Development of Mature Lamellar Bone with a Hematopoietic Compartment in an Aortic Valve Homograft

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Two foci of mature lamellar bone with features of remodeling and with an adjacent hematopoietic compartment were revealed for the first time in an aortic valve homograft by hematoxylin and eosin staining and polarized light microscopy. The valve had been obtained originally from a 52-year-old non-beating-heart donor and implanted as 'fresh antibiotic-preserved' into the left ventricular outflow tract of a 21-year-old man, but was explanted after six years due to valvular insufficiency. The areas close to bone showed the presence of cells resembling osteoblasts, osteoclasts and degenerating chondrocytes. Von Kossa staining disclosed a small area of dystrophic calcification in the vicinity of one bone fragment, whereas the second fragment was accompanied by only weak, diffuse calcification. These findings shows that the formation of ectopic mature bone with secondary development of the hematopoietic compartment can occur in a relatively short time, and suggest that initiators of the process may be present in the grafted valve.

Ectopic valvular ossification during the course of degenerative valve disease is a relatively rare event. The presence of lamellar bone tissue in human cardiac valve was first documented histologically by Rosenstein over a century ago (1). The frequency of ossification in surgically excised native cardiac valves reported in three large retrospective analyses was 13% (aortic and mitral valves) (2), 10.9% (aortic and mitral valves) (3), and 4.4% (aortic valves) (4), while the prevalent pathology - calcification - was found in over 80% of valves.

Although calcification occurs frequently in implanted valve homografts, advanced ectopic ossification has not been reported thus far. Herein is presented a case where, for the first time, mature lamellar bone with hematopoietic elements was identified in an aortic valve homograft.

Case report

A 27-year-old male patient had undergone aortic valve homograft implantation into the left ventricular outflow tract six years previously, following a diagnosis of insufficiency in a congenitally bicuspid aortic valve, without stenosis or calcification. The original homograft had been collected from a 52-year-old non-beating-heart male donor, and implanted as 'fresh antibiotic- preserved'. The patient was admitted due to homograft incompetence, which included valvular insufficiency, endocarditis and ascending aortic aneurysm, and subsequently underwent homograft replacement with a composite valve graft (St. Jude Medical 21A Masters). Following postoperative recovery, the patient was discharged in good health.

Pathology

The examined aortic valve homograft showed significant leaflet reduction and thickening, and also contained two foci of mature lamellar bone accompanied by hematopoietic bone marrow-like areas. The larger focus was located within the wall of the sinus of Valsalva, occupying approximately 8×4 mm of its adluminal surface. The bone was partially exposed, showing a relatively smooth and even surface (4×3 mm), while its remaining part was covered by easily detachable, opaque soft tissue. The smaller focus was present inside the reduced valve leaflet in its hinge area, and extended towards the proximal (attached) part of the leaflet. This lesion was not detected macro-
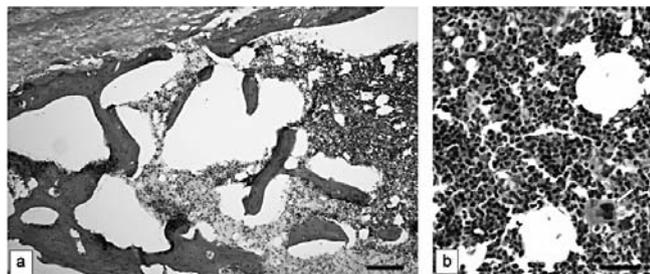
Figure 1: a) Low-power micrograph showing bone trabeculae and hematopoietic compartment in aortic valve homograft. b) Hematopoietic tissue in the valve. The arrow points to a megakaryocyte. Hematoxylin and eosin staining. Scale bars: a) 200 μm; b) 50 μm.
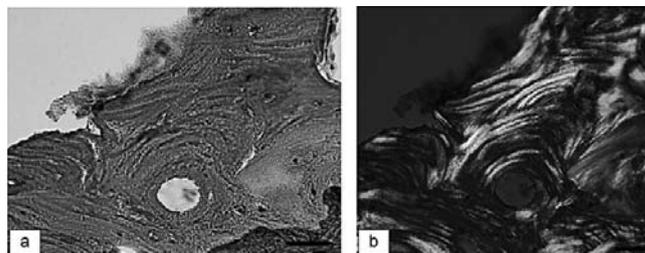

Figure 2: Fragments of the valvular bone viewed by (a) bright-field and (b) polarized light microscopy. A distinct lamellar structure is evident. Both fragments show the presence of concentric, Haversian system-like arrays of bone lamellae, as well as areas with different orientation of parallel lamellae, typical of remodeled bone. Hematoxylin and eosin staining. Scale bars: 50 μm.

scopically, because it was fully covered by thick, soft, partially neointimal, partially highly collagenous tissue.

A light microscopy examination revealed, in both areas, bone tissue composed of spicules and branching trabeculae (40-100 μm thick) with oval and almond-shaped lacunae housing osteocytes. Examination at high magnification revealed the typical structure of lamellar bone with parallel bone lamellae, and a system of canaliculi interconnecting the neighboring lacunae. The intertrabecular spaces were occupied by bone marrow-like areas containing numerous hematopoietic cells and scanty adipocytes, as well as by less-cellular, non-hematopoietic fibrous soft tissue (Fig. 1).

In the thicker regions of both bone fragments, the lamellae were aligned concentrically around hollow canals that were 40-90 μm in diameter, resembling the organization of cross-sectioned Haversian systems with diameters of 120-170 μm (Fig. 2a).

Polarizing microscopy disclosed typical birefringent light and dark bone lamellae with mean thicknesses of 3.4 and 3.6 μm, respectively. In some places, clusters of short, parallel lamellae were oriented at different angles to each other, indicating areas of bone remodeling (Fig. 2b).

In soft tissues adjacent to bone, areas suggesting an active ossification process were observed. These included irregular islets of acidophilic osteoid-like material lined by osteoblastic and occasional osteoclastic cells typical of intramembranous ossification; in other areas, cells resembling degenerating chondrocytes characteristic of endochondral ossification were identified.

Von Kossa staining revealed a small area of dystrophic calcification close to the bone fragment located inside the sinus of Valsalva wall, and also showed prominent neovascularization. Only local, diffuse calcification and limited neovascularization were found in the valve leaflet housing the other bone fragment.

Discussion

To the best of the present authors' knowledge, this is the first report to document mature lamellar bone with features of remodeling and with a hematopoietic component, in a human valved homograft. Osseous metaplasia was described in pulmonary valve homograft implanted in pulmonary position for nine years (5), but the micrograph presented suggested an early phase of ossification. In the present case the process was relatively rapid, with six years being sufficient to develop not only mature lamellar bone but also, secondarily, a hematopoietic component that required bone tissue cells and matrix as obligatory elements of a bone marrow stem cell niche.

In a study of 15 cryopreserved valve homografts (five aortic, 10 pulmonary) implanted in the pulmonary position, Shetty et al. (6) found calcifications in various regions of all explanted grafts. The same group also demonstrated stromal cells expressing a marker of osteoblastic differentiation, core binding factor α1 (RUNX2/Cbfa1), as well as a high expression of osteopontin, osteonectin, RANK and RANKL, indicating the osteogenetic potential of the valve tissue. However, morphologically recognizable bone was not formed, even in the most durable graft (explanted after 16 years). Likewise, in 32 explanted aortic valve homografts (with durability up to 20 years) examined previously by the present authors, bone tissue was not found even in heavily calcified valves (7).

Valvular ossification is regarded as a late event during the course of dystrophic calcification. Hence, it might be expected that an advanced ossification process leading to the formation of mature bone should have occurred in a heavily calcified valve. In the present homograft, however, typical dystrophic

calcification was found microscopically in the vicinity of only one bone fragment, whilst the second fragment was formed in an area showing mild, diffuse calcification. The above-mentioned findings of Shetty et al. (6), as well as the present authors' previous observations (7), suggest that heterotopic bone formation is not a simple consequence of soft tissue calcification.

The processes involved in native aortic valve calcification and ossification can be modified in homograft valves by various host- and donor-related factors (8). The striking question is: Where were the inducers of the ossification process located - in the graft, in the host organism, or in both? The graft was obtained from a man who, at the age of 52 years, was prone to cardiovascular pathologies associated with calcification. Hence, it cannot be excluded that the process had already been initiated in the valve, but at the time of grafting was not sufficiently advanced to be detected macroscopically. The valve was grafted into a young man (aged 21 years) without any previous history of vascular or valvular calcification. According to data reported by Collins et al. (4), this patient could be included in a group with congenitally insufficient rather than stenotic bicuspid aortic valves, and without significant valve calcification. However, this did not prevent bone formation in the graft tissue. It is tempting to suggest, therefore, that local tissue inducers of ectopic ossification are crucial for the process, and can act effectively even if a tissue is transplanted to another organism.

Ossification in transplanted valve homografts seems to be an extremely rare event and is, therefore, difficult to investigate. Studies aimed at the detection of ossification-inducing factors in valves banked for grafting could provide valuable information concerning the mechanism of this process.

Acknowledgements

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