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Review Article

Histopathology of periarticular non-hereditary heterotopic ossification

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ABSTRACT

In the mature adult skeleton, new bone formation is normally restricted to regeneration of osseous tissue at sites of fracture. However, heterotopic ossification, or the formation of bone outside the normal skeleton, can occur within muscle, adipose, or fibrous connective tissue. Periarticular non-hereditary heterotopic ossification (NHHO) may occur after musculoskeletal trauma, following CNS injury, with certain arthropathies, or following injury or surgery that is often sustained in the context of age-related pathology. The histological mechanism of bone development in these forms of heterotopic ossification has thus far been uncharacterized. We performed a histological analysis of 90 bone specimens from 18 patients with NHHO secondary to defined precipitating conditions, including traumatic brain injury, spinal cord injury, cerebrovascular accident, trauma without neurologic injury, and total hip or knee arthroplasty. All bone specimens revealed normal endochondral osteogenesis at heterotopic sites. We defined the order of sequence progression in NHHO lesion formation as occurring through six distinct histological stages: (1) perivascular lymphocytic infiltration, (2) lymphocytic migration into soft tissue, (3) reactive fibroproliferation, (4) neovascularity, (5) cartilage formation, and (6) endochondral bone formation. This study provides the first systematic evaluation of the predominant histopathological findings associated with multiple forms of NHHO and shows that they share a common mechanism of lesion formation.

Contents

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1. Introduction

Non-hereditary heterotopic ossification (NHHO) is the formation of mature bone in non-skeletal tissue, usually found in the broad settings of injury, arthropathy, and aging [1,2]. Many types of injury to the central nervous, musculoskeletal, cutaneous, and cardiovascular systems can lead to ectopic bone formation. NHHO occurs as a clinically severe complication in approximately 20% of individuals following major hip surgery (usually after pelvic and acetabular fractures), and up to 30% of those following spinal cord and traumatic brain injuries [3–17] as well as in patients following cerebrovascular accidents [18,19]. In many cases, periarticular NHHO can lead to significant motion loss at the joint with possible ankylosis and subsequent debilitating functional restrictions in activities of daily living [20–22].

NHHO presents clinically with limitations in joint range of motion following surgery, spinal cord or brain injury, or trauma to musculoskeletal or other connective tissue [1,2]. Periarticular pain, swelling, and general inflammation are followed by restriction in joint mobility and, in some cases, ankylosis of the involved joint [1,2,10,23].

Radiographically, the presence of heterotopic bone lags behind the clinical presentation. At initial presentation, there may be little or no evidence of bone formation on plain radiographs. However, as the lesion begins to consolidate, usually at around 4 to 6 weeks, bone formation becomes evident on plain x-rays with radiographic characteristics similar to normal bone. Technetium bone scanning or positron emission tomography/computed tomography may be used to help diagnose heterotopic lesions at earlier stages and prior to their appearance on plain x-rays [24,25]. While these tests are sensitive, they are not often specific for heterotopic bone formation, and positive results can include infection, hypervascularity, or tumor.

There is little or conflicting information defining the histopathological mechanism(s) of NHHO, and in the past it has been confused with heritable forms of ectopic ossification or malignant neoplasms [26,27]. Histologically, heterotopic ossification has been described as an endochondral or intramembraneous process, or as one with features of both processes [27,28]. With the exceptions of ossification in end-stage cardiac valve disease, and possibly in mature atherosclerotic plaques [29,30], the development of lesions in NHHO has not been clearly elucidated. We undertook the current study in an attempt to clarify the mechanism of bony lesion formation in periarticular NHHO in multiple types of neurologic and musculoskeletal system injuries and conditions.

2. Materials and methods

2.1. Patients

This study was approved by the Office of Regulatory Affairs institutional review board of the University of Pennsylvania. Eighteen patients underwent orthopaedic evaluation in the University of Pennsylvania Health System and were diagnosed with NHHO by clinical and radiographic criteria. All patients had decreased range of motion as a result of heterotopic ossification about the involved joint or joints resulting in varying functional limitations. In neurologically compromised patients, upper extremity ankylosis resulted in significant inability to perform basic activities of daily living. In other patients, for example those undergoing total joint arthroplasty, the heterotopic ossification tended to be less severely limiting. All patients showed evidence of heterotopic bone formation on plain x-rays in varying amounts.

Tissue samples of heterotopic bone were divided into the following groups, according to the specific predisposing medical conditions: cerebrovascular accident (CVA), spinal cord injury (SCI), traumatic brain injury (TBI), non-neurologic trauma and post-arthroplasty.

2.2. Specimen retrieval

Bone samples were collected for preparation after resection under routine operating procedures. The diagnosis and setting of NHHO was known in advance of tissue removal and analysis. All specimens were processed within 36 h of harvesting. An average of 5 intact specimens was obtained per individual, ranging in size from 2 mm to 6.4 cm in longest length (before processing).

2.3. Tissue preparation and basic staining

Tissue samples were fixed in neutral buffered formalin, decalcified, infiltrated and embedded in paraffin, and sectioned at a thickness of 6 μm. Cut samples were then deparaffinized, stained with Harris hematoxylin solution and counterstained with hematoxylin and eosin (H&E) by standard procedures. Other cut tissue sections were stained with Weigert's iron hematoxylin solution and Fast Green stain, and counterstained with Safranin O solution (SAF-O) by standard procedures to confirm the presence of cartilaginous tissue/chondrocytes. All stained slides were examined under light microscopy for histological characteristics of endochondral bone, and for features representative of early and subsequent stages of lesion formation. The sequence of developmental progression in the formation of heterotopic bone was determined by the presence of at least two predominant and distinct cellular elements/tissue types in the same section (e.g., cartilage and bone, fibroproliferative and neovascular tissue, or perivascular and soft tissue lymphocytes). The staging was then determined by overlapping common sections between multiple slides (i.e., any two consecutive stages were always present in the same section and were adjacent to one another). The presence of fibrous tissue, cartilage, and bone as well as the distinct characteristics of other pathohistologic stages were confirmed by two independent examiners (K.L.F. and R.J.P.). At least 30 sections were examined for each specimen and were obtained at multiple levels through the tissue.

2.4. Immunohistochemistry

Immunohistology was performed for the identification of lymphocytes using the mouse monoclonal antibody CD45RA Ab-1 (Lab Vision Corporation, Fremont, CA, USA) and a horseradish peroxidase (HRP)-conjugated universal secondary antibody detection system (Lab Vision Corporation, Fremont, CA, USA) essentially as described by the manufacturer's instructions. Diaminobenzidine (DAB) served as the chromogen/substrate. Briefly, deparaffinized and rehydrated tissue sections were incubated with a hydrogen peroxide blocking solution for 15 min to reduce nonspecific staining due to endogenous peroxidase. The primary antibody was incubated with sections at a dilution of 1:100 for 1 h. The addition of HRP-conjugated secondary antibody and DAB were performed exactly as described by the manufacturer. All washes were performed with phosphate-buffered saline (PBS), except after chromogen/ DAB substrate incubation which substituted deionized water. Sections were counterstained with hematoxylin. Lymphoid follicles from human tonsil were also stained with anti-CD45 antibody and used as a positive control.

3. Results

All samples of heterotopic bone were examined at multiple levels for the presence of chondrocytes and cartilage by the appearance of typical microarchitectural features on H&E stained sections and by characteristic orange to red/purple staining by SAF-O. Fig. 1 shows representative examples of endochondral bone formation in each of the five clinical settings examined. All tissue samples from patients with NHHO, independent of precipitating cause, showed evidence of endochondral bone formation (Table 1). We found no evidence of exclusive formation through an intramembranous process, or of competing or

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