PATHOPHYSIOLOGICAL STUDY

Avascular necrosis of bone in childhood cancer patients: a possible role of genetic susceptibility

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Abstract: With the increasing number of paediatric cancer patients and with their prolonged survival, the evidence of a number of serious complications induced by anticancer therapy is rising. Osteonecrosis (ON) of bone is one of these treatment-related effects with a multifactorial pathogenesis. In the past few years, several polymorphisms of candidate genes with possible role in development of this disorder were studied. We summarized potential risk factors leading to increased susceptibility to osteonecrosis of bone development in cancer patients during childhood and to present current knowledge in the field of genetic aspects of this condition (Ref. 86). Text in PDF www.elis.sk.
Key words: osteonecrosis, avascular necrosis, anticancer treatment, corticosteroids, transplantation, genetic determinants.

Introduction

Childhood cancer patients are at an increased risk of acute, chronic and late complications development. Some of them, such as osteonecrosis of bone, can sometimes be devastating (1–20). Osteonecrosis (ON) is also referred to as avascular necrosis (AVN), “aseptic necrosis,” and “ischemic bone necrosis”. Patients treated for cancer during childhood are more sensitive to this condition than adult population.

The prevalence of AVN in paediatric cancer patients has varied widely from 0.43 % up to 29.5 % (1, 5, 11, 17, 19). This great variation might be explained by the fact that some of these studies relied on self-reports from patients.

Osteonecrosis is a bone disorder occurring more frequently after haematopoietic stem cell transplantation (HSCT) than after conventional chemo- and/or radiotherapy. Cumulative incidence of symptomatic osteonecrosis in 943 childhood cancer survivors was 1.4 % after chemotherapy alone versus 6.8 % after transplantation (p < 0.001). Median time was 2.4 years after diagnosis in the chemotherapy group and 0.9 years after the first transplant in the HSCT group (1). Other authors reported median time from transplantation to diagnosis of osteonecrosis 14 months in their recent study within a cohort of 6244 pediatric and adolescent patients (2).

Osteonecrosis may significantly compromise patient’s quality of life during and after anticancer therapy as evidenced by the fact that 30 % of the patients underwent surgery by 1 year from diagnosis, and about 50 % required surgery by 5 years (16). Although an extensive search was performed to answer the question how to treat paediatric oncology patients with osteonecrosis, the question cannot be answered as there is no evidence-based consensus (18).

Pathogenesis of avascular necrosis in childhood cancer patients

The pathophysiology of osteonecrosis is a multifactorial process. This condition may be induced by temporary or permanent loss of bone vascularity. An ischaemic damage is followed by necrosis of the subchondral bone and degeneration and collapse of the articular joint (6). It is seen mostly in proximal parts of long bones. The most common and also the most severe sites for development of AVN are femoral and humeral heads. It can be found at other bones too, such as small bones of the hands and feet and the jaw. This process can affect one or more bones at one or different times (7–9). AVN often appears after bisphosphonate administration in adults. While bisphosphonates (inhibitors of osteoclastic bone resorption associated with risk of jaw osteonecrosis) have been only recently introduced into treatment of paediatric patients, there is a paucity of data on their long-term effects on AVN development in children (10).

Many other factors such as oxidative stress, genetic predisposition, previous trauma, increased mechanical pressure and...
environment may contribute to disease pathogenesis. Although the precise etiology of AVN has not yet been clearly defined, the use of steroids and hypogonadism are thought to be a principal risk factors for its development (12). High-dose corticosteroid treatment is an essential component of current chemotherapy protocols for treatment many cancers, such as leukemias and lymphomas (6).

The risk factors associated with increased risk of AVN are as follows: age over 10 years at the time of diagnosis, cumulative corticosteroid dose, hypogonadism (as a result of anticancer therapy), Caucasian population, allogeneic transplantation, acute and chronic graft-versus-host disease (aGVHD, cGVHD) after HSCT, radiotherapy (especially older approaches), total body irradiation, bone marrow infiltration, autoimmune diseases, infections, trauma, alcoholism, sepsis, disseminated intravascular coagulation, sickle cell anemia, lower level of albumin, higher lipid levels and body mass index, use of calcineurin inhibitors (cyclosporine and tacrolimus), mycophenolate mofetil, high-dose methotrexate, platinum derivates, cyclophosphamide, bleomycin, doxorubicin, colchicine, L-asparaginase (1, 5–8, 11–16, 21–26).

The main risk factor for AVN development in patients treated for hematological malignancies seem to be prolonged steroid therapy. Glucocorticoids (GSs) can induce proliferation and hypertrophy of bone marrow lipocytes and fat embolism, which can lead to elevated intrasosseous pressure and bone marrow ischemia. Steroids may induce also osteocyte apoptosis and damage of venous drainage of bone marrow (5, 8, 12, 24, 27–31).

Recent studies have shown no significant gender difference in risk of AVN. Osteonecrosis is most likely to manifest during the time that cancer is being treated, but it can also sometimes develop from asymptomatic to symptomatic state after completion of cancer therapy (6, 29, 30).

Genetic determinants of osteonecrosis of bone

Several AVN-predisposing genetic determinants of AVN have been identified recently (21, 27, 32–42). Many authors focused primarily on functional polymorphisms within genes involved in bone cell physiology (bone strength and integrity, bone formation and resorption), vascular system (disruption of vascular supply to the bone, subsequent hypoxia and coagulation defects), oxidative stress, endothelial nitric oxide synthase (eNOS), polymorphisms, DNA oxidation injury in bone after steroids.

Genetic polymorphisms involved in bone cell physiology

Several molecular pathways regulate osteogenesis, bone cells physiology, bone cell resorption and remodeling. The RANK-RANKL-OPG (receptor activator of nuclear factor NFκB, its ligand and osteoprotergerin) pathway plays an important role in the regulation of bone resorption. Some authors show positive association between polymorphisms in these genes and variations in femoral neck compression strength and density (32, 43), but also with AVN development in patients treated with bisphosphonates.

AVN rarely shows familial history, but mutations identified in COL2A1 gene in different families with AVN confirmed, that this gene plays a role in the pathogenesis of AVN (39, 40).

Second group of genes relevant to AVN are factors, which regulate processes such as differentiation and activation of bone remodeling and genes, which regulate balance between osteoblasts and osteoclasts. Many of these are regulators of RANKL and OPG genes. Such factors are proinflammatory interleukines IL-1, IL-4, IL-6, IL-11, IL-17, IL-23, IL-33 tumor necrosis factor TNF-α, transforming growth factor TGF-β, macrophage colony stimulating factor (M-CSF), core binding factor alpha 1 (CBFα-1), peroxisome proliferator-activated receptor gamma (PPARγ) and hormones as parathyroid hormone (PTH), adrenocorticotropic hormone (ACTH), calcitonin (CALC1), vitamin D, vitamin D receptor (VDR), estrogen and many others (33, 44–46).

Some investigators pointed out that increased level of certain interleukins (IL-6, IL-33) in saliva and/or plasma may be an early indicator of AVN development (36, 44, 47). Variations in IL-23 receptor gene (IL23R) were also associated with AVN of the femoral head in Korean population. In this study, 10 SNPs were selected and authors found a significant association of IL23R variants rs4655686, rs1569922 and rs7539625 in 443 patients compared to 273 control subjects (36). Similarly, work of Samara et al in 2012 were focused on cytokine polymorphisms of IL-1α, IL-1R, IL-1RA, IL-4Ra, IL-1β, IL-12, γIFN, TGF-β, TNF-a, IL-2, IL-4, IL-6 and IL-10 genes and their contribution to AVN (44). Their conclusion, based on the manifestation of different allele frequencies (-889 IL-1α, -238 TNF-α, rs1800471 in TGF-β, and -1082 IL-10 in patients group, indicate that the presence of one of the associated alleles or simultaneous carriage of several of them may increase the risk of AVN (44). In a cooperative study of patients with sickle cell AVN, polymorphisms in 66 candidate genes were genotyped and at least one SNP correlation with AVN development were found in each of the BMP6, TGFBR2, TGFBR3, EDN1, ERG, KL, ECE1 genes. Among these, the BMP6 genes were genotyped and at least one SNP correlation with A VN development were found in each of the BMP6, TGFBR2, TGFBR3, EDN1, ERG, KL, ECE1 genes. Among these, the BMP6 (5/14 SNPs) and KL (10/18 SNPs) genes showed strong association, which was replicated in other studies (48). The association of rs267196 and rs267201 of the BMP6 gene with A VN was confirmed as well (49).

The role of other genes such as BMP-2 (bone morphogenetic protein-2), Cbfα-1 (core binding factorα1) or PPAR-γ2 (peroxisome proliferator-activated receptor-γ2) in the pathogenesis of AVN is assumed to be based on the knowledge of its function, but unambiguous results of gene variation associated with AVN are missing (50, 51).

Members of the Annexin protein family (annexins – ANXs) are involved in various physiological processes including signal transduction, calcium metabolism, inhibition of blood coagulation and phospholipase A2, apoptosis, cell adhesion, growth, differentiation and many others (52–54). ANXs are the major components of matrix vesicles and they play a role in cartilage mineralization and probably of the bone, too (56), therefore implication of AXN genes and their polymorphisms was investigated. In cohort of patients with AVN of femoral head, 52 SNPs from three ANX genes (ANXA5, ANXA6, and ANXA11) were genotyped. The results showed association of rs10515644, rs9324679, rs9324677,
Acid phosphatase-1 (ACP1) regulates lipid levels and osteoblast differentiation via Src kinase. ACP1 could affect bone homeostasis and dexamethasone-induced AVN through multiple mechanisms. Polymorphisms in this gene were associated with higher risk of AVN as well as with lower albumin and higher cholesterol levels in blood (15). Kawedia et al (2011) prospectively screened 364 children with acute lymphoblastic leukemia. Four SNPs (rs4241316 and rs10193882 of the SH3YL1, rs12714403 and rs10167992 of the ACP1) in the ACP1-SH3YL1 gene locus were strongly associated with symptomatic AVN in ALL children (15, 27).

From the group of hormones, vitamin D receptor and parathyroid hormone draw the most attention. Despite the known association of BMP6 and IL-6 expression with AVN (46, 49, 50, 55), which are in close regulation process with parathyroid hormone and vitamin D (56). The results of two studies, focused on the confirmation of association between VDR intron 8G/A, rs2228570 with AVN and/or PTH (rs6254) and PTHr (rs1138518), did not show any strong significance (21, 57). Probable efficacy of ACTH in the prevention of human AVN was showed in the study of Zaidi et al (2010).

Genetic polymorphisms involved in the vascular system

In regard to the importance of the vascular system, polymorphisms in the genes functionally linked to blood supply, coagulation and hypoxia were associated with AVN. Prothrombotic condition and/or fibrinolytic defects have been detected in patients with various forms of AVN (58). Several studies focused on the prevalence of prothrombin 20210G/A polymorphism and factor V Leiden (FVL, 1691G/A) in AVN. These studies reported conflicting results regarding the association with AVN (35, 58–65).

The implication of hypoxia inducible factor 1α (HIF-1α) gene and its polymorphisms were analyzed in Korean population and association of variants +41224T>C and +51610C>T was observed as well as the association of haplotype CTCC (+2755C>A, +41224T>C, +45319C>T, +51610C>T) of HIF-1α (I). Ding et al in 2013 confirmed the implementation of HIF-1α in activation of osteogenic genes expression and angiogenic activity by enhanced secretion of VEGF (66). VEGF is another factor, which plays an important role in bone remodeling, suggesting role in AVN as well. Some studies showed that increased expression of VEGF was necessary for reparative processes in later-stage of AVN and its downregulation were detected in the model of primary osteoblasts from femoral head after coinoculation with GCs (67). The association of VEGF level with AVN were also observed in patients treated with GCs (67–69).

Lee et al in 2012 investigated the association between steroid-induced AVN and VEGF variants -2578A/C, -1154A/G, -634C/G, and -405C/G. The frequency of allele -1154A or -1154AA was significantly lower in patients group compared to control population. They also found that the haplotypes CGGC and AGGC might confer an increased risk of steroid induced AVN development (69). Similarly Kim et al (2008) showed an association of promoter variant -634G>C with an increased susceptibility to AVN of femoral head in the Korean population (70). The study of authors Hong et al (2010) analyzed 31 genes and 212 SNPs. Among them, 6 candidate genes: Transferrin (TF), Kinase insert domain receptor (KDR), which belongs to VEGF receptors, Vascular endothelial growth factor C (VEGFC), Insulin-like growth factor binding protein 3 (IGFBP3), Angiotensin I converting enzyme (ACE) and Neuropilin I (NRP1) were significantly associated with AVN of the femoral head. NRP1 plays a versatile role in angiogenesis and is a coreceptor for VEGF. Overall, 9 SNPs showed a significant association with AVN of the femoral head. Those are: rs1880669, rs2692695, rs2718806 of the TF, rs1485766, rs3775203 of the VEGFC, rs2453839 of the IGFBP3 genes and rs4309, rs4344, rs4461142 ACE. Another 6 SNPs showed a protective effect against AVN: rs6837735, rs1870377 of the KDR, rs12573218, rs12358370, rs2269091 of the NRP1 and rs2333496 of VEGFC (71).

The most frequently reported is the association of Plasminogen activator inhibitor-1 (PAI-1), also known as serine protease inhibitor protein (SERPINE1, serpin E1), which is encoded by SERPINE1 gene. The main function of PAI-1 is to maintain the equilibrium of coagulation and fibrinolytic systems. PAI-1 is the main and fast-acting inhibitor of fibrinolysis, it inhibits tissue plasminogen activator (tPA) and urokinase (uPA), the activators of plasminogen. Therefore, it inhibits fibrinolysis and promotes thrombosis (56, 72–74). Increased plasma levels of the PAI-1 are associated with an increased incidence of trombophília and AVN. High levels of PAI-1, caused by PAI-1 gene polymorphism, either as a consequence of treatment with GCs, may lead to disturbance in blood flow to the femoral head and could result in bone AVN (56, 73). French et al with other experts from Children’s Oncology Group in 2008 pointed out the potential of PAI-1 polymorphism, that could predict AVN of bones. From the total number of 980 pediatric patients, 108 patients (11.0 %) after treatment for acute lymphoblastic leukemia (ALL) developed symptomatic AVN. Association of PAI-1 polymorphism (rs6092) with a higher risk of AVN in pediatric patients, who received dexamethasone as a part of treatment for ALL, was confirmed. French et al reported 2.8-fold increased risk of AVN development in patients who have polymorphism in the PAI-1 gene (rs6092, AA/AG genotype) (56). Kim et al in 2011 found a significant correlation of three single nucleotide polymorphisms (SNPs) (4G allele of rs1799889, A allele of rs2227631 and C allele of rs11178) in the PAI-1 gene with higher risk of AVN of the femoral head development (72). In 2013, Zhang et al found a significant association of SNP rs11178 and rs2227631 of PAI-1 gene with steroid induced AVN of femoral head (73). In a meta-analysis of genetic risk factors for glucocorticoid-induced AVN, Gong et al have analyzed 23 publications with 35 candidate genes responsible for AVN. The PAI-1 4G allele in comparison with 5G allele showed a significant association with an increased risk of AVN (75).
Oxidative stress gene polymorphisms

The main hypothesis of AVN development assumes multiple factors operating over time, from which oxidative stress are one of the most important. Kim et al recently published a genetic association study of polymorphisms in the catalse (CAT) gene with the risk of osteonecrosis of the femoral head in the Korean population. They demonstrated that -89A > T, -20T > C, +3033C > T, +14539A>T, +22348C>T, and +24413T>C polymorphisms of the CAT gene were significantly associated with the risk of AVN (76).

Endothelial nitric oxide synthase gene polymorphisms

Nitric oxide (NO) is an important cellular signaling biomolecule involved in a variety of physiologic processes.

NO is catalyzed by NOS enzymes. Polymorphisms in eNOS (endothelial nitric oxide synthase) were studied and association of the TT allele -786T/C, rs1549758 and rs1799983, 27-bp repeat in intron 4, G894T was observed (76, 77, 78).

Genetic polymorphisms involved in DNA oxidation injury in bone after corticosteroids

Considerable evidence supports the fact that glucocorticoids (GCs) are considered as the main cause of AVN development. GCs regulate expression of many target pathways including, matrix metalloproteinases, transcription and growth factors, cytokines, collagen, alkaline phosphatase and many others, through glucocorticoid receptors (GR). Considering the role of GR as mediator in activation of many factors and proteins, GR variability and sensitivity are also considered as a risk factor of AVN. Many studies investigated the prevalence of mutations/public polymorphisms of the NR3C1 gene encoding glucocorticoid receptor associated with increased sensitivity or resistance to glucocorticoids (79–82). In an attempt to associate GR polymorphisms (NR3C1 gene) with AVN, few of them NR3C1 1220A/G, rs6195, rs2282800, rs10052957, rs41423247, rs6198 and rs6189/rs6190 were genotyped in a cohort of AVN patients compared to healthy population, but no association was identified (21, 81, 82).

It is obvious that not all patients treated with GCs are affected with AVN. It is therefore assumed that genetic variability in metabolizing agents (CYP and UGT family) and drug transporters (ABC family) might be crucial. Some authors showed an association between high risk of steroid induced AVN and low activity of CYP enzymes, especially CYP3A (83, 84). These findings were also considered in additional studies. SNPs analysis of the CYP3A4, CYP2D6 and CYP2C19 revealed the association with AVN development (21, 83–85). Different distribution between AVN patients and controls showed 4 SNPs of the CYP2C8 gene and only rs1934951 in this gene was significantly associated with AVN development (86).

Polymorphisms in ABC drug transporters in patients treated with steroids were also studied. The great attention was devoted to ABCB1 C3435T and G2677T/A. Association of ABCB1 SNPs as a predictor of AVN development is ambiguous with both positive and negative AVN association observed (21, 59).

The 677C→T mutation of the 5, 10-methylene-tetrahydrofolate reductase (MTHFR) gene appears to be associated with idiopathic AVN. This mutation was identified as a common cause of MTHFR enzyme deficiency, which leads to hyperhomocysteinemia, associated with an increased risk of thrombosis (35). Zalavras et al observed an association between the MTHFR mutation and idiopathic AVN. They investigated the presence of the 677C→T MTHFR mutation in 66 patients with idiopathic AVN of the femoral head and 300 healthy controls. Homozygosity for the 677C→T MTHFR mutation was present in 26.1 % of patients vs 10 % of controls. The odds ratio was 3.2 (95% confidence interval: 1.2–8.7), which was statistically significant (35).

Conclusion

Pathophysiology of osteonecrosis of bone in childhood cancer patients remains largely unknown and multifactorial. Only a role of glucocorticoids and vascular damage is clearly demonstrated. Recent studies have shown no significant gender difference in the risk of AVN. Although better understanding of associations between polymorphisms in some candidate genes and osteonecrosis is promising, more research is needed to determine whether genetic testing for patients at high risk for developing AVN with early interventions may reduce the morbidity associated with this condition.

References


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