes contribute by encouraging the production of bones, especially under inflammatory conditions. Fibroblasts create immature connective tissue during this process, which can transform to resemble natural bone formation (endochondral ossification), when subjected to continuous stress. Fibroblasts develop into chondrocytes, or cartilage cells, some of which go on to become osteoblasts. Osteoid, or immature bone tissue, starts to form in 1-2 weeks. As calcium

pyrophosphate is gradually replaced by hydroxyapatite, this tissue mineralizes and causes bone to mature in an irregular location.(17)

5.4 HISTOLOGY

Based on its histological structure, HO can be differentiated from other disorders such as osteosarcoma (OS) or myositis ossificans (MO). A crucial characteristic of HO is its zonal pattern of maturation. Immature lesions are hypercellular and contain fibroblasts and spindle cells. (Figure 2)(3) Mature lesions show well-developed lamellar bone with fatty marrow, blood vessels, and look similar to normal bone tissue.(Figure 3)(3) The first stage of the HO process is fibroblastic metaplasia, in which fibroblasts change into chondroblasts and then osteoblasts.(7) Depending on where the HO is located, this may occur after intramembranous or endochondral ossification.

According to histological research, a capsule of compressed muscle and connective tissue frequently surrounds the mature HO. (7)The hypercellular tissue in early HO might be mistaken with soft-tissue malignancies, such as sarcomas. However, HO does not have nuclear abnormalities or abnormal mitoses like OS does. The type of ossification can change depending on the area. For instance, MO rarely manifests cartilage and typically develops through intramembranous ossification. On the other hand, HO in tendons or periarticular tissues is more likely to follow an endochondral pathway, which often begins with the manifestation of cartilage. Low oxygen levels in the surrounding tissue may also encourage the production of HO.(7)

The ossification mechanism is more reliable in genetic HO forms. POH follows an intramembranous pathway, and FOP usually follows an endochondral pathway with significant cartilage production. The addition of DC, which occasionally develops into bone, makes it more complicated. This happens particularly in HO linked to the skin or tendons. (3)

It is important to comprehend the histological phases and forms of HO in order to diagnose the condition, differentiate it from cancer, and determine the best course of treatment,

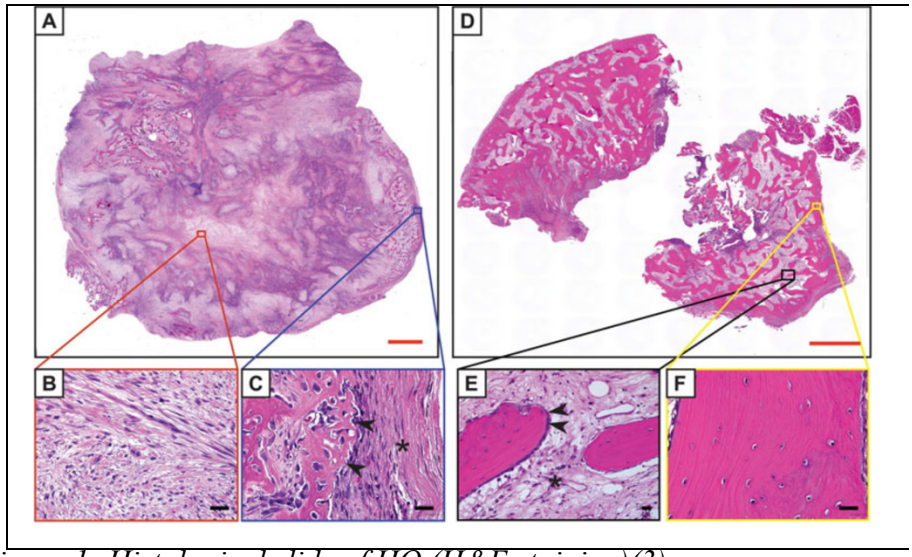

Figure 1: Histological slide of HO (H&E staining)(3)

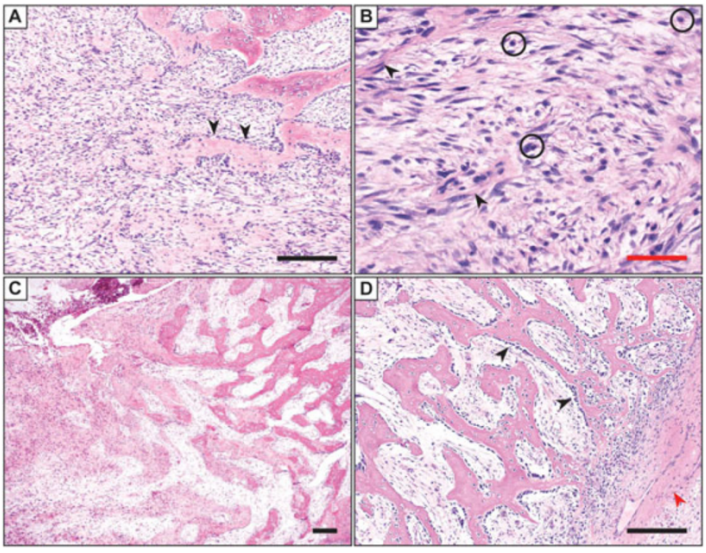

Figure 2: early HO (H&E staining)

***A:** shows a highly cellular lesion made up of spindle-shaped cells and scattered inflammatory cells.* ***B:** Higher magnification reveals these spindle cells in an edematous or fibrous background with small capillary-like vessels and no signs of malignancy.* ***C:** As the lesion matures, a transition from woven to lamellar bone is visible.* ***D:** Osteoblasts line the new bone, and the lesion is often surrounded by a fibrous capsule with blood vessels.(3)*

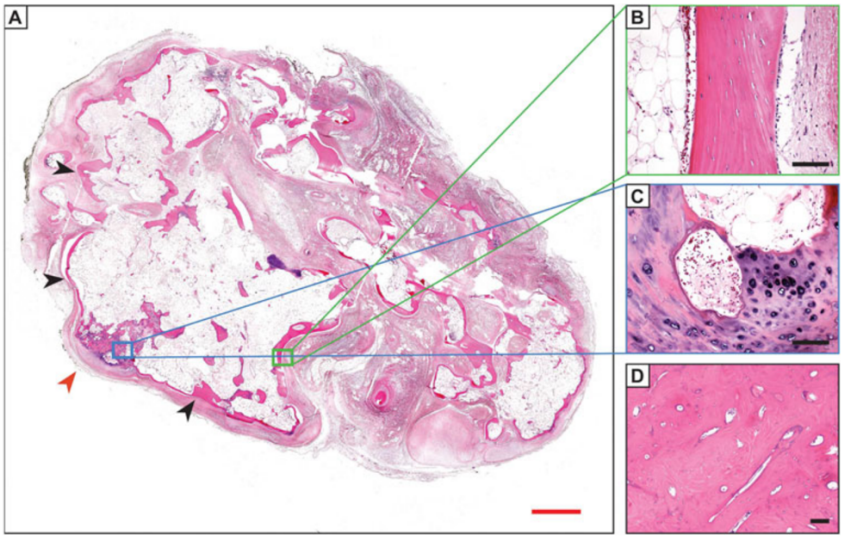

Figure 3: Mature HO (H&E staining)

A: *Mature HO appearance, with foci of endochondral ossification (red arrowhead), a fatty marrow cavity inside (black asterisk), and thicker peripheral bone (black arrowheads).* ***B:*** *features similar to natural cortical bone, a neo-cortex form. On the left side, a lot of adipocytes in the interior.* ***C:*** *Focal metaplastic cartilage and endochondral ossification.* ***D:*** *sclerotic bone(3)*

5.5 CLINICAL RISK FACTORS: MECHANISM OF INJURY

5.5.1 SPINAL CORD AND TRAUMATIC BRAIN INJURY (TBI)

10 % to 53% of patients develop HO after a central neurologic injury, such as SCI or TBI. (Table 1) (4) The relationship between these injuries and formation of ectopic bone is still not completely clear. But studies show that peripheral neurotransmitters affect the formation of osteoblasts.(5) Risk factors that influence the formation of HO after SCI include the type and severity of injury and the level of injury. Low cervical or high thoracic injuries are more prone to develop HO.(5) Complications, such as severe spasticity, impaired cognition, tracheostomy, pneumonia, and urinary tract infections can put the patients at a higher risk. (5) HO after SCI usually forms caudal to the level of injury and around larger joints, mostly the hip.(Figure 4) (2) In comparison in TBI patients HO can form around any large joint, such as hip, knee, elbow, or shoulder. The risk factors for TBI patients are very similar to these of SCI patients. Other factors that could influence the formation of HO could include prolonged immobilization, vascular stasis, edema, and passive manipulation of joints in immobilized patients.

5.5.2 HIP ATRHOPLASTY

HO occurs in approximately 40% of patients following hip arthroplasty.(Table 1) 2%-7% even develop extensive periarticular HO.(3) The patient-related risk factors are not fully clear, but patients with ankylosing spondylitis, Paget's disease, and hypertrophic osteoarthritis have shown to be at higher risk. Surgical factors, such as extended ischemia time, use of cemented implants and type of surgical approach are possibly increasing the risk as well. (6)

5.5.3 FRACTURES

The most studied orthopedic traumas are acetabular and elbow fractures. HO occurs in about 40% of people with surgical fixated acetabular fractures.(2) Risk factors include the surgical approach, concomitant neurologic injury, delayed internal fixation, and use of bone graft and/or bone-graft substitute. Approximately 20% of these patients develop severe restriction in ROM due to the HO. In patients with elbow fractures around 40% develop HO. The most common site of HO in these patients is posteromedial. Concomitant distal humeral fracture, triad injury, Monteggia fracture-dislocation, and trans olecranon fracture-dislocation were linked to more severe HO. For patients at high risk for developing HO prophylaxis and surgical excision should be considered.

5.5.4 THERMAL INJURY

HO is a well-known complication for burn patients. The larger the total body surface area affected the higher is the risk of developing HO. Burns that include >20% body surface area increases the risk immensely. Male-sex and full-thickness injury at or near the joint are other important risk factors. The most frequent location of HO in burn patients is the elbow, then the shoulder and lastly the hip. (Figure 4) The earliest symptom that burn patients usually show is a restriction in ROM. Other symptoms can be pain, erythema, swelling, and palpable bone formation. It is important to differentiate between scar contracture near a joint and HO. (2)

Table 1: Rates of HO according to cause of Trauma(2)

Cause of Trauma	Rate of HO (%)
Thermal burn	0.2-4
Hip arthroplasty	3-90
Neurologic injury	10-53
Spinal cord injury	20

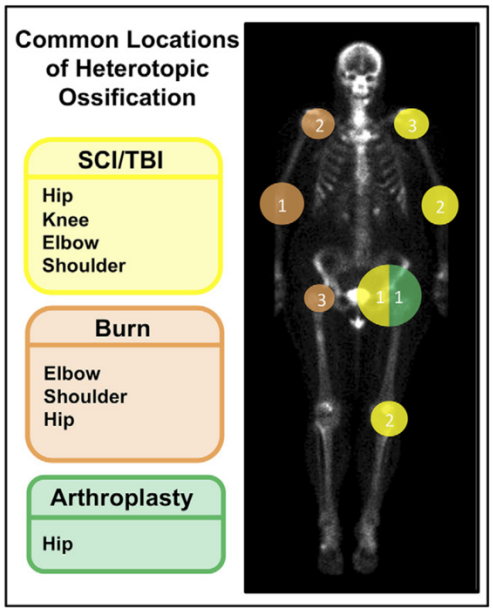

Figure 4: The common locations of HO according to the mechanism of injury.

According to the mechanism of injury, the numbering system, which goes from 1 (highest) to 3 (lowest), shows a declining prevalence of heterotopic ossification at the designated joint.(2)

5.6 GENETIC HO

5.6.1 FIBRODYSPLASIA OSSIFICANS PROGRESSIVA (FOP)

The most common form of genetic HO is FOP. FOP is an extremely rare genetic mutation with a prevalence of 1 in 2 million individuals worldwide. It is a life-threatening disease. Except for a common congenital deformity of the great toes, patients usually appear normal at birth.(Figure 5)

(7) Extraskeletal ossification is a pathological process that usually starts in early childhood and is frequently brought on by mild physical trauma, such as regular immunizations or little falls that occur during play. Known as "flare-ups," these episodes are characterized by excruciating swellings of the soft tissues. Usually beginning in the trunk, ectopic bone production gradually proceeds to spread to the whole body. (8) Heterotopic bone eventually bridges joints, resulting in severe mobility restriction or total loss. With a typical life expectancy of about 40 years, most people with FOP pass away from problems associated with thoracic insufficiency syndrome.

Laboratory tests for FOP patients often show raised serum alkaline phosphatase levels and increasing urinary concentrations of basic fibroblast growth factor during acute flare-ups. Imaging studies are essential for evaluating and tracking the course of disease. Conventional radiographs frequently show ankylosis of neighboring joints because of ectopic bone growth and generally show broad HO without a uniform anatomical distribution.(9) Early, pre-osseous alterations can be detected with MRI, which shows afflicted areas as hyperintense lesions, frequently accompanying soft tissue edema. Compared to MRI and conventional radiography, CT offers a higher degree of spatial resolution and three-dimensional reconstruction, enabling in-depth volumetric examination of ossified regions. Using the Lederson grading scale CT imaging also makes it easier to grade the degree of joint ankylosis.(10)

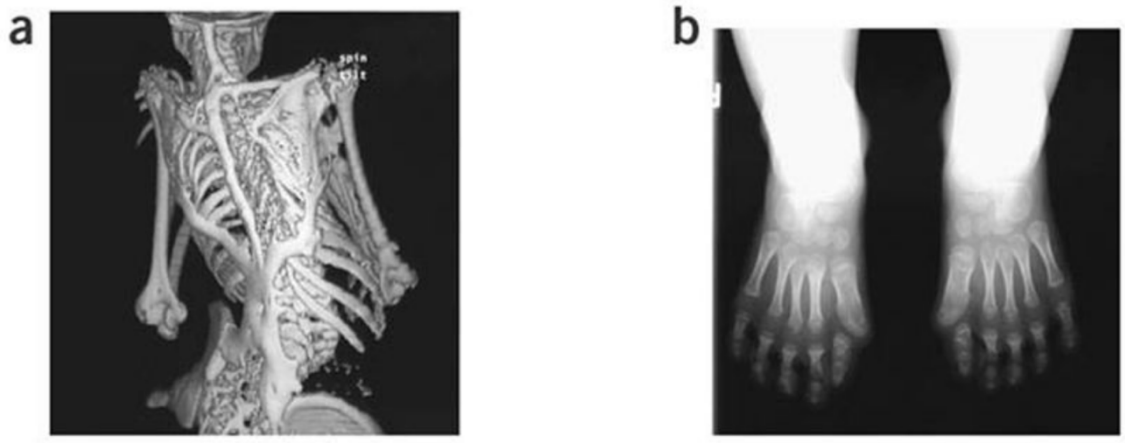

Figure 5: Typical clinical manifestations of FOP.

a: three-dimensional reconstructed CT scan shows a 12-year-old child's back with extensive heterotopic bone growth, typical for FOP. b: anteroposterior radiography of a 3-year-old child's foot on display symmetrical great toe abnormalities.(9)

Since HO usually only starts after flare-ups brought on by mild trauma, inflammation, or other immunological stimuli, this pattern has led to the theory that the onset of HO in FOP is significantly influenced by immune-mediated processes. This is confirmed by the well-established efficacy of anti-inflammatory treatments to reduce the development of HO. To avoid HO production, for example, high-dose corticosteroids have been used prophylactically within the first 24 hours after injury. Additionally, it has been demonstrated that pharmacologic reduction of inflammation or elimination of inflammatory cells dramatically inhibits the growth of ectopic bone in animal models of FOP.(11)

5.6.2 PROGRESSIVE OSSEOUS HETEROPLASIA (POH)

POH is a very rare genetic condition. The early development of HO, usually starting in infancy, is characteristic for POH. (12) POH is a mesenchymal differentiation developmental disorder characterized by progressive HO in cutaneous, subcutaneous, and deep connective tissues during childhood and dermal ossification in infancy. In contrast to FOP POH has cutaneous ossification, asymmetric lesion distribution, unexpected ossification patterns, a majority of intramembranous ossification, and no congenital skeletal abnormalities.(13) Initially presenting as ossification in the dermis and subcutaneous fat, the disease gradually spreads to deeper connective tissues, such as muscle, fascia, tendons, and ligaments, ultimately impairing limb growth and joint mobility. POH's genetic foundation are loss-of-function mutations in the GNAS1 gene. The first clinical symptoms are frequently a maculopapular rash (Figure 6), which is either present at birth or develops in the first few weeks of life and is caused by patchy bone development within the dermis. Crucially, in contrast to other types of HO, POH's ossification process is not brought on by infection or trauma and is not associated with metabolic problems. Instead, it moves past the cartilage intermediate found in endochondral processes by means of intramembranous ossification.(12)

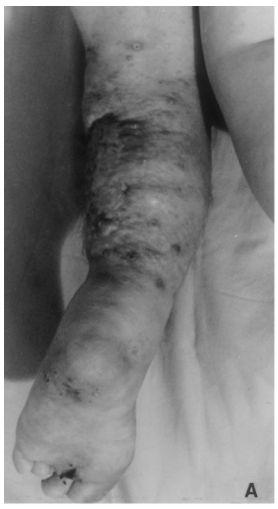

Figure 6: Early manifestation of severe heterotopic ossification in POH.

Severe maculopapular rash. Posterior view of the left leg and popliteal fossa of a 5-year-old child with POH.(13)

5.6.3 DIFFERENCES BETWEEN GENETIC AND ACQUIRED HO

Despite having the same pathway, which is endochondral ossification, genetic and acquired HO differ in the underlying mechanisms and clinical manifestations. (14) Genetic HO, as seen in FOP, is caused by a gain-of-function mutation in the *ACVR1* gene (R206H), leading to hypersensitivity to BMP ligands and resistance to inhibition. In contrast, trauma-induced HO results from external factors such as surgery, musculoskeletal injury, or neurologic trauma, without any underlying genetic mutation. FOP is typically evident in early childhood and is marked by congenital malformations, most notably of the great toes, which is a key diagnostic feature absent in acquired forms.(15) In FOP, HO can occur both at injury sites and spontaneously in tendons and ligaments, whereas trauma-induced HO is often restricted to injured soft tissues, primarily skeletal muscle. Inflammatory “flare-ups” in FOP often affect specific anatomical regions such as the neck, jaw, and shoulders, and may be triggered by minor trauma like vaccination, unlike the localized inflammation seen in acquired HO. Furthermore, while FOP progresses over time to form an ectopic skeleton that fuses with normal bone, acquired HO tends to be localized and once mature non-progressive. This distinction also influences the treatment. In mature HO surgical excision is often the chosen treatment, whereas surgery is contraindicated in FOP due to the risk of exacerbating the ossification. Instead, anti-inflammatory therapies, such as glucocorticoids, are used in FOP to manage flare-ups.(16)

Table 2: Differences between genetic and acquired HO(14–16)

Feature	Genetic HO (FOP)	Acquired HO (Trauma-Induced)
Cause	Mutation in ACVR1 (R206H)	Physical trauma, surgery, burns, or neurologic injury
Inheritance	Autosomal dominant (congenital)	Not inherited
Trigger	Minor trauma or spontaneous (e.g., vaccination, viral illness)	Significant local trauma or inflammation
Progression	Progressive; forms ectopic skeleton that fuses with normal bone	Non-progressive; often limited to injury site
Tissues Involved	Muscles, tendons, ligaments (injury and non-injury sites)	Primarily skeletal muscle near injury
Congenital Features	Malformed great toes (diagnostic hallmark)	None
Inflammatory Flare-Ups	Common; regional pattern (e.g., neck, jaw, shoulders)	Localized to site of trauma only
Ossification Pathway	Endochondral ossification	Endochondral ossification
Treatment Options	Surgery contraindicated; glucocorticoids for flare-ups	Surgical excision possible after maturation
Typical Onset	Early childhood (before age 10)	Any age following trauma
Imaging Characteristics	Diffuse, patterned ossification ('cocoon-like' in POH)	Localized, asymmetric ossification

5.7 CLINICAL PRESENTATION

The stage of nongenetic HO development determines the clinical presentation. During the early/inflammatory stage HO often manifests as localized pain, soreness, and edema. Rapid size growth is a common feature of HO at this period, which could raise clinical suspicion of a soft tissue sarcoma.(3) Later on, when the bone tissue gradually matures, the swelling gets more confined, firmer, and may limit motion if it is close to a joint.

HO most commonly affects the hips, knees, shoulders, and elbows. These symptoms can appear from 3 to 12 weeks after the initial traumatic event.(18) HO could also lead to peripheral nerve entrapment and pressure ulcers. (19) There are various classification systems depending on the location of the symptoms.(2)

5.8 DIAGNOSIS

The first step in diagnosing HO should be physical examination. In the examination signs like swelling, joint stiffness, decreased range of motion, and pain could be detected. Symptoms of nerve damage should also be examined. To establish a definitive diagnosis imaging test are needed. (20)
The initial imaging test performed to identify nongenetic HO is frequently plain radiography. Radiographs have the advantages of being inexpensive and relatively simple to obtain. But they are only able to detect calcifications after 6 weeks and are not able to visualize the anatomic extent of ectopic bone deposition. (2) (18,20)
Therefore, when patients present with symptoms of HO or ossified lesions are showing on x-ray, a second imaging modality should be used and the only therapeutic option usually is surgical intervention, which does not restore the function of the affected site.(20)

On radiographs HO appears phasic and dynamic. The opacities of early lesions may be uneven and lack distinct zonal patterns. Though it normally only affects soft tissue, HO can sometimes adhere to bone, called parosteal HO, which in chronic situations may display a broad-based bony stalk. Extensive ossification in later phases can result in joint ankylosis. Mature intramuscular HO typically has a zonal ossification process and a well-defined, well-developed radiodense mass. Here, radiodensity is most noticeable along the edge of the lesion, giving the mass a calcified shell or outline, known as "eggshell calcification." Tendon and ligament HO often mimic the anatomic structure of the affected tissue. Differentiating calcification from ossification can be difficult. In dermatomyositis HO often appears as a stippled or sheet-like calcification. Genetic forms of HO have distinct patterns on radiographs. FOP often appears as well-circumscribed HO related to a specific muscle, whereas POH shows a “cocoon-like web” from dermis to skeletal muscles. (3)

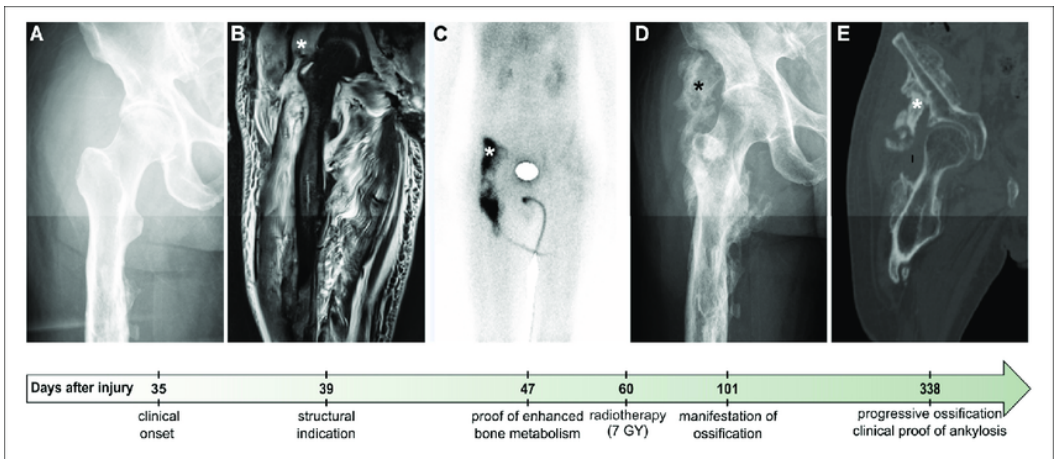

Figure 7: Clinical course of severe heterotopic ossification (HO) following spinal cord injury (SCI)

(A) On day 39 after SCI, initial X-ray shows no clear signs of HO.(B) MRI from the same day reveals a nonspecific, diffuse T2 hyperintense signal near the femoral head, raising suspicion.(C) Scintigraphy performed on day 47 confirms increased bone activity, supporting the diagnosis of HO.(D) Despite a single 7 Gy radiation treatment on day 60, CT later shows clear ossification more than 2 months after symptoms began.(E) By day 408, ankylosis has developed, and surgical resection of the HO is performed after a second 7 Gy radiation treatment the day before.(21)

The most sensitive imaging technique and gold standard for HO early detection is three-phase bone scintigraphy.(22) Incipient HO will be detected by flow studies and blood-pool imaging around 2.5 weeks after injury, and results on delayed scintigrams will turn positive around 1 week later. It will take at least another one to four weeks for radiography results to turn positive in HO.(19) Within 12 months, the majority of bone scan results return to baseline. (19) Serial bone scans have been effective in tracking the metabolic activity of HO, predicting postoperative recurrence, and determining when surgical resection is necessary.(23)Although they have the ability to detect HO early on they are very expensive and low specificity makes it difficult to distinguish between inflammation and early HO.(2)

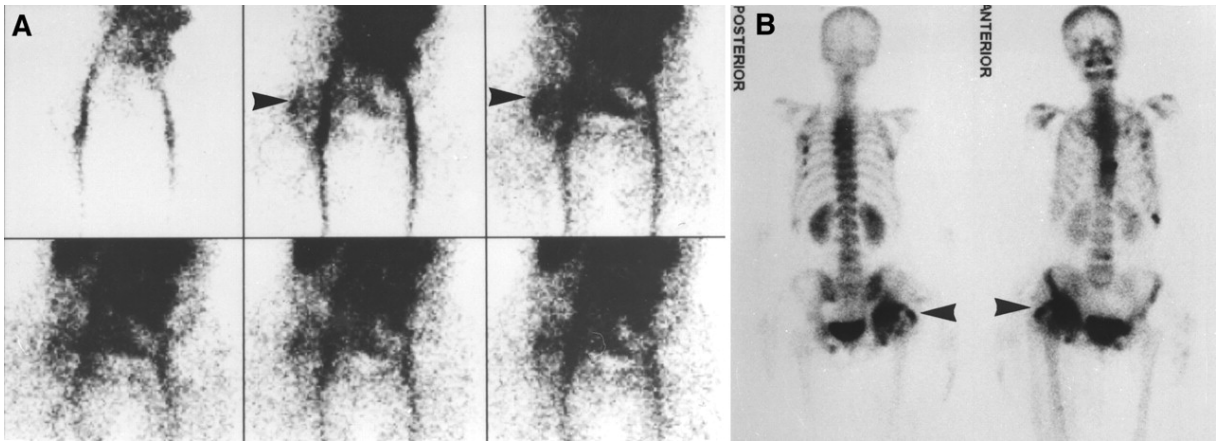

Figure 8: Three phase bone scintigraphy of HO

(A) The flow study shows increased blood flow in the soft tissue outside the right hip (arrowheads). **(B)** The delayed bone scan reveals increased activity in the same area (arrowheads) as well as in multiple healing fractures. (19)

Another imaging modality that can be used in diagnosing HO early on is ultrasonography(US).(24) US has been shown to detect HO earlier than traditional radiographic studies .It can also be used to follow the maturation of HO. According to studies, variations in grey-scale readings on US indicate different stages of HO, which could help with the treatment and modify rehabilitation regimens.(18) US may be more specific than bone scan in differentiating HO from other traumatic, inflammatory, or degenerative diseases of the skeleton.(22)

For preoperative planning computed tomography (CT) can be used to determine important anatomic landmarks, but in some circumstances, magnetic resonance imaging (MRI) may be required, if the surgical field is near anatomical features to assess the extent of local soft-tissue or neurovascular involvement more accurately.(2) MRI can identify elevated tissue density and blood flow during the acute stage of HO. The region may appear similar to or a little brighter than muscle on T1-weighted scans, while inflammation makes it appear noticeably brighter on T2-weighted images. Dark patches surround the margins of all sequences as the disease progresses and calcification develops. MRI results are frequently non-specific and can mimic other disorders during the maturing phase of HO. When HO reaches its mature state, it appears as bright fatty bone tissue with a black border of cortical bone surrounding it on both T1 and T2. This pattern is used for diagnosis, and additional imaging is usually not required. (Figure 9) Because early MRI can reveal distinctive characteristics like "striate" or "checkerboard" patterns and displacement of tissue layers close to the lesion, it is helpful for ruling out other illnesses, like as infections or malignancies. Since HO is frequently confused with osteomyelitis or sarcoma, it is important to recognize these early signs. For diagnosing HO it is only possible to diagnose mature HO on MRI.(22)

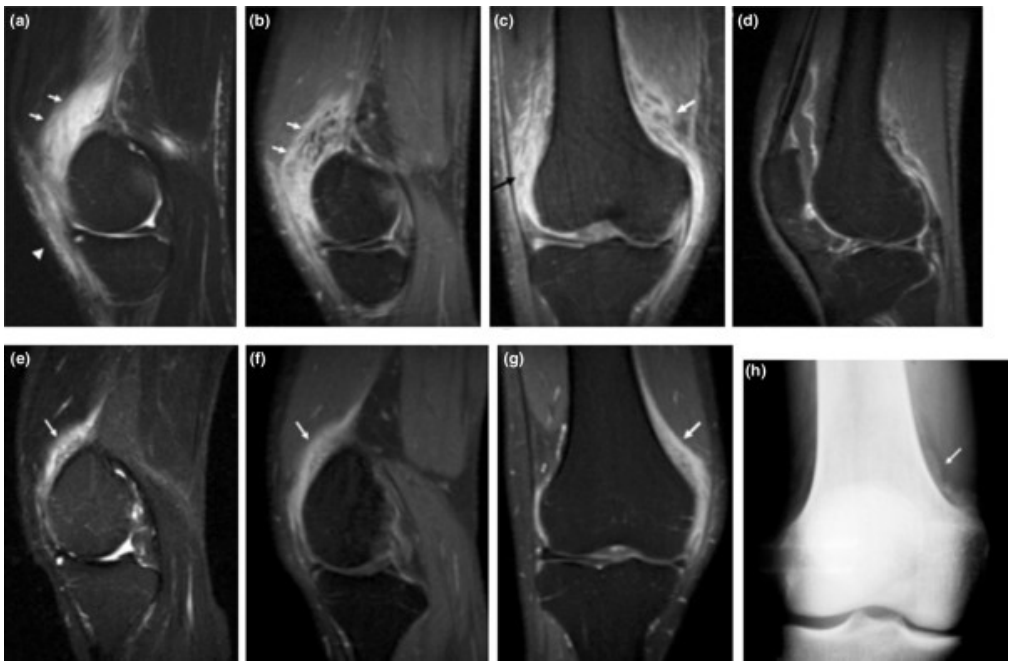

Figure 9: : MRI Findings of Early and Maturing HO in the right knee of a 35-Year-Old Male with TBI.

(a) Sagittal STIR image shows hyperintense signal consistent with early tissue edema and inflammation often seen in the acute phase of heterotopic ossification. (*b, c)* Contrast-enhanced fat-suppressed T1-weighted images in sagittal (b) and coronal (c) planes reveal a "lacy pattern" of enhancement, indicating early vascular and stromal changes. This pattern supports early HO and helps distinguish it from malignancy or infection. *(d)* Mid-sagittal contrast-enhanced T1-weighted image shows joint effusion and synovial enhancement, which can accompany adjacent HO development due to local inflammation. *(e)* On follow-up STIR imaging three weeks later, heterogeneous high signal intensity remains, suggesting ongoing maturation of the lesion. *(f, g)* Sagittal and coronal contrast-enhanced T1-weighted images demonstrate persistent but more organized enhancement, consistent with transition from early HO to a more structured lesion. *(h)* Anteroposterior radiograph of the knee now shows a calcified mass at the location of the vastus medialis, confirming radiographic maturation of HO.(25)

Laboratory studies can also be used for diagnostics.

Alkaline phosphatase (ALP) levels may increase during the first two weeks of injury and then drop to baseline levels within ten to twelve weeks. ALP may be helpful the diagnosis of early HO, although it is nonspecific for osteogenic activity. (22)

Rapidly elevated prostaglandin 24-hour urinary excretion in patients with suspicious symptoms may indicate additional imaging tests.(18)

Although nonspecific, Creatinine Kinase (CK) is typically higher in HO SCI patients and often suggests more involvement of surrounding muscle. (22)

Another indicator that is used is erythrocyte sedimentation rate (ESR). The onset of HO may be indicated by an ESR higher than 35 mm/hr. Another inflammatory marker that may be raised in early HO is C-reactive protein, but they are both non-specific.(26)

5.9 CLASSIFICATION

There are a lot of different classifications for HO in the following a few of them are going to be described in this chapter.

The Brooker classification, which is used for classifying HO in the hip, especially after THA. Taking into account the degree of bone bridging and the effect on joint space and mobility, it distinguishes four classes according to the existence, location, and severity of ectopic bone production surrounding the hip joint.(Table 3)(27) This classification has been criticized because it only uses anteroposterior radiographs, and they are unable to differentiate between overlapping and bridging calcifications.

Della Valle et al. developed a simplified categorization with just three different categories. In particular, it evaluates the degree of bone development and possible constriction of the joint space by classifying HO according to the amount of ectopic bone formations and the spatial relationship between the femur and pelvis.(Table 4) (18)

Schmidt and Hackenbroch created a third, more detailed classification. (18) It categorizes HO based on the region of ossification in relation to the tip of the greater trochanter.

According to the extent and severity of the ossification, as well as the size of the ectopic bone and its relationship to the femur and pelvis, including the possibility of ankylosis.(28)(Table 5)

The Hastings and Graham classification can be used for HO in the elbow. It classifies according to the functional restrictions in the elbow's range of motion (ROM), particularly regarding flexion/extension and supination/pronation, the classification system assesses heterotopic ossification (HO). Patients are categorized based on the degree of range-of-motion restrictions and the existence of ankylosis, which can limit some elbow joint motions. (Table 6)(29)(30)

Table 3: Brooker Classification of HO (25)

Class	Description
1	Islands of bone within the soft tissues about the hip.
2	Bone spurs originating from the pelvis or proximal end of the femur, leaving at least 1 cm between opposing bone surfaces.
3	Bone spurs originating from the pelvis or proximal end of the femur, reducing the space between opposing bone surfaces to less than 1 cm.
4	Apparent bone ankylosis of the hip.

Table 4: Della Valle Classification of HO (18)

Class	Description
1	Absence of HO or islands measuring <1 cm in length.
2	Islands >1 cm or spurs leaving at least 1 cm between femur and pelvis.
3	Spurs leaving <1 cm between opposing surfaces or bony ankylosis.

Table 5: Schmidt and Hackenbroch Classification of HO(28)

Region	Description
1	Heterotopic ossifications strictly below tip of greater trochanter.
2	Heterotopic ossifications below and above tip of greater trochanter.
3	Heterotopic ossifications strictly above tip of greater trochanter.

Grade	Description

A	Single or multiple heterotopic ossifications < 10 mm in maximal extent without contact with pelvis or femur.
B	Heterotopic ossifications > 10 mm without contact with pelvis but with possible contact with femur; no bridging from femur to proximal part of greater trochanter, with no evidence of ankylosis.
C	Ankylosis by means of firm bridging from femur to pelvis.

Table 6: The Hastings and Graham classification of HO (29)

Class	Description
1	Patients with heterotopic ossification (HO) but no functional range of motion (ROM) limitations.
2	Patients with HO and limitations in functional ROM (elbow flexion/extension and/or supination/pronation). This class is subdivided into three categories depending on which plane(s) of motion is affected.
3	Patients with HO and ankylosis present, preventing elbow flexion/extension and/or supination/pronation. This category is subdivided according to which plane(s) of motion is affected.

5.10 DIFFERENTIAL DIAGNOSIS

Early diagnosis of HO can be challenging due to the non-specificity of the patient's signs and symptoms. HO can be clinically and radiographically mimicked by a variety of diseases. When thinking about the diagnosis of HO, it is important to differentiate it from other possible diagnosis. HO should be differentiated from cellulitis, thrombophlebitis, osteomyelitis, or a tumor. The symptoms in the inflammatory stage of the disease are really similar to these diseases.(19) Other possible diagnosis could also be septic arthritis, hematoma, a fracture, or local trauma.(31)
The following is a quick discussion of other certain differentials that need to be considered.

5.10.1 DYSTROPHIC CALCIFICATION

Dystrophic calcification (DC) is a calcification that develops in soft tissue after inflammation or injury. It is well known to occur in cases of collagen vascular disorders such as scleroderma, systemic lupus erythematosus, and dermatomyositis. Organization is the key distinction between DC and HO. As mineralization takes place early in the disease process, DC and HO are essentially identical on plain films, CT, or MRI in the beginning of the disease process. Over several months, DC will continue to exist as amorphous, non-ossified calcifications, while HO will start to organize and ossify into lamellar bone.(18)

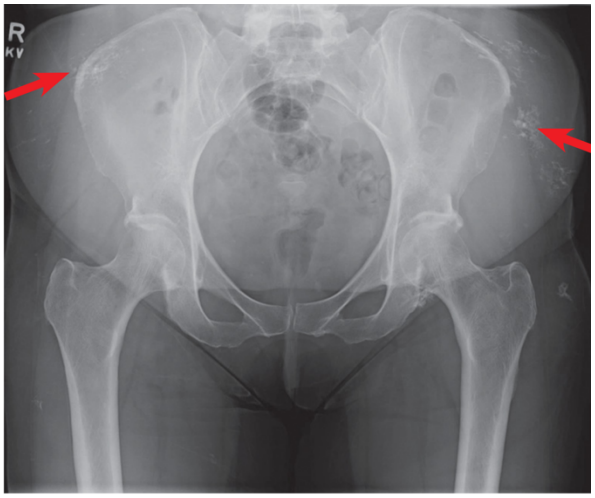

Figure 10: Dystrophy calcification.

Dystrophic calcifications from dermatomyositis are visible in the outer soft tissue (2 red arrows), appearing as hazy, poorly defined areas on the X-ray.(18)

5.10.2 CHONDROCALCINOSIS

Chondrocalcinosis is cartilaginous calcifications that can appear in almost every joint of the body. (32) It is often linked to Calcium Pyrophosphate Deposition Disease (CPDD). (18) On X-Ray it shows up as a thick line parallel to the articular surface in hyaline cartilage, which is also how CPDD can be distinguished from HO, because HO appears as a peripheral circumferential calcific mass with little intra-articular involvement.(18,33)

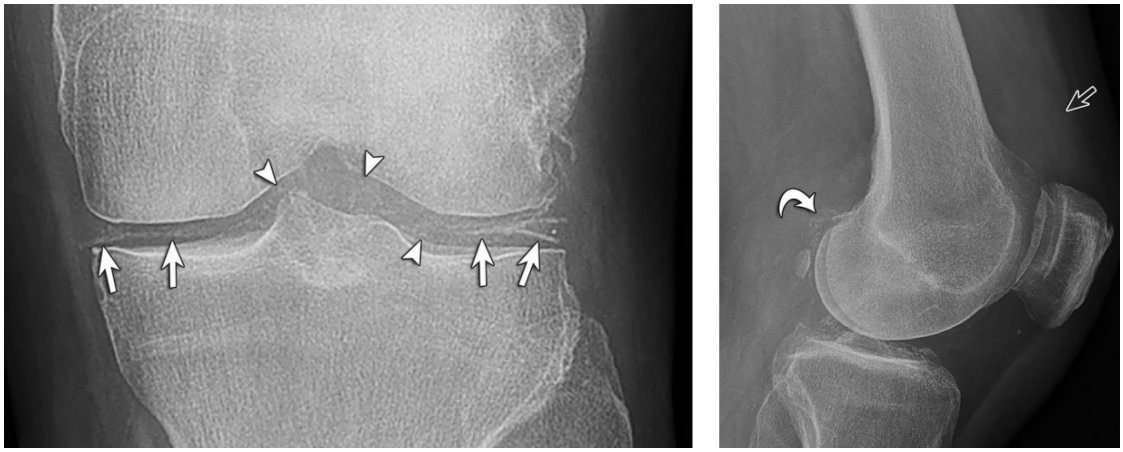

Figure 11: Chondrocalcinosis(34)

5.10.3 TUMORAL CALCINOSIS

Tumoral calcinosis (TC) is a rare clinical and histopathologic syndrome characterized by calcium salt deposition in different peri-articular soft tissue regions.(35) The symptoms can be very similar to these of HO, but they can be differentiated on imaging. TC exhibit peri-articular tissue calcifications that are fluid-filled, lobulated, and cystic.(18)

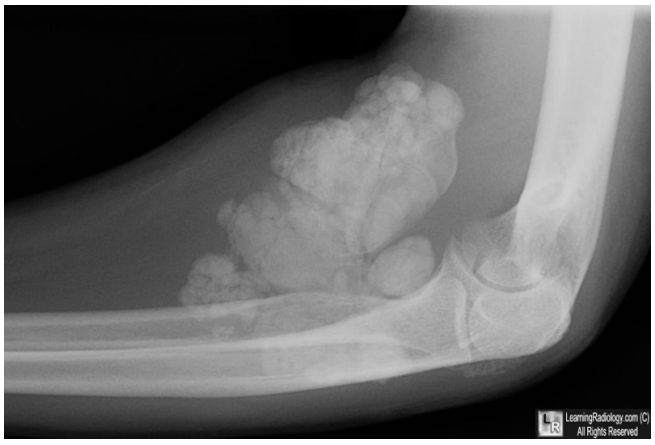

Figure 12: Tumoral calcinosis.

There is a clearly defined, multi-lobular dense area in the soft tissue of the front forearm near the elbow, consistent with calcification. It has a tumor-like appearance, but the underlying bone appears normal.(36)

5.10.4 AVULSION FRACTURES

Avulsion fractures (AF) are bone failures in which soft tissue connected to the bone pulls a portion of bone away from its main body.(37) AF usually exhibit pain, edema, and loss of joint function together with a clear history of trauma. On radiographs they show distinctly defined pieces of bone directly after the trauma, which is the biggest difference to HO. HO can be first seen on radiographs weeks after the initial trauma. On CT, HO presents as a ring of hyperdense cortical bone with a hypodense interior, which also helps to differentiate it from AF.(18)

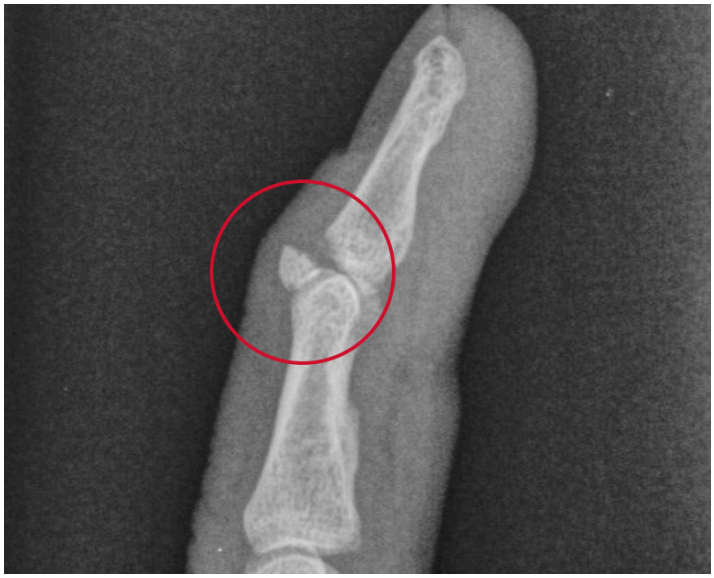

Figure 13: Avulsion fracture(38)

5.10.5 PRIMARY OSTEOSACROMA

The most prevalent form of primary bone sarcoma in children, adolescents, and young adults is osteosarcoma, a rare malignancy.(39) Localized pain and edema are the first symptoms that patients present with. Later it proceeds to joint immobility. On imaging it is usually described as a "sunburst" appearance or as having cloudlike density. CT scan is particularly sensitive to calcification and is effective in showing the amorphous osteoid production in OS, which can help to differentiate it from structured circumferential osteoid formation in HO.(18)

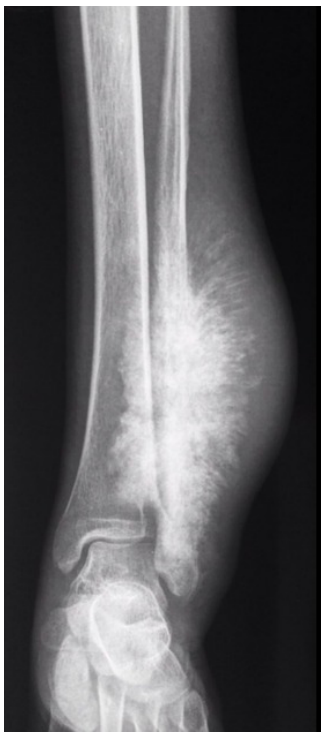

Figure 14: Primary Osteosacroma(40)

5.10.6 TOPHACEOUS GOUT

Crystals of monosodium urate are deposited in the soft tissues and bones both inside and outside of joints, causing tophaceous gout.(41) Patients usually present with acute onset of pain and swelling on the site of deposition.(18) Gouty lesions are characterized by overhanging bone edges that are connected to bony erosions on plain radiography.(41) On x-ray and CT, HO can be differentiated from tophaceous gout by the absence of peripheral soft tissue calcifications, intraosseous erosions, and cortical bone growth.(18)

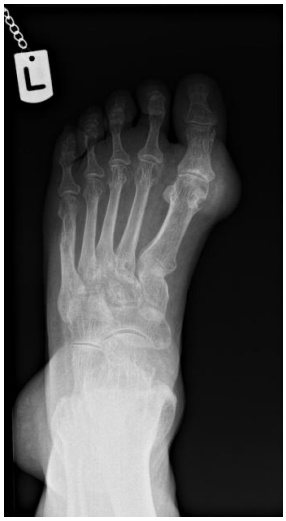

Figure 15: Tophaceous gout.

There are multiple well-defined, punched-out bone erosions with sclerotic edges on both sides, especially near the first MTP joints. Soft tissue swelling and densities near the joints are also present.(42)

5.10.7 CALCIFIC TENDONITIS

Calcific tendonitis is a condition where calcium builds up in tendons, most often affecting the rotator cuff. This results in soreness, swelling, chronic pain when moving, and occasionally restricted joint mobility. Although the precise etiology is still unknown, endocrine abnormalities may be a contributing factor. Near the greater tubercle of the humerus, dense, homogeneous calcium deposits are usually visible on X-rays in AP view with internal and exterior rotation. By showing hyperechoic patches and creating pain when pressure is applied during the scan. calcific tendonitis can also be identified on US. The deposits show up on MRI as bright patches at the tendon attachment site, occasionally with a dark core. On T1-weighted images, these lesions typically lack a distinct shape and a bright center, in contrast to HO.

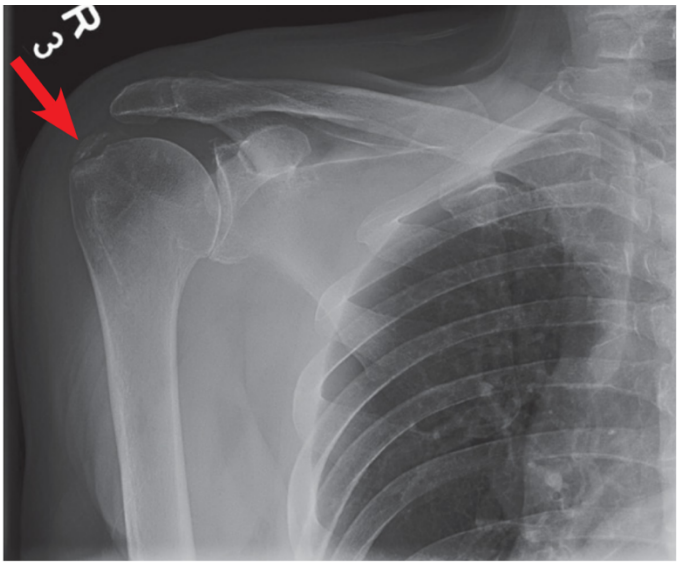

Figure 16: Calcific tendonitis in the right shoulder.

A dense, irregular line of calcification is seen where the supraspinatus tendon attaches to the greater tubercle of the humerus (red arrow)(18)

5.11 PROPHYLAXIS

5.11.1 NSAIDs

The most widely used NSAID for prophylaxis is indomethacin.(26) It can be administered to downregulate prostaglandins, which are thought to be important for cell differentiation into new bone production.(22) Other NSAIDs that have shown good results include ibuprofen, celecoxib, rofecoxib, and meloxicam. For prevention after THA, 75–100 mg of indomethacin daily for 7–14 days after surgery is advised. It is advised that individuals with SCI take 75 mg of indomethacin daily for three weeks. (43)The risk of bleeding must be closely monitored, particularly while chemoprophylaxis for venous thromboembolism is being administered concurrently. Concurrent prevention for gastrointestinal (GI) ulcers is also recommended. Compared to conventional NSAIDs, a selective COX-2 inhibitor at a dose of 20 mg daily can be administered with less GI adverse effects.(26) Indomethacin can also affect the fracture union, because of that it should be used carefully after orthopedic injuries and the risk of long-bone nonunion should be considered.(2)

5.11.2 BISPHOSPHONATE

“Bisphosphonates are antiresorptive agents that induce osteoclast apoptosis and inhibit calcification.”(2) A Bisphosphonate that has been proven to help preventing HO is Etidronate. It has been shown to avoid complications from THA and in patients with SCI. A study demonstrated that if treatment is initiated before HO appears on radiographs, clinically significant HO can be avoided.(22)The recommended course of treatment for HO linked to spinal cord injuries is 20 mg/kg daily for two weeks, followed by 10 mg/kg daily for ten weeks, for a total of twelve weeks. It is advised to take 20 mg/kg daily for one month prior to surgery and then the same dosage for three months after surgery for total hip replacement.(43)

Table 7: Pharmacologic Prophylaxis for Heterotopic Ossification(2,22,43)

Medication	Dosage	Start Time & Notes
Indomethacin	75–100 mg daily	Start within 24 hours post-op for 7–14 days (up to 3 weeks for SCI); monitor for GI bleeding and long-bone nonunion risk
Celecoxib	200 mg/day or 20 mg/day	Start within 24 hours post-op;

	(COX-2 inhibitor)	lower GI side effects than traditional NSAIDs
Ibuprofen, Rofecoxib, Meloxicam	Variable	Alternative NSAIDs with good outcomes; dosing per clinical judgment
Etidronate (Bisphosphonate)	20 mg/kg daily pre-op for 1 month; then 20 mg/kg daily post-op for 3 months	Also: 20 mg/kg/day x 2 weeks + 10 mg/kg/day x 10 weeks in SCI; initiate before radiographic HO signs for best effect

5.11.3 RADIATION

In patients with severe injuries who have a high risk of developing HO, radiotherapy can be used as primary or secondary prevention, either in conjunction with surgical excision or as a preventive measure.(22) Heterotopic ossification rates following THA have been found to range from roughly 5% to 90%; however, following radiation therapy, the frequency dropped to 25%.(2) From 24 hours before surgery until 48 to 72 hours after surgery, radiation therapy can be provided at a dose of 700 to 800 cGy in a single fraction.(2) Potential risk factors could be malignancy, although there are no reported cases, progressive soft tissue contracture, impaired wound-healing, nonunion and inhibited ingrowth of press-fit hip implants after THA. (2)

6 TREATMENT

6.1 OVERVIEW OF TREATMENT STRATEGIES

The management of HO contains two categories. The Prophylaxis for high-risk patients and treatment for HO that has already developed. In the next chapters the treatment for already developed HO is going to be discussed. There is limited consensus regarding the best treatment plans because of the wide variation in the etiology, underlying processes, and risk factors for HO in each individual patient. All these factors must be considered, when choosing the right treatment strategy. Possible options are surgical intervention, non-pharmacological treatment, such as physiotherapy and new innovative treatment strategies.(18)

6.2 SURGICAL INTERVENTION

6.2.1 INDICATION

Surgical excision still remains the only effective treatment for already developed ectopic bone.(18) In Patients who experience functional difficulties, such as pain, joint ankylosis, nerve entrapment, or severely limited range of motion due to HO and when nonoperative modalities of treatment do not result in functional improvement, surgical intervention should be considered. One crucial factor to consider is the timing of the surgical intervention. The timing of intervention should be determined by the degree of bone maturation and the cause of HO. (2) There should be evidence of fully developed and mature HO to reduce intraoperative complications and the recurrence of HO by allowing the lesion to mature and the patient's tissue to heal.(18) Contraindications for surgery would be signs of infection, like fever, swelling, erythema, or non-union, or impaired motor-control.(44) Before surgery the serum alkaline phosphatase levels should also be normal. (Table 5) (19)

HO after traumatic event may be resected at six months; spinal cord injury related HO at one year; and traumatic brain injury HO is removed at 1.5 years.(45) Some people advise early excision following orthopedic interventions in particular because of the relative preservation of tissue planes that are crucial for distinguishing ectopic bone from normal callus and scar at the location of a recent operation. (2)

Table 8: Criteria for Recommending Surgical Removal of Heterotopic Ossification(19)

Criterion	Description
1	Significantly limited range of motion for involved joint (e.g., hip should have 50° range of motion); for most patients, progression to joint ankylosis is the most serious complication of heterotopic ossification.
2	Absence of local fever, swelling, erythema, or other clinical findings of acute heterotopic ossification.
3	Normal serum alkaline phosphatase.
4	Return of bone scan findings to normal or near normal; if serial quantitative bone scans are obtained, there should be a sharply decreasing trend followed by steady state for 2–3 months.

6.2.2 SURGICAL APPROACH

The surgical approach depends highly on the anatomical location, surrounded nerve and tissue involvement, and extend of the HO.

For HO excision, both open and arthroscopic techniques have been reported. For individuals who have had previous fractures or in situations where nerve structure may be abnormal due to previous surgeries, an open approach is generally advised. An open technique that allows for nerve decompression is the most effective way to treat nerve involvement.(20)

Arthroscopic surgery can be used for excision of HO for example in the shoulder.(46) Revision arthroplasty and surgical ossification excision are the gold standard treatments for grades III and IV HO after THA. (47) The goal of the surgery should be to achieve full excision of the ectopic bone, because incomplete resection of the HO is linked to recurrence, and soft tissue release. (3)(48)

6.2.3 COMPLICATIONS

Bleeding is one of the main post-operative complications of surgical HO excision.(22) The risk of bleeding can be reduced by embolizing the feeding artery to the HO mass, according to multiple case reports. For interventional radiology to do embolization the day before surgery, preoperative contrast-enhanced three-dimensional CT can show the HO and any supplying arteries extending to the HO.(49) Other possible complications would be osteomyelitis, cellulitis, delayed wound healing and potential recurrence of HO. (19) According to reports, complications from acetabular fractures occur at a rate of 33.3% and include recurrence of heterotopic ossification, sciatic nerve damage, femoral head osteonecrosis, and intraoperative femoral neck fractures.(2)

6.2.4 POSTOPERATIVE CARE

It is important to immediately start with prophylaxis post operation, to try and prevent HO recurrence(47). A combination of NSAIDs and Radiotherapy should be prescribed. A study showed that after excision, secondary therapies such as medication, ROM therapy, and radiotherapy were successful. In both TBI and SCI populations, the postoperative therapy of etidronate and indomethacin significantly decreased the recurrence of HO and improved the ROM.(43)

6.3 PHYSIOTHERAPY

The impact of physical therapy on HO is debated. Excessive or poorly timed mobility may worsen HO, according to some research, while early postoperative exercise may promote recovery and

lower the risk of HO. Early joint immobilization may trigger or worsen the HO, whereas passive movement or extended immobilization during inflammation may encourage it. On the other hand, while prolonged bed rest may hinder microcirculation and encourage ossification because of aberrant stem cell differentiation, controlled movement aids in blood flow and tissue regeneration during the final stages of recovery.(50) Physical therapy is important for preserving joint movement, improving function, and preventing ankylosis. (5)(7)Research has shown that in some animal models immobilization may inhibit the HO formation by disrupting mechanotransduction signaling, collagen structure, and promoting adipogenesis. (51)However other studies have also demonstrated that repetitive stretching can increase osteogenic activity. Despite these concerns, gentle physical therapy within a pain-free range has shown positive effects on maintaining or even improving the mobility without increasing the ectopic bone formation. This involves using structured workout regimens and constant passive motion. But important details about the optimal timing, intensity, frequency, and methods of exercise are still unclear.(7)

6.4 INNOVATIVE TREATMENT

The ultimate objective of HO research is to prevent, stop, or even reverse the disease completely, even though there are a number of therapy approaches available to control symptoms and limit its course. However, finding universal treatment targets is extremely difficult due to the variety of triggers and mechanisms driving acquired HO. On the other hand, genetic HO, especially FOP, offers a useful model for comprehending important cellular and molecular processes related to ectopic bone growth. Experimental strategies that directly target these pathways have been devised and evaluated in clinical settings as well as in vivo in animal models. FOP has been the subject of most human research. By blocking the BMP signaling system, more especially the ACVR1/ALK2 receptor, studies have shown encouraging outcomes.

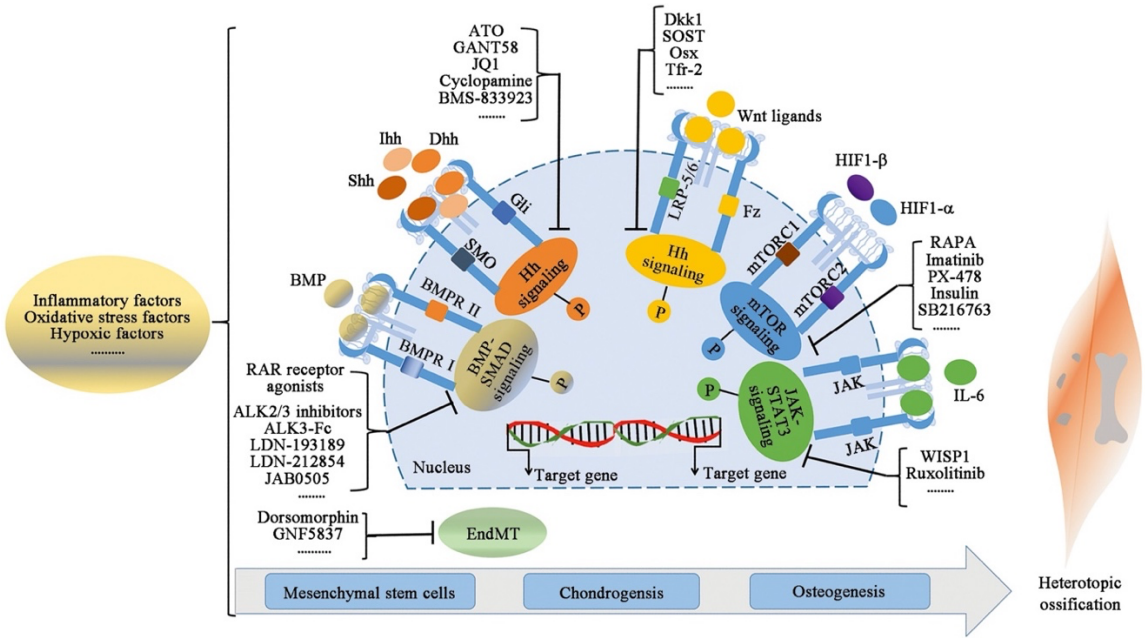

Figure 17: An overview of innovative treatment approaches for HO using several signaling pathways(52)

6.4.1 RAR SIGNALING INHIBITORS

Retinoids affect bone and cartilage formation through retinoic acid receptors (RARs). There are three types of RARs. α, β, and γ, these play a role in inhibiting cartilage development (chondrogenesis). Research has shown that retinoid agonists can block bone formation and potentially treat HO. In 1998, isotretinoin (a non-selective RAR agonist) helped reduce HO in patients with FOP, but it had a lot of side effects. As a result, the use of more focused RAR agonists became more popular. (48)In 2010, a study showed that NRX195183, a selective RAR-α agonist, helped prevent HO in mice by affecting gene expression involved in bone and cartilage formation. (53)More recently palovarotene, an RAR-γ agonists, showed strong results in preventing HO with minimal side effects. Palovarotene blocks pathways involved in bone formation and promotes normal tissue repair. (54)In 2022, it became the first RAR-γ agonist approved by Health Canada for treating HO in children over the age of 8 years, females over the age of 10 years and for males diagnosed with FOP.(48)

6.4.2 HEDGEHOG SIGNALING

Hedgehog (Hh) signaling is important for normal bone growth and repair, especially in forming cartilage and bone from stem cells. When Hh signaling becomes too active, it can lead to bone-related diseases like bone tumors, arthritis, and HO.(55) The Hh pathway is controlled by two proteins, Patched, which blocks the pathway, and Smoothened, which activates it. The three main Hh proteins in humans are Sonic, Indian, and Desert Hh.(56) Studies have shown that HO can be

caused due to overactive Hh signaling, especially in people with certain gene mutations like GNAS1. This mutation leads to higher Hh activity.(48) Recent research shows that Hh signaling works with the YAP protein and creates a self-activating loop that increases abnormal bone growth. Different types of HO, including genetic and injury-related HO, can be prevented by blocking YAP or Hh, without causing damage to healthy bones.(51) Examples for that would be GLI inhibitors like arsenic trioxide (ATO) and GANT58, which were able to reduce HO in mice. As well as JQ1, a drug that blocks a related protein (BRD4), also reduced HO in a mouse model(57), and Cyclopamine and BMS-833923, which block the SMO protein, helped to prevent bone-forming signals, and reduced abnormal bone growth.(58)(56) Taking everything into account, inhibiting the Hh pathway is a promising treatment option for HO.

6.4.3 ENDOTHELIAL-TO-MESENCHYMAL TRANSITION (EndMT) PATHWAY INHIBITORS

More recently researcher shows that EndMT also plays a role in diseases like fibrosis, cancer, and HO. (59)In 2010, Medici et al. showed that EndMT contributes to HO in patients with FOP. They found that blood vessel cells (vascular endothelial cells) in inflamed areas could transform into cartilage and bone cells through EndMT. This change was triggered by inflammation and specific signaling proteins like TGF-β2 and BMP4, which activate the ALK2 receptor. These transformed cells went on to form ectopic abnormal bone through both intramembranous and endochondral ossification.(51) Because of this, blocking EndMT has become a promising target for HO treatment. EndMT is regulated mainly by proteins from the TGF-β and BMP families. (59)While BMP2, BMP4, and TGF-β2 promote EndMT, others like BMP7 and VEGF can block it.(51) A TGF-β-blocking antibody (1D11) showed reduced HO in mice. Suppressing the TGF-β2 receptor in stem cells also prevented HO. (60)Other studies showed that increasing SMAD7, a protein that inhibits TGF-β signaling, could block EndMT and reduce HO in tendon injury models. (61)(62)New evidence suggests that the nervous and hormonal systems may also influence HO by affecting EndMT. (63)Neurotrophin-3 (NT-3) can promote HO by triggering EndMT(63), but drugs like dorsomorphin and GNF58 can block this process.(64) Melatonin also encourages EndMT and HO formation, while the drug 4-P-PDOT (an MT2 receptor blocker) can stop this effect. These findings show that EndMT is also promising for new HO treatments.(65)

6.4.4 BMP/SMAD SIGNALING INHIBITORS

BMPs are part of the TGF-β superfamily and are key regulators of bone and cartilage formation. BMPs activate specific receptors (ALK2, ALK3, ALK6), which then activate SMAD proteins

(Smad1/5/8) that control genes involved in bone growth. When this signaling increases, it can lead to abnormal bone growth.(66) For example, people with FOP have mutations in the ALK2 gene, which causes increased BMP signaling. High levels of BMPs are also found in trauma-related HO, and blocking BMP receptors has been shown to reduce this abnormal bone formation.(67) To treat HO, several drugs targeting BMP signaling have been developed. The first small molecule to inhibit BMP receptors ALK2, ALK3, and ALK6 was Dorsomorphin in 2007. **(51)**A more effective version of dorsomorphin is LDN-193189. It blocks SMAD1/5/8 activation and reduces HO in mouse models. LDN-212854 is newer drug that mainly targets ALK2 and can controll both injury-related and spontaneous HO.(51) RK-71807 is a new promising drug with better oral absorption than the others.(68) ALK3-F blocks BMP ligands, reduces bone growth, inflammation, and improves stem cell function more effectively than earlier drugs. Other potential treatments could be Dasatinib and Quercetin combination. This drug combination reduces BMP signaling.(69) Metformin could also be used, because it blocks HO through the AMPK pathway(70), as well as, Tamoxifen, which affects the TGF- β signaling through estrogen receptor activity.(71)

6.4.5 WNT/ β-CATENIN SIGNALING INHIBITORS

The Wnt/β-catenin signaling pathway helps control the bone metabolism. When Wnt proteins bind to cell surface receptors (Frizzled and LRP5/6), they activate β-catenin, which promotes the growth and development of osteoblasts and chondrocytes, partly by increasing the Runx2 protein.(72) Wnt/β-catenin signaling often becomes overactive in patients with HO. Trauma-related HO is associated with high β-catenin and Runx2 levels. Studies show that blocking Runx2 reduces β-catenin and prevents HO. (73)KIF26B is another protein that affects HO by limiting only osteogenesis by influencing the Wnt/β-catenin signaling.(74)

Other inhibitors of Wnt/β-catenin signaling pathway are for example Dickkopf-1(Dkk1) and sclerostin (SOST). In patients with ossification of the posterior longitudinal ligament (OPLL), Dkk1 levels were lower than normal, and lab studies showed that Dkk1 can stop the growth and bone-forming activity of these ligament cells.(75) Studies showed that Osx (Sp7) helps to activate Dkk1 and SOST directly, which also reduce Wnt signaling.(72) Reduce HO in animal studies were found with transferrin receptor-2 (Tfr-2) It activates another pathway (p38-MAPK), which increases the production of SOST. This shows that controlling the Wnt pathway, by increasing Dkk1 or SOST, could be a useful to prevent or treat HO.(51)

6.4.6 HYPOXIA-INDUCIBLE FACTOR 1(HIF-1)/MAMMALIAN TARGER OF RAPAMYCIN (mTOR) SIGNALING INHIBITORS

mTOR is a key protein that helps cells respond to signals from inter- and extracellular. It is composed of mTORC1 and mTORC2. It is essential for normal bone growth. mTOR activates the growth of osteoblasts.(76) If mTOR is blocked there is reduced bone formation. One important part of mTORC1 is the protein Raptor, which helps to respond to signals like hypoxia, growth factors, and stress. One of the downstream targets of mTOR is HIF-1α, a protein that helps cells adapt to hypoxia and inflammation. HIF-1α plays a key role in promoting cartilage cell growth. (77)Early stages of HO have shown high levels of HIF-1α. Removing HIF-1α in animal models reduced HO formation.(51) Rapamycin is a drug, which originally was used to prevent organ transplant rejection. It blocks mTOR activity and as a result reduced HO in several animal models. It works by interfering with multiple processes.(78) In lab studies showed that rapamycin also reduces ectopic bone growth in FOP patients. However, in a small clinical study two FOP patients didn't show reduced ectopic bone growth with rapamycin.(79) This shows that it might not work for every patient. Other promising drug are imatinib, which is used for leukemia treatment, and PX-478.

6.5 PROGNOSIS

HO can lead to a lot of different complications, such as decreased joint function and mobility, peripheral nerve entrapment, and pressure ulcers. While up to 70% of cases remain asymptomatic, more severe outcomes such as ankylosis, vascular compression, and lymphedema have also been reported.(80)Surgical excision is often pursued in symptomatic cases and has shown good outcomes. On average, the surgery is performed 3.6 years after the initial injury.(26) Studies have shown that hip ROM often improves significantly after surgery. The mean ROM in hip HO is 24.3° prior to the surgery and often improves significantly 6 months postoperatively(81). However, there are various risk with surgical excision, such as bleeding, infection, and recurrence of HO.

In the case of elbow HO, complete restoration of the pre-injury function is very rare, but there are notable improvements in the ROM. Most published data show an average gain of 50 to 110 degrees in the elbow's arc of motion. (20)A mean improvement of 67 ° (range 13–131°) was identified in a systematic review of 626 elbows.(82) The results differ depending on the kind of injury. Patients with traumatic brain injury showed a mean of 109°, burn patients a mean of 88°, and trauma patients exhibited the biggest improvement. Despite these improvements, complications following elbow HO excision are not rare. (20)

7 EXAMPLE CASE

7.1 CASE HISTORY

A case reported by Vanden Berge et al. (2022) described a 67-year-old female with a medical history of psoriasis, diabetes mellitus, and prior breast cancer. She presents to a clinic with bilateral knee pain. She underwent a right total knee arthroplasty (TKA) in 2007, followed by two manipulations under anesthesia due to postoperative stiffness. In 2008, due to ongoing mobility issues and a flexion contracture, she decided to get a revision TKA at another hospital. After the revision TKA her knee motion initially improved (0°–100°) under aggressive physical therapy. Due to insurance limitations she had to stop the therapy and her knee motion worsened again.

In 2011, her right knee motion was reduced to 10°–45°.She had no signs of infection, but the significant stiffness persisted. In 2021, thirteen years post-revision, she came to the clinic again. Her right knee was locked in a mild flexion, but she was able to walk short distances with a limp and minimal pain. Performed radiographs showed extensive HO covering the right knee, including the collateral ligaments, anterior and posterior capsules, with bridging bone formation. (Figure 5 and Figure 6).There was no evidence of prosthetic loosening or infection. The left knee showed tricompartmental osteoarthritis, worse in the medial compartment, and was managed conservatively with physical therapy, NSAIDs, and injections. (Figure 7) Imaging also revealed lumbar spondylosis and enthesopathic changes in the pelvis. Laboratory results were unremarkable, and bone density scans indicated osteopenia. Given the absence of pain and the chronic nature of the HO, the patient decided to proceed conservative management of the right knee, understanding that her range of motion was unlikely going to improve.

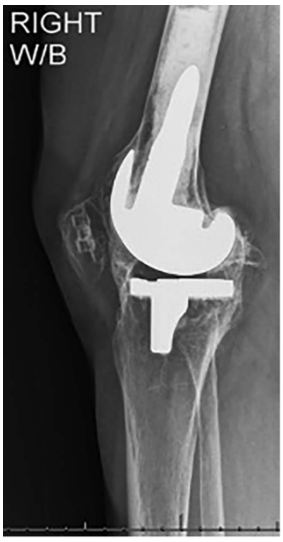

Figure 18: radiograph of the right knee (lateral)

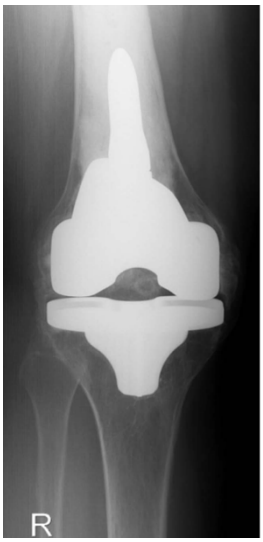

Figure 19: radiograph of the right knee (AP). Showing extensive HO

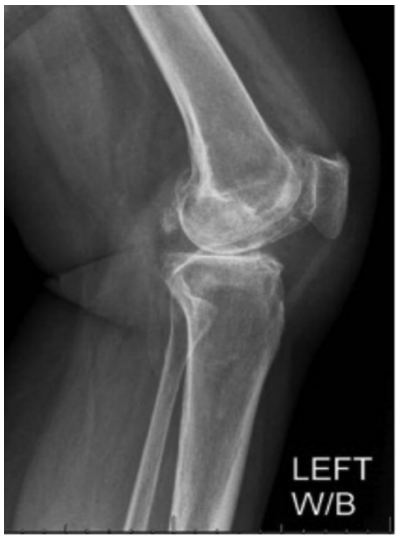

Figure 18: Left knee (lateral). Tricompartmental osteoarthritis with joint line narrowing

7.2 DISCUSSION

The difficulties in treating HO following TKA are demonstrated by this example. The 67-year-old woman developed severe HO in her right knee, even though she underwent revision TKA. The early improvement with physical therapy shows how beneficial it can be. Later, radiographs revealed widespread HO surrounding the knee joint, damaging several crucial soft tissues and ligaments. It's interesting to note that the patient's primary issue was restricted movement and not the pain. This also shows how different HO symptoms can be. In the end the patient decided to not undergo another surgery, in which the ectopic bone could have been removed. She decided to continue conservative treatment, which probably won't improve her symptoms. This instance demonstrates how, even years after surgery, HO can result in persistent mobility issues. It also highlights the significance of early diagnosis, ongoing physical therapy and especially prophylactic treatment with radiotherapy and medications, such as NSAIDs, after these kinds of surgeries. Even though the primary treatment for adult HO is surgery, not all patients decide for this approach, when considering the possible complications and possible recurrence of HO after surgery. In such cases it is important to educate the patient about all possibilities and complications that come with it. If the patient denies surgery, it is important to perform regular checkups and personalized treatment. Thinking about these cases it is even mor important to fully understand the mechanism of HO and developing new treatment and prophylaxis strategies.

8 EXTENSIVE SUMMARY

HO can either result from uncommon genetic disorders or acquired causes, such as physical trauma or surgery. The degree of the trauma and the patient are two elements that affect acquired HO. It usually results from occurrences like fractures, joint replacement procedures, or neurological injuries. On the other hand, certain gene mutations that result in ectopic bone production in soft tissues via distinct biological pathways produce hereditary forms such as FOP and POH. The pathophysiology of HO is still not completely clear. It involves a complex interaction between inflammation, stem cell differentiation, and signaling pathways like BMP/Smad, Wnt/β-catenin, Hedgehog (Hh), EndMT, and mTOR/HIF-1α. The recruitment and development of mesenchymal stem cells into bone-forming cells are facilitated by these pathways. Clinically, HO presents with pain, swelling, reduced joint mobility, and in severe cases, ankylosis or nerve entrapment. Diagnosis relies on physical examination, imaging (X-ray, CT, MRI, ultrasound, bone scintigraphy), and laboratory markers such as alkaline phosphatase. The gold standard imaging module for diagnosing HO is three phase bone scintigraphy. Several classification systems, for example Brooker or Hastings and Graham classification, have been developed to help with

diagnosing and treatment planning. Prophylactic treatment for patients at high-risk of developing HO include NSAIDs (e.g., indomethacin), bisphosphonates (e.g., etidronate), and radiation therapy. For established HO, surgical resection remains the main treatment. Surgery should be performed when the ectopic bone has fully matured to reduce the risk of recurrence. Postoperative care includes physical therapy and immediate prophylaxis with NSAIDs and RT. However, complications like infection, nerve injury, and recurrence are common. The effectiveness of Physiotherapy is controversial. Aggressive and wrong timed exercise may worsen the HO, but controlled, gentle and pain-free therapy can preserve mobility and reduce spasticity. Evidence suggests that timing, intensity, and technique are critical to optimizing outcomes. Innovative Treatment options that target molecular pathways are being explored and could be very helpful in treating ho, especially genetic forms like FOP. These include RAR agonists (e.g., palovarotene), BMP inhibitors (e.g., dorsomorphin, LDN-193189), Hh and EndMT pathway blockers, and mTOR/HIF-1α inhibitors (e.g., rapamycin). Although many of these therapies are still in experimental stages, they offer hope for preventing or reversing HO in the future. Overall, this thesis highlights the complexity of HO and emphasizes the need for individualized, multidisciplinary approaches in diagnosis, prevention, and treatment, with a growing emphasis on targeted molecular therapies.

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FIGURES:

TABLES: