Spinal Cord (2002) 40, 313–326

www.nature.com/sc

Clinical Review

Neurogenic heterotopic ossification in spinal cord injury

AA van Kuijk*,[2], ACH Geurts[1] and HJM van Kuppevelt[1]

[1]*Department of Rehabilitation Medicine, Sint Maartenskliniek, Nijmegen, The Netherlands;* [2]*University Medical Centre St Radboud, Nijmegen, The Netherlands*

Neurogenic heterotopic ossification (NHO) is a frequent complication in spinal cord injury (SCI) that is often difficult to treat. This review emphasizes the incidence, risk factors and clinical signs of NHO in SCI patients. Although the exact pathophysiology underlying NHO in neurologic patients is not yet understood, different pathogenic mechanisms have been proposed in the literature. A selection of the most important theories will be given and discussed. Moreover the different diagnostic, therapeutic, and preventive methods currently used in NHO management after SCI will be reviewed.
Spinal Cord (2002) **40,** 313–326. doi:10.1038/sj.sc.3101309

Keywords: neurogenic heterotopic ossification; spinal cord injury

Introduction

Neurogenic heterotopic ossification (NHO) is a frequent complication in spinal cord injury (SCI). It is characterized by the formation of new extra osseous (ectopic) bone in soft tissue surrounding peripheral joints in patients with neurologic disorders. In SCI patients, the incidence ranges from 10–53% depending on the study design, the methods of detection (radiologically or by clinical symptoms), and the diagnostic criteria used.[1–28] Moreover, in the early literature, no attempt was made to separate cases with ectopic bone formation associated with trauma or decubitus from true ectopic ossification not associated with a history of (local) trauma or infection. In non-traumatic myelopathies the incidence of NHO seems less compared to traumatic SCI ranging somewhere between 6–15%.[6,14,19,29,30] NHO is less common in children than in adults with an incidence generally reported between 3–10%.[31,32] The clinical symptoms in children do not differ from those reported in adults, but are less pronounced compared to adult patients. Moreover, spontaneous regression of NHO is more often reported in children and young adults than in older adults.[31,32]

The clinical spectrum of NHO ranges from an incidental finding on X-rays to severe limitation of the range of motion and even complete ankylosis of peripheral joints. In the majority of cases, the extent of NHO is minimal and only diagnosed as a radiographic finding. However, in 20–30% of the SCI patients clinically significant NHO is present with a reduction of joint range of motion, whereas in 3–8% of the SCI patients ankylosis develops.[7,8,10,12,19,20,22–24,27,29,33] NHO always occurs below the level of the SCI, most commonly at the hip (70–97%).[4,5,7,8,10,12,14,15,18,19,22,24,26,28,34–37] Other body segments including the knee, elbow, shoulder, hand and spine (in decreasing incidence) may be involved.[7,10,12,14,22,23,33,36,38–42] Incidentally, NHO has been reported after soft tissue surgery in SCI patients.[43,44]

Although NHO may develop even several years after SCI, it is generally diagnosed between 1 to 6 months post-injury with a peak incidence at 2 months[7,8,10,12,19,22,45–50] Although NHO may begin well before the clinical signs become evident, the initial signs are often seen within the first 3 weeks after SCI.[3,4,7,9,39,51] The most common clinical findings are a decreased joint range of motion and a peri-articular swelling due to interstitial oedema of the soft tissues.[1,7,8,10,30,36,39,45,52,53] In patients with sensory sparing, the first symptom may be pain in the affected area.[10,54] Peri-articular erythema and warmth may also occur, sometimes accompanied by a low-grade fever.[55] Spasticity may increase secondary to the NHO development.[53] Reduction of hip joint movement and spasticity may lead to loss of an adequate sitting position,[51] pressure sores, and related pain complaints[52] and may also compromise transfers and activities of daily living. Although not commonly reported in SCI patients, ectopic ossification has been associated with compression of vascular structures and nearby peripheral nerves.[56–61] The relatively low

*Correspondence: AA van Kuijk, University Medical Centre St Radboud, Huispost 720, PO Box 9101, 6500 HB Nijmegen, The Netherlands

incidence of neuropathies found in SCI patients may be due to the sensory loss in (complete) SCI patients, so that they will not experience painful paraesthesias associated with nerve compression. Moreover, progression of NHO sufficient to compress nerves and nearby vascular structures and thereby causing clinical symptoms may result in ankylosis prior to possible neurovascular compression.

Pathophysiology

NHO originates in the connective tissues and may be contiguous with the skeleton, but does not involve the periosteum. When near a joint, it leaves the joint space and capsule preserved.[34–36,48,62] Muscle fibres are not primarily involved in the process, but can be incorporated in or compressed by the fibrosing and calcifying soft tissues leading to local muscle necrosis.[8,34,48]

NHO begins as an area of oedematous, inflammatory reaction coinciding with an increased bloodflow in the affected soft-tissues.[48] First, an exsudative cellular infiltration is seen, then fibroblastic proliferation occurs followed by osteoid formation, and subsequent deposition of bone matrix. Primitive osteoid is deposited as small masses within an area of fibroblastic mesenchymal reaction early within the first 2 weeks at first in the periphery. Osteoblasts produce tropocollagen, which polymerizes to form collagen, and secrete alkaline phosphatase (AP). The AP lyses pyrophosphate, a compound that prevents calcium deposition. Thus, by inactivating the pyro-phosphate near the developing ectopic bone matrix, AP allows calcium to precipitate and the bone matrix to mineralize.[63–65]

The mineralization process of the soft tissues consists of an amorphous calcium phosphate phase, which is gradually replaced by enlarging hydroxyapatite crystals.[48] The centripetal pattern of maturation that is seen in the following weeks is the basis of the zone phenomenon described by Ackerman.[66] The zone phenomenon is characterized by a thin outer zone in the surrounding muscle that encloses a broader intermediate zone. In the intermediate zone, areas of immature bone are lined by osteoblasts, while in the outer margins of this zone mature bone forming a well-demarcated outer trabecular rim is already present. The intermediate zone surrounds the central zone that consists of an undifferentiated highly cellular proliferation of fibroblasts with haemorrhage and muscle necrosis. As the lesion matures, the peripheral rim of the intermediate zone becomes radiographically opaque due to progressive mineralization.[67–69] The entire sequence of bone maturation is usually completed within 6–18 months.[10,35,52,70] Mature NHO resembles normal bone, both histologically and radiologically.[71,72] and consists of cancellous bone with Haversian canals, cortex, blood vessels and bone marrow, although with a minor amount of hematopoesis.[34,36,39,48,73]

According to Chalmers *et al*[74] three conditions must be met for the formation of ectopic ossification: the presence of osteogenic percursor cells, an inducing agent, and a permissive environment. Although the precise causal mechanism for NHO is still unknown, humoral, neural and local factors probably all play a role in its pathophysiology (Figure 1). There is either a migration of distant mesenchymal cells to the area involved, with subsequent transformation of these cells into osteoblasts, or a transformation of the local mesenchymal cells directly into osteoblasts.[52,63,75–77] Whether these cells migrate at random or in response to some chemotactic factor is still not known, but the importance of several factors has been suggested in the transformation of mesenchymal cells into osteoblasts.[77,78]

Humoral factors

Recent work with the serum of traumatic brain injured (TBI) and SCI patients seems to indicate that humoral mechanisms may be involved.[79,80] In the study of Binder,[79] the serum of a TBI patient increased the osteoblast growth factor activity in foetal rats. Kurer *et al*[80] incubated sera from SCI patients with and without NHO 4–7 months post injury and sera from healthy control subjects with human osteoblasts in tissue culture. An increase in osteoblast stimulating factors was found only in the SCI groups and the activity was more pronounced in the group of SCI patients with NHO compared to patients without NHO. Renfree *et al*[26] incubated sera from SCI and TBI patients throughout the first 12 weeks post-injury with osteoblasts from foetal rats. They observed a significant rise in serum mitogenic activity during the period after the injury in both patient groups. However, no significant differences were seen when patients who developed NHO were compared to other patients and healthy control subjects. Because their findings did not support the existence of a humoral factor that *directly* stimulates osteoblast proliferation within the first 12 weeks post injury, Renfree *et al*[26] hypothesized that the rise in mitogenic activity seen in their patients may *indirectly* play a role in the bone inductive process.

Although humoral factors may play a role in the osteo-inductive process their origin and their biological characteristics have not actually been identified in SCI patients. Yet, in experimental studies concerning the *in vitro* induction of ectopic bone, an osteo-inductive protein released from demineralized bone tissue could be identified.[81] This factor was called bone morphogenic protein (BMP).[77,82–84] It is, therefore, possible that bone resorption and collagen degradation in acute SCI patients may well release (still unidentified) osteo-inductive factors.[85]

Neuro-immunological factors

The neural influence on NHO development cannot be disregarded taking into account its high incidence in neurologic disorders and the confinement of the NHO to the body regions with neurologic deficits. Dejerine[1]

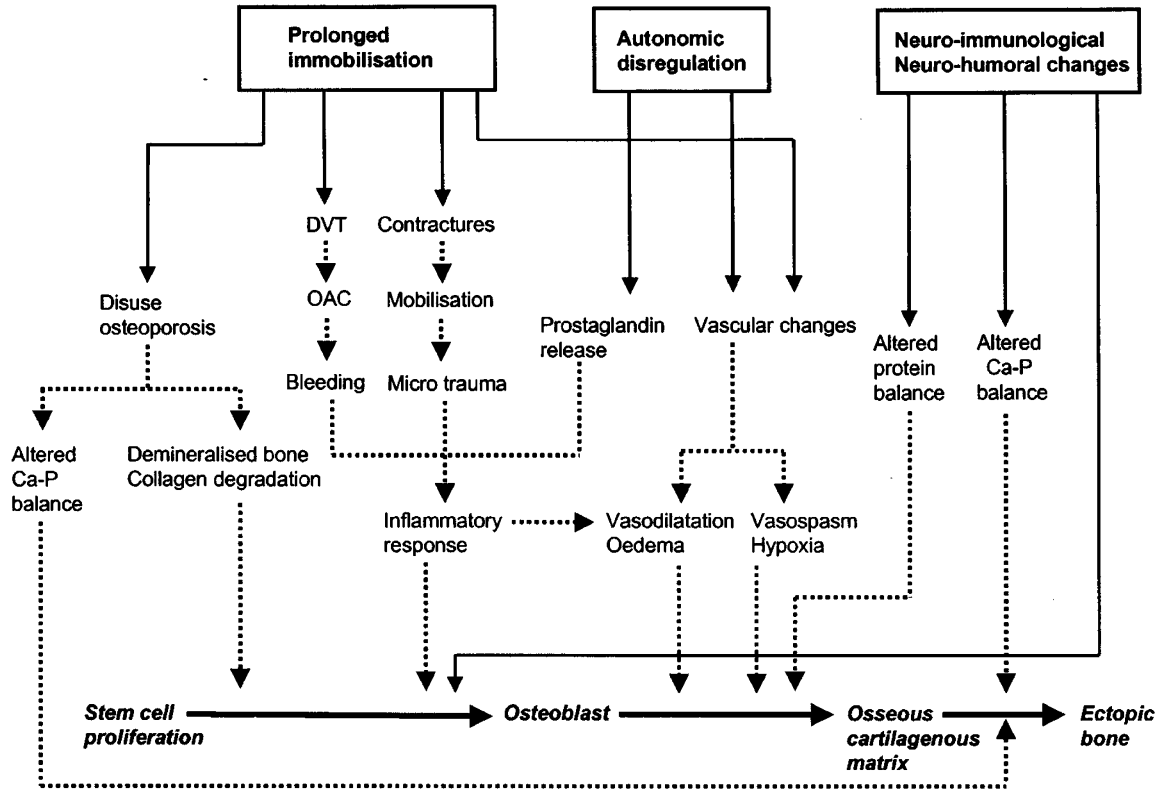

Figure 1 Visual approach to the pathophysiology of NHO in SCI patients. OAC: oral anti-coagulant. DVT: deep venous thrombosis

was one of the first to suggest that damage to the intermedio-lateral sympathetic columns of the traumatized spinal cord might predispose to NHO through autonomic dysregulation. Secondary to an altered balance within the autonomic nervous system, a diversity of metabolic and vascular changes may occur.[1,3,36,46,48,73,86] Indeed, several studies have shown that the initial stage of NHO is characterized by local microvascular alterations such as an increased vascularity, venous haemostasis and arterio-venous shunting in the involved tissues.[36,48,87] These modifications in the blood perfusion and oxygen levels of the soft tissues might play a critical role in the formation of NHO,[36,87,88] although it remains unclear whether these changes are secondary to or a causal factor in the NHO. Whether a disruption of the neuro-immunological pathways could result in an abnormal balance between osteoblast and osteoclasts and the subsequent induction of NHO is also still hypothetical.[89] Yet the presence of interstitial oedema by itself, whether due to autonomic dysregulation,[1] hypersensitivity, or hypoproteinemia[6] may add to a permissive environment by facilitating pathological calcification of the osteoid.

Local factors

The local factors that may predispose to NHO are venous thrombosis or haemostasis, (local) infection, decubitus ulcers and (micro) trauma. These factors may lead to tissue damage and subsequent inflammatory reactions causing oedema and tissue hypoxia[12] and may predispose to ectopic bone formation either by providing a permissive environment or by releasing humoral factors through the inflammatory process. In animal studies it has been suggested that eg prostaglandin E2 (PGE2) and interleukin-1 can induce a dose dependent increase in the subperiostal lamellar bone formation[90] and that subcutaneous injections of PGE2 in growing rats can induce heterotopic bone formation.[91,92] Measurement of 24-h PGE in SCI patients with NHO showed abnormal 24-h PGE2 urinary excretion as long as the bone-scan had not stabilized.[27] However, the mechanism by which prostaglandins may induce ectopic bone formation in SCI patients remains unclear and there are well-known difficulties in the interpretation of PGE2 urinary excretion measurements in men presenting with a positive urinary sperm count or in patients with lower urinary tract infections. Both groups account for a substantial amount of the SCI population.

Risk factors

Several risk factors have been associated with NHO. In general, no association was found between NHO and race or gender,[13,19] although in some studies a slightly

higher incidence has been suggested for (young) male patients.[10,13,14,17,24,47] A genetic predisposition for NHO based on human lymphocyte antigen (HLA)-typing has also been suggested. The human leukocyte antigen system (HLA) consists of a series of glycoprotein molecules that appear on the surface of virtually all nucleated cells. The major histocompatibility complex (MHC) is located on the short arm of the sixth chromosome and regulates a large and complex portion of the body's immune system and possibly other aspects of cell proliferation. The MHC contains a number of loci, more specifically the classic HLA-antigens (HLA-A, HLA-B, and HLA-C).[93] Some authors have suggested an association between HLA B18 and HLA B27 on the one hand and NHO on the other hand,[94–96] whereas others could not corroborate this association.[93,97–99]

The degree of completeness of SCI seems to be more important than the level of the lesion. Although Catz *et al*[37] did not find a relationship between radiologically diagnosed NHO and the severity of the motor deficits, other authors have reported that complete transverse SCI is more commonly associated with NHO than incomplete SCI with relative risks (RR) reported between 2.0–4.2.[1,13,14,19,21,22,24,88] Also, NHO is less frequently reported (<5%) in patients with lumbosacral or conus-cauda lesions, which regain or retain ambulation.[88]

Other clinical factors associated with NHO are the presence of pressure sores,[6,21,22,24,30,47] urinary tract infections or renal stones,[3,4,6,24,100] deep venous thrombosis (DVT),[24] severe spasticity,[22] and (micro) trauma. An area of soft tissue damage due to pressure ulceration with subsequent oedema may predispose to the development of ectopic ossification.[101] On the other hand, pressure sores may occur secondary to NHO due to a decrease in eg hip range of motion that affects sitting position and alters pressure patterns. As a result, body weight is unequally distributed among the tubera and pressure ulceration may evolve, mostly contralateral to the NHO affected side.[11]

An infected urinary tract could serve as a source of antigenic material precipitating an immune response that triggers subsequent NHO.[89] On the other hand, it may be that demineralization of bone accompanied by a loss of calcium and a subsequent loss of collagen as is seen in the acute phase of SCI[102,103] is associated with an increased risk of developing both urinary tract stones, osteoporosis and NHO.[3,4,17,100] As yet, the conclusion seems justified that the relatively high incidence of urinary tract infection and stone formation in SCI patients with NHO is not well understood from a pathophysiological perspective.[21,22]

Patients in the acute phase of traumatic SCI are known to be hypercoagulable and at risk of developing thromboembolic complications. In several studies an association was found between DVT and NHO, with RR reported between 1.8 and 2.0 for patients with DVT compared to patients without DVT. Colachis *et al*[25] found a 5.3% co-incidence of DVT and NHO. Although the authors routinely screened all SCI patients on admission for DVT, they did not routinely screen for NHO at the same time and hence, there may have been an underestimation of the real incidence of NHO in this study. In all cases the DVT was diagnosed prior to the NHO and the NHO activity was present at the same side of the body. Although these findings suggest that DVT may be a possible risk factor for NHO, Perkash *et al*[104] emphasized in their report of three chronic SCI patients with acute NHO the possibility of a *secondary* hypercoagulable state. They reported that the NHO activity correlated well with the increased coagulation parameters. As the NHO activity decreased, the coagulation parameters returned to near baseline values suggesting that NHO may alter blood coagulation and thereby predispose to DVT. Another possible mechanism by which NHO may predispose to DVT is a compression of vascular structures by local oedema as well as by the expanding ectopic bone mass.[56,57,59,61,105,106] As a consequence, it can also be concluded that the precise pathophysiological relationship between DVT and NHO has yet to be established.

Controversy also exists regarding the possible association between NHO and spasticity. In some studies NHO is more commonly seen in SCI patients with spasticity and more extensively in those with severe spasticity (RR 0.17–2.0).[21,22,30] Moreover, ectopic ossification seems to be rare in flaccid limbs or in limbs not affected by spasticity.[17,21,52] Although such observations may suggest that spasticity predisposes to the development of ectopic ossification, the direction of this putative causality may easily be reversed such that a developing ectopic mass leads to an increase in spasticity.[6,7,10,14]

As for the role of (micro) trauma, mechanical stress to the musculotendinous apparatus may arise either from vigorous passive exercises[9,17,46] or from loss of mobility and muscle imbalance causing peak pressure on soft tissue areas. Mechanical stress causes local micro-trauma that may induce ossification either indirectly through an inflammatory response or directly by releasing osteoblast-stimulating factors. Results from several case series revealed that the time-interval between the SCI and the beginning of passive movement exercises plays a vital role in the risk of developing NHO.[6,9,51,107] In the study of Daud,[107] clinically apparent NHO only occurred in SCI patients in whom the start of passive motion exercises was delayed until 7 or more days after the SCI. These results were supported by animal studies.[108,109] Studies performed by Izumi[110] and Michelson[108,109] demonstrated that heterotopic bone could be produced in rabbits by forced passive movements in paralytic limbs that had been immobilized for a certain period. It is, therefore, tempting to speculate that (forced) passive movements following a period of immobilization may easily result in shear and tear of soft tissues leading to an increased risk of developing NHO.[111,112]

In conclusion, apart from the existence and completeness of SCI and the probable role of (micro) trauma, the reports of other possible risk factors and their presumed causal relationship with NHO are still inconclusive.

Diagnosis

NHO is primarily diagnosed based on clinical signs and likelihood. The early symptoms must be differentiated from arthritis,[113] thrombophlebitis, DVT, cellulitis, soft-tissue haematoma, complex regional pain syndrome (CRPS),[114] and soft tissue tumour.[8,35,36,105,115–118] Elevated serum alkaline phosphatase (SAP) levels may be of value in differentiating early NHO from other inflammations, as SAP may be markedly elevated during active osteogenesis.[16] Ultrasonography can be used for the early identification of clinically suspected NHO and for differentiating NHO from DVT, a developing pressure sore, infection, or tumor.[60,65,69,112,119] The use of phlebography to differentiate between NHO and DVT can be misleading. A large mass of ectopic bone can distort and compress vascular structures, causing venographic findings that mimic venous thrombosis.[60,61,106] In order to differentiate between NHO and arthritis due to rheumatic disease, synovial fluid analyses can be performed. In contrast with arthritis due to rheumatic disease, synovial fluid analysis in NHO reveals a low white blood cell count, high protein levels, low viscosity and no crystals.[39,48,113,120]

Laboratory examinations

It has been shown that routine blood chemistry, eg the determination of isolated urinary and serum calcium, is of little value in the diagnosis and monitoring of NHO.[16,48,65,72,100,121–123] Also an elevated erythrocyte sedimentation rate may reflect the initial 'inflammatory' phase of NHO, but is very aspecific.

The SAP consists of a series of iso-enzymes which are sensitive, but non-specific indicators of heterotopic ossification. They are found in several human tissues including the skeleton, the liver, the intestinal mucosa, and the placenta.[64] The various iso-enzymes can be separated by electrophoresis. Electrophoretical studies performed with the sera of NHO patients revealed that elevated SAP levels reflect the activity of the ossification process.[16,116,124] When new bone is actively deposited, the SAP levels are elevated. As soon as the ossification has stopped, the enzyme levels return to normal.[16,48,70,72,125] In general, SAP levels start to rise on average 7 weeks before the first clinical signs of NHO become apparent, exceed normal levels 1 week later and reach peak levels 3 weeks after the appearance of the first clinical signs.[65,70] Thereafter, the SAP levels gradually decline to reach normal values at about 5 months post onset. However, extensive bone formation may lead to prolonged elevation of SAP levels,[39,48] whereas a minor amount of bone formation may not lead to SAP elevation at all. Normalization of the SAP levels does not constitute adequate proof of stabilization of the osteogenic process[45,48,49,52,62] nor does the height of the SAP levels accurately correlate with either the peak activity in the bone formation or the number or amount of NHO lesions.[100] Therefore, SAP levels are generally considered of limited value for judging NHO maturity, as well as for detecting possible recurrence or reactivation. Nonetheless, SAP levels may play a role in diagnosing NHO, since the persistent elevation of both inorganic phosphate and SAP levels increases the likelihood of active heterotopic bone formation.[122]

The determination of urinary collagen metabolite excretion has also been advocated in SCI patients with NHO. Normally, the urinary excretion of collagen related glycosides is an indication of the tissue origin of the collagen being currently degraded. A degradation of bone collagen will produce a large increase in the excretion of gal-hydroxylysine and a smaller increase in the glu-gal hydroxylysine excretion. The reverse will be true in the case of skin collagen degradation as seen in decubitus.[100] In SCI patients with NHO the gal-hydroxylysine excretion in urine was found to be higher than in SCI patients with NHO, and did not return to control values until the bone turnover had stabilized.

Also the urinary concentration of hydroxyproline, which is another collagen metabolite, was found to parallel the level of SAP in SCI patients with NHO.[72,48] The peak in the hydroxyproline excretion is related to a specific polypeptide fraction that reflects the collagen degradation.[48,122] Yet, the urinary hydroxyproline levels appear to be elevated in nearly all tetra en paraplegic SCI patients, which makes it difficult to differentiate between the general influence of SCI and the specific influence of bone formation in patients with early NHO development. Moreover, although urine hydroxyproline levels are associated with SAP levels, they are unreliable parameters in determining NHO maturation.[48]

Longitudinal studies are necessary to determine the precise temporal relationship between an increase in the characteristic urinary glycoside excretion and the appearance of clinical signs of bone formation. Until now, measurements of collagen metabolites derived from bone, connective tissue, or muscles have been abandoned, because of their unspecificity and methodological difficulty.[36]

Radiological examination

In the early stages of NHO, the bone formation mainly consists of osteoid that shows a high uptake of osteotropic radionuclides through which it is readily detectable by three-phase 99m Technetium bone scanning.[15,16,28,39,70,126–132] The first phase of the three-phase bone scan is the period immediately after the intravenous injection of the radionuclides and detects areas of increased blood blow, which is an early indicator of the inflammation process (the

dynamic blood flow phase). The second phase (the static blood pool phase), identifies areas of increased blood pooling several minutes after the injection. The third phase (the static bone phase) determines the degree of osseous uptake of the labelled radionuclides several hours after the injection. The blood flow and blood pool phases of the bone scan are able to detect NHO as early as 2.5 weeks after SCI and are followed by a positive static bone phase 1–4 weeks later.[28,129] Bone scans return to normal as the NHO matures usually within 6–18 months after its first clinical signs.[70] Compared to plain radiography, bone scanning is a more sensitive diagnostic test for early NHO, but radiography is more specific. A disadvantage of the three-phase bone scan is its low specificity leading to potential difficulty in discriminating NHO from other inflammatory, traumatic, or degenerative processes of the skeleton, eg fracture, bone tumor, metastasis, or osteomyelitis which all show increased osteoblastic activity and thereby increased uptake of osteotropic radionuclides.[126,127,132–134] Moreover, it requires expensive specialist equipment and the use of ionising radiation. Yet bone scanning may reach a fair specificity once clinical signs and likelihood have given rise to reasonable suspicion of NHO.[28] In monitoring NHO maturation, the three-phase bone scan seems the most sensitive tool and currently the 'golden standard'.[49,126,129,132] Nonetheless, the criteria for maturity rating on the bone scans remains a topic of discussion. Whether a qualitative assessment eg a continuous decrease in the uptake or a steady state in the radionuclide accumulation, or a quantitative assessment such as specific uptake ratios should be considered as the best maturity index remains unclear.

The sonographic diagnosis of NHO depends on the age of the lesion, the rate of bone formation and the degree of mineralization.[133] The first finding on ultrasound (US) examination of a focal, elongated hypoechoic mass is unspecific and is also seen in muscle tears, abscess, and soft tissue tumor. The muscle fibres and soft tissues surrounding the lesion may appear compressed, but the interface is smooth and there is no evidence of invasion. Thereafter, the development of a centripetal pattern of maturation can be seen. Initially, the intermediate zone contains foci of echodense islands and will not be uniformly reflective. Later, the foci of echodense islands rapidly become confluent[68,69,135] and the complete zone phenomena can be seen sonographically. As the NHO matures, the peripheral rim of the intermediate zone becomes more reflective on US due to increased mineralization[67–69] and 4–6 weeks after the first clinical signs the NHO behaves like corticated bone. At that time, the US beam is totally reflected and the NHO can also be seen radiographically.[133,69] Mechanical forces can modify the appearance of the zone phenomena. Therefore, the sensitivity and specificity of US for diagnosing NHO strongly depend on the experience of the radiologist. Serial sonography allows differentiation from muscle tears, soft tissue haematoma, abscess, thrombosis and soft tissue tumor.[67,68,119,135,136] Sonography has the advantage of the possibility of bedside application, is relatively cheap, and requires no radiation.

The earliest radiographic sign of NHO is an increased density of the peri-articular soft tissues due to oedema.[39,47,48] Gradually, the image shows flocculent densities as calcium precipitates. The maturation process proceeds rapidly with increased delineation of the soft tissue mass and the formation of bony cortex and trabeculations. The skeleton underlying the NHO may show variable degrees of demineralization, but the underlying bone configuration and the joint surfaces remain unaffected.[18] NHO will become evident on plain radiography upon accumulation of minerals about 2–6 weeks after the three-phase bone scan first becomes positive.[70,129,137] In several studies radiographic signs of NHO were found on average 1–10 weeks after the first clinical signs.[19,36,47] Plain radiography may be positive even in asymptomatic patients as early as 4.5 weeks after the SCI. For monitoring NHO activity, plain radiography may be valuable as long as the X-rays show modification from one examination to the next.[48] However, with a large block of ectopic bone, interpretation of the X-rays is difficult because immature elements may be obscured by mature bone.[47] Therefore, radiography is generally considered of little value for judging NHO maturity,[47] as well as for detecting possible recurrence or reactivation.[49]

CT scanning allows a better visualization of the ectopic bone in relation to the soft tissues.[118,138] The CT scan may aid in the development of a surgical plan,[139] especially three-dimensional tomography, eg in order to avoid areas of immature bone.[140] MRI scanning is the best technique to define the extent of the soft tissue oedema, but has no role in the early diagnosis of NHO. The appearance of soft tissue swelling is aspecific and the lack of signal from calcification prevents differentiation from other inflammatory processes.[118]

The vascular changes seen immediately after SCI and more frequently in the case of concomitant NHO can be visualized by angiography. They consist of opening of the arteriovenous shunts, early venous return, and locally increased vascularity, which can easily be determined by angiography.[48,141] However, the persistence or absence of venous return is not a valid indication of the degree of maturity. As ectopic bone matures, the pathological angiographic findings regress, in particular the vasodilatation and blood pooling without necessarily returning to normal.[48,141] Complications of angiography are haematoma and subsequent scar tissue formation. As yet, angiography has no role in diagnosing or monitoring NHO.

Treatment

Disodium etidronate (EHDP)

Once NHO has been diagnosed, patients can be treated with sodium etidronate, which is a disphosphonate.

Diphosphonates are structural analogues of the naturally occurring inorganic pyrophosphates, which play an important role in the calcium-phosphate metabolism. Unlike the natural pyrophosphates, diphosphonates are almost completely stable to biochemical degradation. EHDP has been most widely used in patients with ectopic bone formation. *In vitro* EHDP strongly binds with hydroxyapatite, the main inorganic constituent of bone, thereby blocking the transformation of amorphous calcium phosphate into hydroxyapatite crystals, without inhibiting the formation of bone matrix.[142–145] In animal studies and experimental models of ectopic ossification, it has also been demonstrated that EHDP can reduce the number of bone forming cells and alter their cell morphology.[146,147] EDHP might also have an anti-inflammatory effect probably by affecting the production of interleukin-1.[148]

In SCI patients EDHP has mainly been used to block ectopic bone formation in the early phase after the clinical diagnosis of NHO. Stover[62] treated patients on average 56 days post-SCI with EHDP 20 mg/kg/day for the first 2 weeks, followed by 10 mg/kg/day for the next 10 weeks. At the start of the EHDP treatment radiographic evidence of NHO was found in 25% of the placebo-group and in 22% of the EHDP group. After the treatment 41% of the placebo group and 30% of the EHDP group showed radiographic evidence of NHO. At its best EHDP may have slowed down the mineralization process. The authors suggested that for an optimal effect EHDP should be started at an earlier phase of NHO before significant amounts of ectopic bone have been formed. Finerman *et al*[29] treated patients with clinical signs but without radiographic evidence of NHO according to Stover's[62] treatment protocol on average 112 days post-SCI. At the end of the treatment period of 12 weeks, 6% of the EHDP group demonstrated NHO on X-rays, of which only 2% was clinically significant. In the placebo group, 27% demonstrated radiographic evidence of NHO, of which 13% was clinically significant. However, after cessation of treatment, the incidence of NHO increased in the EHDP group, although the lesions were less extensive than in the placebo group. Since the majority of NHO is radiographically diagnosed within the first 6 months after SCI,[52,149,70] Garland *et al*[20] stated that EHDP should be administered for at least a 6-month period. Although EHDP seems to block the mineralization process to a certain extent, it does not influence the formation of the osteoid matrix. After cessation of EHDP administration, the bone matrix already formed may undergo an uninhibited mineralization, the so-called 'rebound ossification'.[52,65]

Some of the differences seen in therapeutic response to EHDP between patients may be due to variations in the bioavailability of EHDP after absorption by the gut. Because of the low solubility of EHDP in water, absorption in the intestinal tract can fluctuate from 1–10%. To avoid such variations and to improve the bioavailability of EHDP, Banovac *et al*[137] administered EHDP intravenously in SCI patients with early clinical signs of NHO and a positive I and II phase on the bone scan on average 28 days post-SCI using high dosages over a prolonged time period: 300 mg for 3 days, followed by 20 mg/kg/day orally for 6 months. A prompt reduction in the peri-articular swelling was seen during the first 48 h. However, after the treatment period, no radiographic differences with regard to extent of NHO were found between the EHDP patients and a control group treated with 20 mg/kg/day orally for 2 weeks followed by 10 mg/kg/day for 6 months. In the subgroup without radiographic evidence of NHO (N = 12), only two patients (15%) developed minimal radiographic evidence of NHO at the end of the follow-up period on average 11 months post onset of therapy. Banovac[28,150] repeated his study in asymptomatic patients with a positive third phase on the three-phase bone scan. Only one patient developed NHO to a minimal extent.

Side effects of EHDP include hyperphosphatemia. In animal studies EHDP induced osteomalacia and spontaneous fractures have been reported. However, these complications have not been found in patients after total hip arthroplasty (THA) nor in SCI patients. Moreover, animal studies suggest that three to five times higher dosages are needed to induce such complications compared to the dosages currently used in SCI patients.[151] The main reasons for patients to discontinue EHDP treatment are gastro-intestinal symptoms such as nausea, diarrhoea, and abdominal distress.

Non-steroidal anti-inflammatory drugs (NSAID)

The basic knowledge about the prophylactic treatment of ectopic bone formation by NSAID comes from animal studies and studies in patients undergoing THA.[152–157] The effect of NSAID has been attributed to the inhibition of the inflammation process and the suppression of mesenchymal cell proliferation.[52] Anti-inflammatory drugs inhibit the release of prostaglandins and related substances, thereby reducing the stimulation of the formative and resorptive phases of the bone remodelling.[152–157] Moreover, NSAID are known to inhibit the differentiation of the mesenchymal cells into osteogenic cells and to reduce ectopic ossification in soft tissues in experimental conditions.

In the only randomised clinical trial (RCT) performed in SCI patients, Banovac *et al*[158] allocated patients to either indomethacin 75 mg/day or placebo for 3 weeks from admission to a rehabilitation centre on average 20 days after their injury. There was a significantly higher incidence of NHO diagnosed by bone scan in the placebo group (65%) compared to the indomethacin treated group (25%). In the indomethacin treated patients with NHO the onset of symptoms was delayed on average 31.7 days compared to the placebo treated patients with NHO on average 19.2 days. Also, the inflammatory symptoms, ie swelling, erythema, and fever were less pronounced.

No side effects were reported, although gastrointestinal complications eg dyspepsia and gastric ulceration or bleeding, and delayed fracture healing have been reported in other studies.[152,153] Moreover indomethacin is also known to have an antithrombotic effect and may alter blood coagulation especially with the concomitant use of other anticoagulants to prevent deep vein thrombosis (DVT).

Other types of medication

Two other types of medication have been used in the treatment of NHO after SCI. Calcitonin has been used in an uncontrolled study with inconclusive results.[159]

Warfarin, which is an anti-coagulant that inhibits vitamin-K-dependent synthesis of calcium binding proteins such as osteocalcin has been used in a historic cohort of 227 SCI patients, of whom 33 had been treated with a low dose (prothrombin time 1.5–2) for the prevention of DVT.[23] The authors did not observe clinically significant NHO as long as 10 years after SCI in the Warfarin treated group, whereas in the other patients clinically significant NHO developed in 15%. Although methodologically non-optimal, this study suggests a possible inhibiting effect of Warfarin on NHO, yet the nature of this effect remains to be elucidated.

Glucocorticoids, such as prednisolone can decrease the formation of bone and scar tissue *in vitro*. Animal studies showed that prednisolone decreased the inflammatory changes and fibrosis in rabbits caused by forceful manipulation after prolonged immobilization and therefore, also decreased ectopic bone formation.[154] However, until now, glucocorticoids have not been used in the treatment of NHO in SCI patients.

Low-field irradiation

The exact mechanism by which irradiation affects NHO formation remains unknown, although it is suggested that irradiation may disrupt the differentiation process of pluripotent mesenchymal cells into osteoblasts.[160–164] Irradiation may also decrease pain perception associated with the tissue inflammation surrounding the NHO, or it may induce the ablation of pain receptors.[164] Sautter-Bihl *et al*[165] treated 20 SCI patients with low field irradiation with 10 Gy in single fractions of 2 to 2.5 Gy. In 15 patients irradiation was given as a primary treatment to prevent NHO, whereas in seven patients it was used after operative NHO resection to prevent NHO recurrence. Four of the seven operated SCI patients, received a THA. During the follow-up period of 12 weeks, none of the 22 patients showed progression of their NHO. In the five patients followed for 44 months, the hip joint range of motion and sitting position did not deteriorate. In a second study, the same author[166] treated 49 SCI (70 joints) with low field irradiation with 8 to 10 Gy in 2–25 single dose fractions. In 58 joints, irradiation was the primary treatment after the first clinical signs of NHO, whereas in 12 joints irradiation was performed after surgical resection of the NHO. In 71% no progression of the incipient ossification or post-operative recurrence of NHO was seen during a follow-up period of on average 11 months.

Complications of radiation therapy include delayed wound and bone healing, osteonecrosis,[167] as well as the risk of developing radiation-induced sarcoma. Although these complications are not mentioned in relation to dosages less than 30 Gy delivered within a 3-week period,[168,169] they certainly limit the use of radiation therapy as the primary prevention of NHO, when a relatively long life expectancy and the frequent involvement of multiple joints in SCI patients are taken into account.[170]

Surgical resection

The indications for surgical resection of NHO are improvement of joint motion, eg to achieve an adequate sitting or standing position,[49,116,171] reduction of spasms, and prevention of pressure sores.[172–175] However, operative resection of NHO has been associated with severe complications and poor outcome.[171] These complications include deep (2–5%)[174,175] or superficial infection (7–38%),[45,49,174,175] post-operative haemorrhage (5–38%),[45,173] and excessive intra-operative bleeding requiring (multiple) blood transfusions (9–83%).[49,174–176] Moreover, intra- and post-operative fracturation of bone may occur in the case of severe osteoporosis. Stover[174] has reported intra-operative fracturation of the femoral head in 5% and post-operative (spontaneous) fracturation in 3–16% of the SCI patients operated for NHO around the hip joint. Since other authors mention a higher percentage (29%)[175] of SCI patients with femoral heads too brittle to preserve during operation, the real incidence of this problem may be even higher. NHO resection is also associated with a poor outcome due to a high recurrence rate. The post-operative recurrence of NHO depends on the definition used and the follow-up period. Radiographic signs of NHO recurrence have been mentioned in 82–100% of the operated SCI patients,[45,49,173,174] although clinically significant NHO would develop in only 17–58% of the cases.[10,45,49,173,174,176] It has been suggested that the recurrence after resection can be reduced if NHO is removed only once the first and second phase of the bone scan have normalized.[10,52,115,129,132] However, such a maturity rating does not guarantee that NHO recurrence is unlikely. Garland *et al*[49] reported that still 36% recurrence of clinically significant NHO was found in SCI patients with mature NHO on the bone scan. It was not so much the maturity that was associated with the risk of NHO recurrence, but rather the extent of the NHO preoperatively. There was also no correlation between NHO recurrence and the time post SCI or NHO onset.[49,174,177] Recently McAuliffe *et al*[170] and Freebourn[177] reported three patients that had successfully undergone early resection of NHO, that is

within 7–11 months post SCI. Earlier surgery may be warranted to prevent fibrous ankylosis, muscle contractures, and severe disuse osteoporosis to decrease the intra-operative fracturation rate. Hence, although many experts advise a time period of 12–18 months between NHO diagnosis and resection, no adequate studies are available to corroborate this advice or to give an indication of the most effective timing for the surgery.

Different prophylactic measures have been advocated to reduced the NHO recurrence post surgery. As for THA, both irradiation and indomethacin seem to be effective in reducing the occurrence of heterotropic ossification after THA,[152,153,160–163] whereas EHDP has been abandoned due to its ineffectiveness on bone matrix formation and the high risk of 'rebound' ossification.[142,146] In SCI patients EHDP,[174] NSAID, irradiation,[163,165,166,170,175] or a combination of treatments[177–179] have all been recommended to reduce the NHO recurrence after surgery, however, no controlled studies are available.[163,165,166,170,174–179]

Primary prevention

Since the pathophysiology of NHO is poorly understood, the only preventive treatment possible is the early identification and adequate treatment of the putative risk factors. Through adequate nursing management the incidence of urinary tract infections, decubitus ulcers, and DVT may be reduced, and thus, the risk of developing NHO. Although in the early literature aggressive passive physiotherapy has been recommended to improve joint mobility and to counteract ankylosis in the case of contractures, it is now generally accepted that SCI patients profit from early, regular, and cautious joint mobilization to prevent more rigorous exercises with the risk of (micro) trauma to the peri-articular tissues becoming necessary. When gentle passive movements of the large peripheral joints are started and maintained from the day of the injury, the joint capsules are kept as supple as possible, muscles will not easily shorten and contractures will not readily develop, so that NHO may be prevented. This insight takes into account the highly 'vascular' state of the paralysed area and the concomitant use of anticoagulants (to prevent DVT), that may predispose to haematoma and secondary NHO, particularly during the rehabilitation phase after SCI.[19]

Various, more specific prophylactic measures have been proposed to prevent NHO, including the use of diphosphonates, NSAID, and more recently irradiation. The latter two methods have been successful to some degree in preventing heterotopic ossification eg after THA,[142,152,153,160–163,180] but no controlled studies are available in SCI patients.

Conclusion

NHO is a frequent complication in SCI. Although the precise pathophysiology of NHO is still unknown, humoral,[26,79–85] neural,[1,3,6,36,46,48,73,86–89] and local factors[12,27,90–92] probably all play a role in the formation of ectopic bone. Apart from the completeness of SCI[1,13,14,19,21,22,24,88] and the possible role of (micro) trauma,[6,9,17,46,51,107–112] reports of possible risk factors like DVT, pressure ulceration, infection, and spasticity[6,7,10,14,21,22,24,30,47,89] and their presumed causal relationship with NHO are inconclusive. Studies on experimental bone induction have emphasized the role of neuro-immunological and humoral factors. The role of these factors is currently also studied in the pathophysiology of CRPS and shoulder–hand syndrome and seems to be a promising area of further research in the pathophysiology of NHO. Until now, most studies on experimental bone induction have been performed in animal models without SCI. To enhance our knowledge of the pathophysiology of NHO in SCI, research needs to be done in SCI patients as well as in animal models with SCI.

The diagnosis of NHO is primarily based on clinical signs and likelihood. It is the concurrence of persistent elevated SAP levels with characteristic clinical signs and, ultimately, positive radiographic findings that is essential for definitively diagnosing NHO.[122] Sonography may also play a role in the early diagnosis of NHO, however its sensitivity and specificity greatly depend on the experience of the radiologist.[67–69,133] The three-phase bone scanning is the most sensitive technique for diagnosing NHO and, although generally aspecific, may reach a fair degree of specificity once clinical signs and likelihood have given rise to reasonable clinical suspicion.[28,49,70,126,129,132] For monitoring NHO activity, plain radiographs and elevated SAP levels may be valuable as long as they show modification from one examination to the next, but currently the three-phase bone scan seems to be the 'golden standard'. CT and MRI scanning are mainly used to aid in the development of a surgical plan.[118,138,140]

Several studies have documented that early diagnosis is of crucial importance in the treatment of NHO.[29,65] Waiting for late clinical or radiographic symptoms of NHO may allow a significant amount of ectopic bone to be deposited before treatment is initiated. Although EHDP inhibits the mineralization of bone, it does not influence the formation of osteoid.[142–145] Hence, after cessation of EHDP, the bone matrix already formed may still undergo mineralization as soon as the drug has been cleared from the tissues, especially in the first 6 months after SCI, the so-called 'rebound' ossification.[52,65] Therefore, in the treatment with diphosphonates prolonged administration up to 6 months seems to be of crucial importance.[20,28,137,150] Yet, there is also some evidence that with the late application of EHDP, the severity of the lesions remains less compared to placebo treatment.[29,62] More recently, NSAID and irradiation have been used in SCI to prevent NHO occurrence. The effects of both NSAID and irradiation have been attributed to the suppression of the differentiation

process of the mesenchymal cells into osteoblasts.[52,152–157,160–164] Irradiation may also decrease pain perception association with the soft tissue inflammatory reaction surrounding the NHO, or it may result in the ablation of pain receptors.[164] NSAID may also exert an effect through the inhibition of the inflammatory response.[152–157] However, the exact mechanisms by which NSAID or irradiation prevent NHO development remain unknown. Both treatments, however, do not influence the ectopic bone already formed, and are, therefore, effective only in the early stages of NHO.[158,165,166] More controlled studies are needed to determine the effectiveness and complication rates of different (prophylactic) treatments for NHO in SCI patients.

The indication for surgery is to improve joint range of motion to achieve adequate posture, reduction in spasms, and prevention of pressure sores.[49,116,171,175] Operative resection of NHO is generally associated with severe complications and poor outcome.[45,49,71,173–176] It has been suggested that the recurrence of NHO after resection can be reduced, if the NHO is removed during a radionuclide steady stage, however, this policy is not supported by unequivocal clinical evidence.[10,52,115,129,132] Although most authors advise a delay of 12 to 18 months between NHO diagnosis and surgical resection, until now no adequate controlled studies are available to corroborate such advice. Hence, there is no consensus on the most effective timing for surgery.

To date there is no satisfactory prevention of NHO and its prophylaxis is mainly based on the early identification and adequate treatment of putative risk factors, such as DVT, pressure ulcers, urinary tract infection and spasticity. Yet, there is ample empirical evidence that regular and cautious mobilization of the large peripheral joints should be recommended, from the day of the injury, to keep the joint capsules as supple as possible and to maintain adequate muscle length. With such an approach contractures will not readily develop and NHO related to traumatizing mobilization might be prevented.

References

1 Dejerine A, Ceillier MA. Para-ostéo-arthropathies des paraplégiques par lésion médullaire: Étude clinique et radiographique. *Ann Méd* 1918; **5:** 497–535.
2 Heilbrun N, Kuhn WG. Erosive bone lesions and soft tissue ossifications associated with spinal cord injuries (Paraplegia). *Radiology* 1947; **48:** 579–593.
3 Abramason AS. Bone disturbances in injuries to the spinal cord and cauda equina. Their prevention by ambulation. *J Bone Joint Surg* 1948; **30(A):** 982–988.
4 Liberson M. Soft tissue calcifications in cord lesions. *JAMA* 1953; **11:** 1010–1013.
5 Lodge T. Bone, joint and soft tissue changes following paraplegia. *Acta Radiol* 1956; **46:** 435–445.
6 Damanski M. Heterotopic ossification in paraplegia: A clinical study. *J Bone Joint Surg* 1961; **43(B):** 286–299.
7 Hardy AG, Dickson JW. Pathological ossification in traumatic paraplegia. *J Bone Joint Surg* 1963; **45(B):** 76–87.
8 Freehafer AA, Yurick R, Mast WA. Para-articular ossification in spinal cord injury. *Med Serv J Can* 1966; **22:** 471–478.
9 Silver JR. Heterotropic ossification. A clinical study of its possible relationship to trauma. *Paraplegia* 1969; **7:** 220–230.
10 Wharton GW, Morgan TH. Ankylosis in the paralyzed patient. *J Bone Joint Surg* 1970; **52A:** 105–112.
11 Hassard GH. Heterotropic bone formation about the hip and unilateral decubitus ulcers in spinal cord injury. *Arch Phys Med Rehabil* 1975; **56:** 355–358.
12 Stover SL, Hataway CJ, Zeiger HE. Heterotopic ossification in spinal cord injured patients. *Arch Phys Med Rehabil* 1975; **56:** 199–204.
13 Scher AT. The incidence of ectopic bone formation in posttraumatic paraplegic patients of different racial groups. *Paraplegia* 1976; **14:** 202–206.
14 Hernandez AM *et al*. The para-articular ossifications on our paraplegics and tetraplegics. A survey of 704 patients. *Paraplegia* 1978; **16:** 272–275.
15 Prakash V, Lin MS, Perkash I. Detection of heterotopic calcification with 99m Tc Pyrophosphate in spinal cord injury patients. *Clin Nucl Med* 1978; **3:** 167–169.
16 Furman R, Nicholas JJ, Jivoff L. Elevation of the serum alkaline phosphatase coincident with ectopic bone formation in paraplegic patients. *J Bone Joint Surg* 1970; **52(A):** 1131–1137.
17 Goldman J. Heterotopic ossification in spinal cord injuries. *Physiotherapy* 1980; **66:** 219–220.
18 Blane CE, Perkash I. True heterotopic bone in the paralyzed patient. *Skeletal Radiol* 1981; **7:** 21–25.
19 Knudsen L, Lundberg D, Ericsson G. Myositis ossificans circumscripta in para-/tetraplegics. *Scand J Rheum* 1982; **11:** 27–31.
20 Garland DE, Alday B, Venos KG, Vogt JC. Diphosphonate treatment for heterotopic ossification in spinal cord injury patients. *Clin Orthop* 1983; **176:** 197–200.
21 Lal S, Hamilton BB, Heineman A, Betts HB. Risk factors for heterotopic ossification in spinal cord injury. *Arch Phys Med rehabilitation* 1989; **70:** 387–390.
22 Bravo-Payno P *et al*. Incidence and risk factors in the appearance of heterotopic ossification in spinal cord injury. *Paraplegia* 1992; **30:** 740–745.
23 Buschbacher R *et al*. Warfarin in prevention of heterotopic ossification. *Am J Phys Med Rehabil* 1992; **71:** 86–91.
24 Wittenberg RH, Peschke U, Bötel U. Heterotopic ossification after spinal cord injury; epidemiology and risk factors. *J Bone Joint Surg* 1992; **74(B):** 215–218.
25 Colachis SC, Clinchot DM. The association between deep venous thrombosis and heterotopic ossification in patients with acute traumatic spinal cord injury. *Paraplegia* 1993; **31:** 507–512.
26 Renfree KJ *et al*. Evaluation of serum osteoblast mitogenic activity in spinal cord and head injury patients with acute heterotopic ossification. *Spine* 1994; **19:** 740–746.
27 Schurch B, Capaul M, Vallotton MB, Rossier AB. Prostaglandin E_2 measurements: Their value in the early diagnosis of heterotopic ossification in spinal cord patients. *Arch Phys Med Rehabil* 1997; **78:** 687–691.

28 Banovac K, Gonzalez F. Evaluation and management of heterotopic ossification in patients with spinal cord injury. *Spinal Cord* 1997; **35:** 158–162.
29 Finerman GAM, Stover SL. Heterotopic ossification following hip replacement or spinal cord injury. Two clinical studies with EHDP. *Metabl Bone Dis Rel Res* 1981; **4/5:** 337–342.
30 Taly AB *et al*. Heterotopic ossification in non-traumatic myelopathies. *Spinal Cord* 1999; **37:** 47–49.
31 Garland DE *et al*. Spinal cord insults and heterotopic ossification in the pediatric population. *Clin Orthop* 1989; **245:** 303–310.
32 Kluger G, Kochs A, Holthausen H. Heterotopic ossification in childhood and adolescence. *J Child Neurol* 2000; **15:** 406–413.
33 Frankel HL. Traumatic quadriplegia with ectopic ossification. *Proc R Soc Med* 1971; **64:** 222–223.
34 Miller LF, O'Neill CJ. Myositis ossificans in paraplegics. *J Bone Joint Surg* 1949; **31(A):** 283–294.
35 Venier LH, Dutunno JF. Heterotopic ossification in the paraplegic patient. *Arch Phys Med Rehabil* 1971; **52:** 475–479.
36 Chantraine A, Minaire P. Para-osteo-arthropathies. A new theory and mode of treatment. *Scand J Rehabil Med* 1981; **13:** 31–37.
37 Catz A *et al*. Is the appearance of peri-articular new bone formation related to local neurological disability? *Paraplegia* 1992; **30:** 361–365.
38 Lynch C, Point A, Weingarden SI. Heterotopic ossification in the hand of a patient with spinal cord injury. *Arch Phys Med Rehabil* 1981; **62:** 291–293.
39 Nicholas JJ. Ectopic bone formation in patients with spinal cord injury. *Arch Phys Med Rehabil* 1973; **54:** 354–359.
40 Cope R. Heterotopic ossification. *South Med J* 1990; **83:** 1058–1064.
41 Bell RB, Wallace CJ, Swanson HA, Brownell AKW. Ossification of the lumbosacral dura and arachnoid following spinal cord trauma. Case report. *Paraplegia* 1995; **33:** 543–546.
42 Good AE, Solsky MA, Gulati SM. Heterotopic ossification simulating acute arthritis. A patient with stable, chronic neurologic disease. *J Rheumatol* 1983; **10:** 124–127.
43 Röhl K, Bosse A, Bötel U. Ungewöhnliche lokalisation einer heterotopen Ossifikation im bereich einer Laparotomienarbe. *Unfallchirung* 1993; **96:** 662–664.
44 Rubayai S, Ambe MK, Garland DE, Capen D. Heterotopic ossification as a complication of the staged total thigh muscles flap in spinal cord injury patients. *Annals Plast Surg* 1992; **29:** 41–46.
45 Hsu JD, Sakimura I, Stauffer ES. Heterotopic ossification around the hip joint in spinal cord injured patients. *Clin Orthop* 1975; **112:** 165–169.
46 Nechwatal E. Die Vermeidung Heterotoper Ossifikationen. Ein zentrales Problem bei der Fruehbehandlung von Querschnittgelaehmten. *Z Orthop* 1972; **110:** 590–596.
47 Tibone J, Sakimura I, Nickel VL, Hsu JD. Heterotopic Ossification around the hip in spinal cord-injured patients. A long term follow-up study. *J Bone Joint Surg* 1978; **60(A):** 769–775.
48 Rossier AB *et al*. Current facts on para-osteo-arthropathy (POA). *Paraplegia* 1973; **11:** 36–78.
49 Garland DE, Orwin JF. Resection of heterotopic ossification in patients with spinal cord injuries. *Clin Orthop* 1989; **242:** 169–176.
50 Kim SW, Wu SY, Kim RC. Computerized quantitative radionuclide assessment of heterotopic ossification in spinal cord patients. *Paraplegia* 1992; **30:** 803–807.
51 Roche MB, Jostes FA. Ectopic bone deposits. A paraplegic complication. *Am J Surg* 1948; **75:** 633–636.
52 Garland DE. A clinical perspective on common forms of acquired heterotopic ossification. *Clin Orthop* 1991; **263;** 13–29.
53 Blankenship LD, Strommen JA. 27-year-old man with a swollen leg. *Mayo Clin Proc* 2000; **75:** 977–980.
54 Rosin AJ. Ectopic calcification around joint of paralyzed limbs in hemiplegia, diffuse brain damage and other diseases. *Ann Rheum Dis* 1975; **34:** 499–505.
55 Tow AP, Kong KH. Prolonged fever and heterotopic ossification in a C4 tetraplegic patient. Case report. *Paraplegia* 1995; **33:** 170–174.
56 Colachis SC, Clinchot DM, Venesy MD. Neurovascular complications of heterotopic ossification following spinal cord injury. *Paraplegia* 1993; **31:** 51–57.
57 Varghese G, Williams K, Desmet A, Redford JB. Nonarticular complication of heterotopic ossification: a clinical review. *Arch Phys Med Rehabil* 1991; **72:** 1009–1013.
58 Bradleigh LH *et al*. Deep venous thrombosis associated with heterotopic ossification. *Arch Phys Med Rehabil* 1992; **73:** 293–294.
59 Haselkorn J, Britell CW, Cardenas DD. Diagnostic imaging of heterotopic ossification with coexistent deep-venous thrombosis in flaccid paraplegia. *Arch Phys Med Rehabil* 1991; **72:** 227–229.
60 Yarkony GM, Lee MY, Green D, Roth EJ. Heterotopic ossification pseudophlebitis. *Am J Med* 1989; **87:** 342–344.
61 Paul SM, Hammerman A, Stoddard E. Heterotopic ossification causing extrinsic compression of the deep venous system of the lower extremity (abstract). *Arch Phys Med Rehabil* 1988; **69:** 760.
62 Stover SL, Niemann KMW, Miller III JM. Disodium Etidronate in the prevention of postoperative recurrence of heterotopic ossification in spinal cord injury patients. *J Bone Joint Surg* 1976; **58(A):** 683–688.
63 Jensen LL, Halar E, Little JW, Brooke MM. Neurogenic heterotopic ossification. *Am J Phys Med* 1987; **66:** 351–363.
64 Bolger JT. Heterotopic bone formation and alkaline phosphate. *Arch Phys Med Rehabil* 1975; **56:** 36–39.
65 Buschbacher R. Heterotopic ossification. A review. *Crit Rev Phys Rehabil Med* 1992; **4:** 199–213.
66 Ackerman LV. Extra osseous localized non-neoplastic bone and cartilage formation. *J Bone Joint Surg* 1958; **40(A):** 279–298.
67 Peck RJ, Metreweli C. Early myositis ossificans: a new echographic sign. *Clin Radiol* 1988; **39:** 586–588.
68 Fornage BD, Eftekhari F. Sonographic diagnosis of myositis ossificans. *J Ultrasound Med* 1989; **8:** 463–466.
69 Cassar-Pullincino VN *et al*. Sonographic diagnosis of heterotopic bone formation in spinal cord patients. *Paraplegia* 1993; **31:** 40–50.
70 Orzel JA, Rudd TG. Heterotopic bone formation. Clinical, laboratory, and imaging correlation. *J Nucl Med* 1985; **26:** 125–132.

71 Nollen AJG. Effects of ethylhydroxydiphosphonate (EHDP) on heterotopic ossification. *Acta Orthop Scand* 1986; **57:** 358–361.
72 Kewalramani LS, Orth MS. Ectopic ossification. *Am J Phys Med* 1977; **56:** 99–121.
73 Benassy J, Mazabraud A, Diverres J. L'ostéogenésè neurogène. *Rev Chir Orthop* 1963; **49:** 95–116.
74 Chalmers J, Gray DH, Rush J. Observations on the induction of bone in soft tissues. *J Bone Joint Surg* 1975; **57(B):** 36–45.
75 Buring K. On the origin of cells in heterotopic bone formation. *Clin Orthop* 1975; **110:** 293–302.
76 Wlodarski KH. Bone histogenesis mediated by non-steogenic cells. *Clin Orthop* 1991; **272:** 8–15.
77 Puzas JE, Miller MD, Rossier RN. Pathologic bone formation. *Clin Orthop* 1989; **245:** 259–281.
78 Urist MR, Hay P, Dubuc F, Buring K. Osteogenic competence. *Clin Orthop* 1969; **64:** 194–220.
79 Binder SM *et al*. Evidence for a humoral mechanism for enhanced osteogenesis after head injury. *J Bone Joint Surg* 1990; **72(A):** 1144–1149.
80 Kurer MHJ, Kohker MA, Dandona P. Human osteoblast stimulation by sera from paraplegic patients with heterotopic ossification. *Paraplegia* 1992; **30:** 165–168.
81 Bridges JB, Pritchard JJ. Bone and cartilage induction in the rabbit. *J Anatomy* 1958; **92:** 28–38.
82 Ekelund A, Brosjö O, Nillson SS. Experimental induction of heterotopic bone. *Clin Orthop* 1991; **263:** 102–112.
83 Mohan S, Baylink DJ. Bone growth factors. *Clin Orthop* 1991; **263:** 30–48.
84 Sawyer JR, Myers MA, Rosier RN, Puzas JE. Heterotopic ossification. Clinical and cellular aspects. *Calcif Tissue Int* 1991; **49:** 208–215.
85 Smith R, Triffit T. Bones in muscles. The problem of soft tissue ossification. *Q J Med* 1986; **61:** 985–990.
86 Major P, Resnick D, Greenway G. Heterotopic ossification in paraplegia: A possible disturbance of the paravertebral venous plexus. *Radiology* 1980; **136:** 797–799.
87 Lotta S, Scelsi L, Scelsi R. Microvascular changes in the lower extremities of paraplegics with heterotopic ossification. *Spinal Cord* 2001; **39:** 595–598.
88 Abramson DJ, Kamberg S. Spondylitis, pathological ossification, and calcification associated with spinal cord injury. *J Bone Joint Surg* 1949; **31(A):** 275–283.
89 Rush PJ. The rheumatic manifestations of traumatic spinal cord injury. *Semin Arthritis Rheum* 1989; **19:** 77–89.
90 High WB. Effects of orally administered prostaglandin E2 on cortical bone turn over in adult dogs; a histomorphometric study. *Bone* 1987; **8:** 363–373.
91 Jee WSS, Ueno K, Deng YP, Woodbury DM. The effects of prostaglandin E2 in growing rats; increased metaphyseal hard tissue and cortico-endosteal bone formation. *Calcif Tissue Int* 1985; **37:** 148–157.
92 Jee WSS *et al*. The role of bone cells in increasing metaphyseal hard tissue in rapidly growing rats treated with prostaglandin E2. *Bone* 1987; **4:** 171–178.
93 Garland DE, Alday B, Venos KG. Heterotopic ossification and HLA antigens. *Arch Phys Med Rehabil* 1984; **65:** 531–532.
94 Minaire P, Betuel H, Girard R, Pilonchery G. Neurologic injuries, paraosteoarthropathies, and human leukocyte antigens. *Arch Phys Med Rehabil* 1980; **61:** 214–215.
95 Minaire P, Betuel H, Pilonchery G. Syteme HLA chez les blesses medullaires atteints de para osteo-arthropathies neurogenes. *Nouv Presse Med* 1978; **7:** 3044.
96 Larson JM *et al*. Increased prevalence of HLA-B27 in patients with ectopic ossification following traumatic spinal cord injury. *Rheum Rehabil* 1981; **20:** 193–197.
97 Hunter T *et al*. Histocompatibility antigens in patients with spinal cord injury or cerebral damage complicated by heterotopic ossification. *Rheumatol Rehabil* 1980; **19:** 97–99.
98 Weiss S *et al*. Histocompatibility (HLA) antigens in heterotopic ossification associated with neurological injury. *J Rheumatol* 1979; **6:** 88–91.
99 Seignalet J *et al*. HLA and neurogenic paraosteoarthropathies. *Tissue antigens* 1983; **21:** 268–269.
100 Rodriguez GP, Claus-Walker J, Kent MC, Garza HM. Collagen metabolite excretion as a predictor of bone- and skin-related complications in spinal cord injury. *Arch Phys Med Rehabil* 1989; **70:** 442–444.
101 Heilbrun N, Kuhn WG. Erosive bone lesions and soft tissue ossifications associated with spinal cord injuries (Paraplegia). *Radiology* 1947; **48:** 579–593.
102 Pilonchery G, Minaire P, Milan JJ, Revol A. Urinary elimination of glycosaminoglycans during the immobilisation osteoporosis of spinal cord injury patients. *Clin Orthop* 1983; **174:** 230–235.
103 Pietschmann P *et al*. Increased serum osteoclacin levels in patients with paraplegia. *Paraplegia* 1992; **30:** 204–209.
104 Perkash A *et al*. Persistent hypercoagulation associated with heterotopic ossification in patients with spinal cord injury long after injury has occurred. *Paraplegia* 1993; **31:** 653–659.
105 Orzel JA, Rudd TG, Nelp WB. Heterotopic bone formation and lower extremity swelling mimicking deep-venous disease. *J Nucl Med* 1984; **25:** 1105–1107.
106 Gutknecht DR. Heterotopic ossification and deep venous thrombosis: Concurrence (?), bleeding complications, and caval interruption. *South Med J* 1992; **85:** 1244–1246.
107 Daud O, Sett P, Burr RG, Silver JR. The relationship of heterotopic ossification to passive movements in paraplegic patients. *Disab Rehabil* 1993; **15:** 114–118.
108 Michelsson JE, Rauschning W. Pathogenesis of experimental heterotopic bone formation following temporary forcible exercising of immobilised limbs. *Clin Orthop Rel Res* 1983; **178:** 265–272.
109 Michelsson JE, Granroth G, Andersson LC. Myositis ossificans following forcible manipulation of the leg. A rabbit model for the study of heterotopic bone formation. *J Bone Joint Surg* 1980; **62(A):** 811–815.
110 Izumi K. Study of ectopic bone formation in experimental spinal cord injured rabbits. *Paraplegia* 1983; **21:** 351–363.
111 Hossack DW, King A. Neurogenic heterotopic ossification. *Med J Aust* 1967; **7:** 326–328.
112 Snoecx M, De Muynck M, Van Laere M. Association between muscle trauma and heterotopic ossification in spinal cord injured patients. Reflections on causal relationships and diagnostic value of ultrasonography. *Paraplegia* 1995; **33:** 464–468.
113 Yue CC, Regier A, Kushner I. Heterotopic ossification presenting as arthritis. *J Rheum* 1985; **12:** 769–772.
114 Lefkoe TP, Cardenas DD. Reflex sympathetic dystrophy of the lower extremity in tetraplegia: case report. *Spinal Cord* 1996; **4:** 239–242.

115 Wharton G. Heterotopic ossification. *Clin Orthop* 1975; **112:** 142–149.
116 Couvée LMJ. Heterotropic ossification and the surgical treatment of serious contractures. *Paraplegia* 1971; **9:** 89–93.
117 Yarkony GM, Bass LM, Keenan III V, Meyer PR. Contractures complicating spinal cord injury: Incidence and comparison between spinal cord center and general hospital acute car. *Paraplegia* 1985; **23:** 265–271.
118 Laurin NR, Powe JE, Pavlovsky WF, Driedger AA. Multimodality imaging of early heterotopic bone formation. *Can Assoc Radiol J* 1990; **1:** 93–95.
119 Bodley R, Jamous A, Short D. Ultrasound in the early diagnosis of heterotopic ossification in patients with spinal injuries. *Paraplegia* 1993; **31:** 500–506.
120 Baron M, Stern J, Lander P. Heterotopic ossification heralded by knee effusion. *J Rheum* 1983; **10:** 961–964.
121 Kim SW *et al*. Serum alkaline phosphatase and inorganic phosphorus values in spinal cord injury patients with heterotopic ossification. *Paraplegia* 1990; **28:** 441–447.
122 Chantraine A, Nusgens B, Lapiere CM. Biochemical analysis of heterotopic ossification in spinal cord injury patients. *Paraplegia* 1995; **33:** 398–401.
123 Chantraine A, Very JM, Baud CA. A biophysical study of posttraumatic ectopic ossification. A case report. *Clin Orthop* 1990; **255:** 289–292.
124 Mead S, Cain HD, Kelly RE, Liebgold H. Periarticular calcification in paraplegics: attempted treatment with disodium edetate. *Paraplegia* 1963; **1:** 62–68.
125 Nechwatal E. Early recognition of heterotopic calcifications by means of alkaline phosphatase. *Paraplegia* 1973; **11:** 79–85.
126 Tanaka T *et al*. Quantitative assessment of para-osteoarthropathy and its maturation on serial radionuclide bone images. *Radiology* 1977; **123:** 217–221.
127 Suzuki Y, Hisada K, Takeda M. Demonstration of myositis ossificans by 99m Tc pyrophosphate bone scanning. *Radiology* 1974; **31:** 663–664.
128 Tyler JL, Derbekyan V, Lisbona R. Early diagnosis of myositis ossificans with Tc-99m diphosphonate imaging. *Clin Nucl Med* 1984; **9:** 256–258.
129 Freed JH, Hahn H, Menter R, Dillon T. The use of three phase bone scan in the early diagnosis of heterotopic ossification and in the evaluation of Didronel therapy. *Paraplegia* 1982; **20:** 208–216.
130 Drane WE. Myositis ossificans and the three-phase bone scan. *Am J Roentgenol* 1983; **142:** 179–180.
131 Nagaraj N, Elgazzar AH, Fernandez-Ulloa M. Heterotopic ossification mimicking infection. Scintigraphic evaluation. *Clin Nucl Med* 1995; **20:** 763–766.
132 Muheim G, Donath A, Rossier AB. Serial scintigrams in the course of ectopic bone formation in paraplegic patients. *Am J Roentgenol* 1973; **118:** 865–869.
133 Thomas EA, Cassar-Pullicino VN, McCall IW. The role of ultrasound in the early diagnosis and management of heterotopic bone formation. *Clin Radiol* 1991; **43:** 190–196.
134 Vento JA, Sziklas JJ, Spencer RP, Rosenberg RJ. Reactivation of Tc-99 MDP uptake in heterotopic bone. *Clin Nucl Med* 1985; **10:** 206–207.
135 Kramer FL, Kurtz AB, Rubin C, Goldberg BB. Ultrasound appearance of myositis ossificans. *Skeletal Radiol* 1979; **4:** 19–20.
136 Scola FH, Parzaile JR. Ultrasonographic diagnosis of heterotopic ossification mimicking deep vein thrombosis. *J Clin Ultrasound* 1991; **19:** 55–57.
137 Banovac K, Gonzalez F, Wade N, Bowker JJ. Intravenous disodium etidronate therapy in spinal cord injury patients with heterotopic ossification. *Paraplegia* 1993; **31:** 660–666.
138 Zeanah WR, Hudson TM. Myositis ossificans. Radiologic evaluation of two cases with diagnostic computed tomograms. *Clin Orthop* 1982; **168:** 187–191.
139 Amendola MA *et al*. Myositis ossificans circumscripta. Computer tomographic diagnosis. *Radiology* 1983; **149:** 775–779.
140 Bressler EL, Marn CS, Gore RM, Hendrix RW. Evaluation of ectopic bone by CT. *Am J Roent* 1987; **148:** 931–935.
141 Yaghmai I. Myositis ossificans. Diagnostic value of arteriography. *Am J Roent* 1977; **128:** 811–816.
142 Lindholm TS, Bauer FCH, Rindell K. High doses of the diphosphonate EHDP for the prevention of heterotopic ossification. An experimental and clinical study. *Scand J Rheumatol* 1987; **18:** 33–39.
143 Russell RGG. Diphosphonates and polyphosphates in medicine. *Br J Hosp Med* 1975; 297–313.
144 Russell RGG, Fleish H. Pyrophosphate and diphosphonates in skeletal metabolism. Therapeutic, clinical and therapeutic aspects. *Clin Orthop Rel Res* 1975; **108:** 241–263.
145 Russell RGG, Smith R. Diphosphonates. Experimental and clinical aspects. *J Bone Joint Surg* 1973; **55(B):** 66–86.
146 Plasmans CM, Kuypers W, Slooff TJJH. The effect of Ethane-1-hydroxy-1, 1-disphosphonic acid (EHDP) on matric induced ectopic bone formation. *Clin Orthop* 1978; **132:** 233–243.
147 Hu HP *et al*. The effect of biphosphonate on induced heterotopic bone. *Clin Orthop* 1991; **272:** 259–267.
148 Mahay PR, Urist MR. Experimental heterotopic bone formation induced by bone morphogenic protein and recombinant human interleukin-1b. *Clin Orthop* 1988; **237:** 236–244.
149 Garland DE. Clinical observations on fracture and heterotopic ossification in the spinal cord and brain injured populations. *Clin Orthop* 1988; **233:** 86–101.
150 Banovac K. The effect of etidronate on late development of heterotopic ossification after spinal cord injury. *J Spinal Cord Med* 2000; **23:** 40–44.
151 Flora *et al*. The long-term skeletal effects of EHDP in dogs. *Metab Bone Dis Rel Res* 1981; **4/5:** 289–300.
152 Kjaersgaard-Andersen P *et al*. Indomethacin for prevention of heterotopic ossification. *Acta Othop Scand* 1993; **64:** 639–642.
153 Kjaersgaard-Andersen P, Schmidt SA. Total hip arthroplasty. The role of anti-inflammatory medications in the prevention of heterotopic ossification. *Acta Orthop Scand* 1990; **263:** 78–86.
154 Ahrengart L, Lindgren U, Reinholt FP. Comparative study on the effects of radiation, indomethacin, prednisolone, and ethane-1-hydroxy-1,1-disphosphanate (EHDP) in the prevention of ectopic bone formation. *Clin Orthop* 1988; **229:** 265–273.

155 Nilsson OS, Bauer HCF, Brosjö O, Törnkvist H. Influence of indomethacin on induced heterotopic bone formation in rats. Importance of length of treatment and of age. *Clin Orthop* 1986; **207:** 239 – 245.
156 Törnkvist H, Nilsson OS, Bauer HCF, Lindholm TS. Experimentally induced heterotopic ossification in rats influenced by anti-inflammatory drugs. *Scand J Rheumatol* 1983; **12:** 177 – 180.
157 Sudmann E, Bang G. Indomethacin induced inhibition of Haversian remodelling in rabbits. *Acta Orthop Scand* 1979; **50:** 621 – 627.
158 Banovac K, Williams JM, Patrick LD, Haniff YM. Prevention of heterotopic ossification after spinal cord injury with indomethacin. *Spinal cord* 2001; **39:** 370 – 374.
159 Naftchi NE, Viau AT, Sell GH, Lowman EW. Spinal cord injury: effect of thyrocalcitonin on periartricular bone formation in three subjects. *Arch Phys Med Rehabil* 1979; **60:** 280 – 283.
160 Ayers DC, Pelligrini VD, Evarts CM. Prevention of heterotopic ossification in high-risk patients by radiation therapy. *Clin Orthop* 1991; **263:** 87 – 93.
161 Coventry MB, Scalon PW. The use of radiation to discourage ectopic bone. A nine-year study in surgery about the hip. *J Bone Joint Surg* 1981; **64(A):** 201 – 208.
162 Deflitch CJ, Stryker JA. Postoperative hip irradiation in prevention of heterotopic ossification. Causes of treatment failure. *Radiology* 1993; **188:** 265 – 270.
163 Pelligrini VD, Gregoritch SJ. Preoperative irradiation for prevention of heterotopic ossification. *J Bone Joint Surg* 1996; **78(A):** 870 – 881.
164 Schaeffer MA, Sosner J. Heterotopic ossification: Treatment of established bone with radiation therapy. *Arch Phys Med Rehabil* 1995; **76:** 284 – 286.
165 Sautter-Bihl ML *et al*. The radiotherapy of heterotopic ossification in paraplegics. *Strahlenther Onkol* 1995; **171:** 454 – 459.
166 Sautter-Bihl ML, Hültenschmidt B, Liebermeister E, Nanassy A. Fractionated and single-dose radiotherapy for heterotopic bone formation in patients with spinal cord injury. A phase-I/II study. *Strahlenther Onkol* 2001; **177:** 200 – 205.
167 Van Kuijk AA, Van Kuppevelt HJM, Van der Schaaf DB. Osteonecrosis after treatment for heterotopic ossification in spinal cord injury with the combination of surgery, irradiation, and an NSAID. *Spinal Cord* 2000; **38:** 319 – 324.
168 Brady LW. Radiation induced sarcomas of bone. *Skel Radiol* 1972; **4:** 72 – 78.
169 Kim JH *et al*. Radiation induced soft tissue and bone sarcoma. *Radiology* 1978; **129:** 501 – 508.
170 McAuliffe JA, Wolfson AH. Early excision of heterotopic ossification about the elbow followed by radiation therapy. *J Bone Joint Surg* 1997; **79(A):** 749 – 755.
171 Armstrong-Ressy CT, Weiss AR, Ebel A. Results of surgical treatment of extraosseous ossification in paraplegia. *New York State Med* 1959; **59:** 2548 – 2553.
172 Aanholt van PCTh, Martina JD, Eisma WHE. Ectopische botvorming, diagnostiek en behandeling. *NTvG* 1991; **9:** 380 – 384.
173 Stover SL, Hahn HR, Miller III JM. Disodium etidronate in the prevention of heterotopic ossification following spinal cord injury (preliminary report). *Paraplegia* 1976; **14:** 146 – 156.
174 Stover SL, Niemann KMW, Tulos J. Experience with surgical resection of heterotopic bone in spinal cord injury patients. *Clin Orthop* 1991; **263:** 71 – 77.
175 Meiners T, Abel R, Böhm V, Gerner HJ. Resection of heterotopic ossification of the hip in spinal cord injured patients. *Spin Cord* 1997; **35:** 443 – 445.
176 Gacon G, Deidier CH, Rhenter JL, Minaire P. Possibilités du traitement chirurgical des para-ostéoarthropathies neurogènes. Étued critique de 70 cas opérés. *Rev Chir Orthop* 1978; **64:** 373 – 390.
177 Freebourn TM, Barber DB, Able AC. The treatment of immature heterotopic ossification in spinal cord injury with the combination surgery, radiation therapy and NSAID. *Spinal Cord* 1999; **37:** 50 – 53.
178 Biering-Sørensen F, Tøndevold E. Indomethacine and disodium etidronate for the prevention of recurrence of heterotopic ossification after surgical resection. *Paraplegia* 1993; **31:** 513 – 515.
179 Ellerin BE *et al*. Current therapy in the management of heterotopic ossification of the elbow: a review with case studies. *Am J Phys Med Rehabil* 1999; **78:** 259 – 271.
180 Nilsson OS. Heterotopic ossification. *Acta Orthop Scand* 1998; **69:** 103 – 106.