Microvascular obstruction in avascular necrosis

Immunohistochemistry of 14 femoral heads

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14 femoral heads with late stage avascular necrosis of different etiologies were histologically examined, with special attention to vascular structures. Decalcified slices were stained with hematoxylin-eosin, safranin-O, van Gieson stain, and Martius Scarlet Blue. Immunohistochemical techniques with antibodies against Factor VIII, and Ulex Europeus Lectin were used to visualize the endothelium of the blood vessels. 5 distinct zones of the necrotic femoral head could be identified. The necrotic zone contained areas with richly vascularized connective tissue. In the transitional zone, several areas with intravascular aggregations of newly formed and older fibrin clots were noticed, mainly on the venous side of the vascular system. Other small vessels were collapsed, with a few endothelial cells clumped together in the center of a concentric fibrous tissue. We suggest that obstruction to the venous outflow due to intravascular thrombosis as well as to perivascular fibrosis is important in the pathogenesis of non-traumatic avascular necrosis of the femoral head.

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Intravascular blockage due to coagulation has been suggested as the main pathomechanism in the development of non-traumatic avascular necrosis of the femoral head of various etiologies (Jones 1985, 1992, Arnoldi 1994). However, the nature and the exact location of blood flow obstructions remain controversial (Hungerford and Lennox 1985, Spencer and Brookes 1988, Ohzono et al. 1992).

We examined the histology of femoral heads with late-stage nontraumatic avascular necrosis of various etiologies. We put special emphasis on intraosseous intravascular obstruction and vascular and perivascular architecture, to elucidate the pathogenesis of avascular necrosis and to correlate the histological findings with previous measurements of intraosseous pressure and microvascular blood flow.

Patients and methods

14 femoral heads of 12 patients (9 men, 3 women) with nontraumatic avascular necrosis were obtained at the time of total hip replacement. The average age of the patients was 45 (32–58) years. The etiology was considered idiopathic in 3 patients, due to corticosteroids in 5, and alcohol-induced in 4 patients. All cases were stage 3 or 4 (Ficat 1985).

Immediately after removal, the femoral head was cut in the coronal plane, fixed for at least 2 weeks in neutral formalin, decalcified in formic acid, embedded in paraffin and cut into 10 µm thick sections. The sections were stained with hematoxylin and eosin (HE) and with safranin-O to evaluate changes in cartilage structure and to detect areas with fibrocartilaginous metaplasia in the deeper part of the head.

Martius Scarlet Blue (MSB) was used to visualize intravascular aggregations of erythrocytes and fibrin thrombi of different ages. By this method, erythrocytes and newly formed thrombi are stained bright yellow, older thrombi scarlet and old, established thrombi are stained blue. The structure of the wall of the vessels was examined using van Gieson stain.

Following protease treatment for 15 min, sections were incubated with Factor VIII antibody (DAKO AO82) and further processed by a Dualink technique (E432 and E433). Following the same proteolysis, other sections were incubated with Ulex Europaeus Lectin (DAKO X921) and anti-ULEX Europaeus Lectin (DAKO B279). Binding was visualized using carbazole.

For comparison, the femoral heads of 4 patients with fresh fractures of the femoral neck due for hemiarthroplasty, with no signs of avascular necrosis or other degenerative hip disorders, were stained similarly and examined histologically.

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Results

5 different zones could be distinguished histologically in most of the heads.

1) The hyaline cartilage usually had a normal smooth surface and a uniform thickness. Penetration of well-vascularized fibrous tissues from the osteochondral junction into the cartilage, however, was not uncommon (Figure 1). Cloning of chondrocytes was only occasionally observed, as also was duplication or lack of the tidemark.

2) The subchondral zone, consisting of a narrow rim of dead bone attached to the hyaline cartilage, had irregular patches of cartilage of fibrocartilaginous character. This area was separated from the deeper layer by a fracture, which also was visible macroscopically and radiographically as the so-called crescent line (Figure 2).

3) The necrotic zone, or the sequester, had less than 50 percent osteocytes in the lacunae of the trabeculae, and sparse osteoblastic and osteoclastic activity. A characteristic feature in this area was replacement of the normal marrow fat and hematopoietic tissues by more or less primitive mesenchymal cells, forming loose and fibrous connective tissue and with shreds of metaplastic fibrocartilage. Often, these invasive replacement tissues were well vascularized (Figure 3).

4) The transitional zone was located between the sequester and normal cancellous bone. This zone was well vascularized, and vigorous osteoblastic and osteoclastic activity was observed. The arterial and arteriolar walls appeared to consist of smooth muscle and well arranged elastic membranes in the arteries. The lumen of the arteries was inconspicuous. However, veins and venules were often dilated, and MSB staining showed that these vessels contained tightly packed erythrocytes and fibrin thrombi of different ages (Figure 4). Similar occlusions were found in the vessels of the haversian canals (Figure 5). Visualization of endothelial cells by means of antibodies against Factor VIII and Ulex Europaeus revealed an unremarkable endothelial lining of these arteries and veins (Figure 6).

Furthermore, in the marrow space of the transitional zone, several small groups of endothelial cells were observed, surrounded by a concentric fibrous tissue, with little or no residue of the original vessel wall. The lumen of these altered vessels was either inconspicuous with 1 or 2 erythrocytes inside, or it had completely disappeared.

5) In the zone of normal cancellous bone deep to the necrosis, areas with vascular obstructions and perivascular fibrous tissues, as described above,

Figure 1. Osteonecrotic femoral head with penetration of well-vascularized fibrous tissue from the marrow into the cartilage (MSB, ×25).

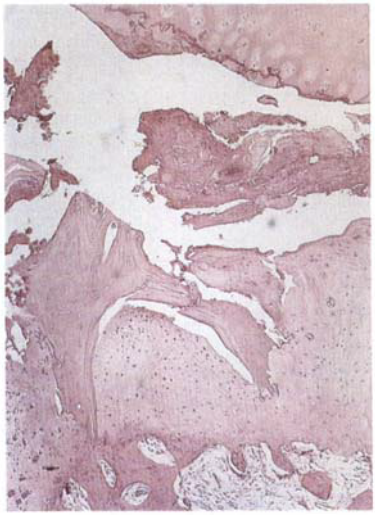

Figure 2. The subchondral fracture line ("crescent lesion") with a narrow zone of dead bone attached to hyaline cartilage. Below the fracture, dead trabeculae interspersed with fibrocartilage are seen (HE, ×25).

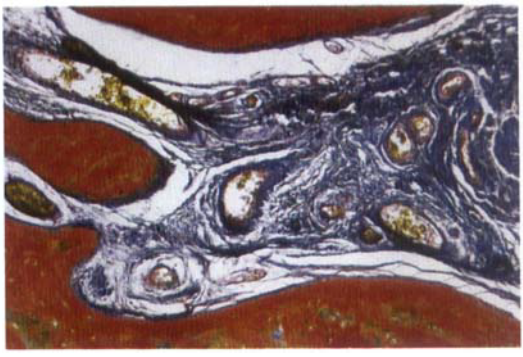

Figure 3. Necrotic zone with dead trabeculae and invasion of primitive well-vascularized mesenchymal tissues (MSB, ×100).

Figure 4. Marrow from the transitional zone showing osteoblastic lining. In the marrow space, arteries of different sizes and two dilated veins with recent thrombi are visible (MSB, ×160).

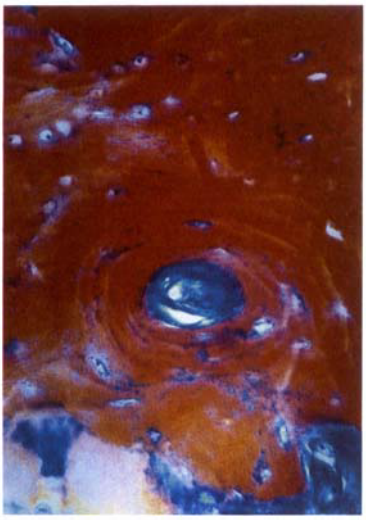

Figure 5. Haversian canal from transitional zone containing dense fibrous tissue with small lucent area just beneath the center (MSB, ×250).

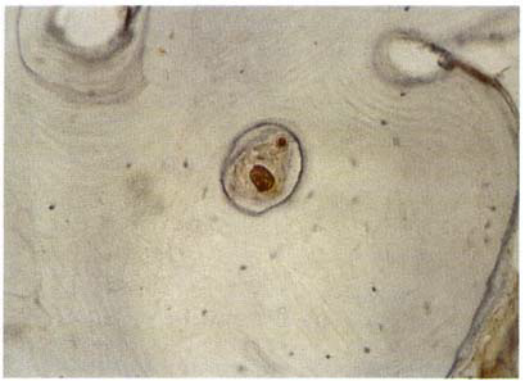

Figure 6. Haversian canal with partly preserved vessels (Factor VIII antibody, ×250).

were often observed, even at some distance from the transitional zone.

The number of cases in this study was too limited to analyze the differences in number and age of intravascular obstructions in the various etiological groups.

In the control heads, the histological appearance was that of normal cancellous bone, with no degeneration of hematopoietic tissue or fatty bone marrow. There was no obstruction in the vascular system and no invasion of replacement tissues or metaplastic cartilage in the deeper parts of these femoral heads.

Discussion

The pathogenesis of nontraumatic avascular necrosis remains elusive. Hungerford (1981) and Hungerford and Zizic (1983) suggested an increase in the size of the intraosseous extravascular marrow tissue as the main pathogenetic pathway. This expansion would increase the intraosseous pressure and thus cause compression of the flexible intraosseous vessels, with eventual ischemia and death of the bone cells. This hypothesis was supported by an increase in the average size of the lipocytes of the marrow fat that was found in patients with nontraumatic avascular necrosis by Solomon (1981) and in corticosteroid-treated rabbits by Wang et al. (1977).

Spencer and Brookes (1988) found obstruction of the arterial supply in the joint capsule on postmortem microangiography of human hips. Recently, interruption of the intracapital arteries due to damage in the vascular wall was noted in the vicinity of the necrotic lesion (Ohzono et al. 1992). Jones (1985, 1992) reported evidence of intravascular thrombosis of the intraosseous arterioles, probably initiated by lipid embolism and endothelial damage. He also suggested that occlusive thrombi were not always found at the time of specimen or postmortem examinations of the necrotic femoral heads, as an accelerated fibrinolytic response might reopen the occluded circulation. However, this presumption does not seem to have further clinical or experimental support.

We found a widespread obstruction to the femoral head blood flow on the venous side of the vascular system. These histological observations in late stage nontraumatic osteonecrosis were not reported until recently (Arnoldi 1994), probably because special stains have not been used before. However, the vascular obstructions in the haversian canals might equal the "bony plugs" that were observed by Catto (1976) in HE-stained sections.

We noted that the richly vascularized transitional zone contained vessels with a preserved endothelial layer, but with dilatations on the venous side, probably caused by thrombi in other areas. The venous outflow was further impaired by thrombi and by perivenous concentric fibrosis, which considerably reduced the lumen of the veins and venules, and the obstruction of the venous outflow might partly be due to perivascular growth of primitive mesenchymal tissues, stimulated by a decrease in the intraosseous oxygen tension (Stern et al. 1966).

Similar alterations of the vessels were observed in the primitive mesenchymal replacement tissues in the necrotic lesion. In some places, these tissues appeared as expansions from the granulation tissue of the transitional zone, in others as an invasion from the synovial attachment at the osteochondral juncture or from the fovea.

Obstruction of the microvascular outflow on the venous side, rather than obstructions of the arterial inflow of the femoral head, explains the increased intraosseous pressure that is generally found in necrosis of the femoral head (Hungerford 1979, Camp and Colwell 1986, Hauzeur et al. 1987, Lausten and Mathiesen 1990). Further, obstruction of the venous outflow explains the decreased microvascular blood flow (Lausten and Arnoldi 1993) and the decreased partial pressure of oxygen (Kiær et al. 1990) that have also been found in the necrotic femoral head.

Consequently, we suggest that an important feature in the pathogenesis of avascular necrosis of the femoral head is obstruction of the venous outflow due to obstructions of the intraosseous venous system, especially in the transitional zone at the border of the sequester.

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References

Arnoldi C C. Vascular aspects of degenerative joint disorders. Acta Orthop Scand (Suppl 261) 1994; 65:1–82.

Camp J F, Colwell C W Jr. Core decompression of the femoral head for osteonecrosis. J Bone Joint Surg (Am) 1986; 68 (9): 1313–9.

Catto M. Pathology of aseptic necrosis. In: Aseptic necrosis of bone (Ed. Davidson J K). Excerpta Medica, Amsterdam, Oxford 1976: 3–100.

Ficat R P. Idiopathic necrosis of the femoral head: early diagnosis and treatment. J Bone Joint Surg (Br) 1985; 67: 3–9.

Hauzeur J P, Pasteels J L, Orloff S. Bilateral non-traumatic aseptic osteonecrosis in the femoral head. An experimental study of incidence. J Bone Joint Surg (Am) 1987; 69 (8): 1221–5.

Hungerford D S. Bone marrow pressure, venography and core decompression in ischemic necrosis of the femoral head. In: The Hip. Proceedings of the seventh open scientific meeting of the Hip Society. C V Mosby, St. Louis 1979; 218–37.

Hungerford D S. Pathogenetic considerations in ischemic necrosis of bone. Can J Surg 1981; 24 (6): 583–90.

Hungerford D S, Lennox D W. The importance of increased intraosseous pressure in the development of osteonecrosis of the femoral head. Orthop Clin N Am 1985; 16: 635–54.

Hungerford D S, Zizic T M. Pathogenesis of ischemic necrosis of the femoral head. In: The Hip. Proceedings of the eleventh open scientific meeting of the Hip Society. C V Mosby, St. Louis 1983; 249–62.

Jones J P. Fat embolism and osteonecrosis. Orthop Clin N Am 1985;16 (4): 595–633.

Jones J P. Intravascular coagulation and osteonecrosis. Clin Orthop 1992; 277: 41–53.

Kiær Th, Pedersen N W, Kristensen K D, Starklint H. Intraosseous pressure and oxygen tension in avascular necrosis and osteoarthritis of the hip. J Bone Joint Surg (Br) 1990; 72: 1023–30.

Lausten G S, Arnoldi C C. Blood perfusion uneven in femoral head osteonecrosis. Acta Orthop Scand 1993; 64 (5): 533–6.

Lausten G S, Mathiesen B. Core decompression for femoral head necrosis. Acta Orthop Scand 1990; 61 (6): 507–11.

Ohzono K, Takaoka K, Saito S, Saito M, Matsui M, Ono K. Intraosseous arterial architecture in nontraumatic avascular necrosis of the femoral head. Clin Orthop 1992; 277: 79–88.

Solomon L. Idiopathic necrosis of the femoral head: Pathogenesis and treatment. Can J Surg 1981; 24: 573–8.

Spencer J D, Brookes M. Avascular necrosis and the blood supply of the femoral head. Clin Orthop 1988; 235: 127–40.

Stern B, Glimcher M J, Goldhaber P. The effect of various oxygen tensions on the synthesis and degradation of bone collagen in tissue culture. Proc Soc Exp Biol Med 1966;121: 869–72.

Wang G J, Sweet D E, Reger S I, Thompson R C. Fat cell changes as a mechanism of avascular necrosis of the femoral head in cortisone-treated rabbits. J Bone Joint Surg (Am) 1977; 59: 729–35.