Original Investigation

Analog Method for Radiographic Assessment of Heterotopic Bone in Fibrodysplasia Ossificans Progressiva

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Rationale and Objectives: Severe progressive multifocal heterotopic ossification (HO) is a rare occurrence seen predominantly in patients who have fibrodysplasia ossificans progressiva (FOP) and is difficult to quantitate owing to patient-, disease-, logistical-, and radiation-related issues. The purpose of this study was to develop and validate a scoring system based on plain radiographs for quantitative assessment of HO lesions in patients with FOP.

Materials and Methods: Institutional review board approval was obtained from the University of Pennsylvania, and all data comply with Health Insurance Portability and Accountability Act regulations. The University of Pennsylvania Institutional Animal Care and Use Committee approved the use of mice in this study. First, we used a mouse model of FOP-like HO to validate a semiquantitative analog scale for estimating relative heterotopic bone volume. Second, we used this validated scale to estimate the relative amount of HO from a retrospective analysis of plain radiographs from 63 patients with classic FOP. Finally, the scale was applied to a retrospective analysis of computed tomographic images from three patients with FOP.

Results: In the FOP-mouse model, the observed rating on the analog scale is highly correlated to heterotopic bone volumes measured by microcomputed tomography ($R^2 = 0.89$). The scoring system that was applied to radiographs of patients with FOP captured the clinical range of HO typically present at all axial and appendicular sites. Analysis of computed tomographic scans of patients with FOP found that observed radiograph ratings were highly correlated with HO volume ($R^2 = 0.80$).

Conclusions: The scoring system described here could enable practical, quantitative assessment of HO in clinical trials to evaluate new treatment modalities, especially for FOP. The development of the six-point analog scale described here provides and validates a much-needed, reproducible, and quantifiable method for describing and assessing HO in patients with FOP. This scale has the potential to be a key descriptor that can inform patients with FOP and clinicians about disease progression and response of HO lesions to interventions and treatments.

Key Words: Heterotopic bone; fibrodysplasia ossificans progressiva.

INTRODUCTION

Fibrodysplasia ossificans progressiva (FOP) (Mendelian Inheritance in Man #135100) is a severely disabling heritable disorder characterized by congenital malformations of the great toes and progressive heterotopic ossification (HO) that forms qualitatively normal bone at extraskeletal sites (1). During the first decade of life, affected children experience episodic exacerbations of painful soft tissue swellings (flare-ups), often precipitated by soft tissue injury, intramuscular injections, viral infection, muscular stretching, falls, or muscular fatigue (1,2). These flare-ups transform skeletal muscles, tendons, ligaments, fascia, and aponeuroses into heterotopic bone, rendering movement restricted or impossible. Classic FOP is caused by a recurrent activating mutation (c.617G>A; R206H) in the gene encoding activin A receptor type I/activin-like kinase 2 (*ACVR1/ALK2*), a bone morphogenetic protein type I receptor (3). At present, there is no definitive treatment, but emerging drug development offers the possibility of beneficial interventions (4–8).

Although multiple classification systems grade the severity of focal HO (9–13), none is applicable to progressive multifocal

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HO, nor does any adequately measure volumetric bone by plain radiographs. A simple method for quantifying extraskeletal bone formation would be useful to assess the effectiveness of evolving treatments, especially for FOP, where rigid immobility because of progressive multifocal HO often precludes serial quantitative computed tomographic (CT) scans at major urban medical centers.

We developed an analog scoring system of HO based on radiographic appearance and relative size (Table 1). For early lesions, the score is based on the former, whereas for more advanced lesions, the score is based on the latter. The purpose of this study is to develop and validate a scoring system based on radiographs for semiquantitative assessment of HO lesions in patients with FOP.

MATERIALS AND METHODS

Animals and Injury-induced HO Model

The University of Pennsylvania Institutional Animal Care and Use Committee approved the use of mice in this study. A transgenic mouse model of FOP-like HO containing a constitutively active (ca) ACVR1/ALK2 allele flanked by loxP sites (caALK2 mice) was used in all experiments (4,7,14). To induce expression of caALK2, 50 μL of 0.9% NaCl solution containing 5×10^{10} genome copies of recombinant adenovirus-expressing Cre recombinase (University of Pennsylvania Vector Core) and cardiotoxin (10 μM solution; Sigma-Aldrich, St. Louis, MO) was injected into the left hind limb musculature of mice at 3 weeks of age. Tissues were recovered 7, 10, and 14 days post injection ($n = 10$, 10, and 14, respectively).

Microcomputed Tomography

Microcomputed tomography was performed on the adenovirus-cre/cardiotoxin injected legs of caALK2 mice post mortem. A Scanco vivaCT 40 (Bruettisellen, Switzerland) was used to determine the volume of heterotopic bone and obtain a two-dimensional image of the medial view of the sagittal plane of the limb analogous to radiographs. Scanning was performed using a source voltage of 55 kV, a source current of 142 μA, and an isotropic voxel size of 10.5 μm. Skeletal and HO bone was differentiated from "non-bone" by an upper threshold of 1000 Hounsfield units and a lower threshold of 150 Hounsfield units. A recent study demonstrated the usefulness of using micro-CT as an imaging tool for the evaluation of HO in animal models (15).

Patients

Classic FOP was diagnosed by clinical criteria and confirmed by mutation analysis (c.617G>A; R206H in the gene *ACVR1/ALK2*). Plain radiographs from 63 patients with FOP and CT scans from a further three patients with FOP (two boys, one girl; ranging from 8 to 13 years of age) were retrospectively reviewed in accordance with institutional review board approval at the University of Pennsylvania.

Assessment of Radiographs

To determine if the HO scale could be used to estimate the amount of HO seen on plain radiographs, films available in the FOP film library of The Center for Research in FOP and Related Disorders from patients with FOP were retrospectively reviewed. Each HO lesion seen on radiographs was classified according to the six-point analog scale described in Table 1 by two raters (FSK and RJP) independently blinded to prior classification to determine inter-rater reliability of the scoring system.

Clinical CT Imaging and HO Volume Analysis

Retrospective examination of CT scans (obtained as part of a comprehensive spinal deformity analysis) from three patients with FOP was performed as a gold standard for comparative radiographic analysis on concomitant two-dimensional images. Two scans had been performed on Siemens-manufactured CT machines at the Children's

TABLE 1. Analog Scale to Measure HO on Plain Radiographs in Patients with FOP*

0	No HO
1	Single or multiple spicules (punctate) or islands (non-contiguous) of HO
2	Coalescing islands or reticular complexes of heterotopic bone
3	Single contiguous HO having longest dimension ≤one-half of the diameter of the reference normotopic bone** in any projection
4	Single contiguous HO with longest dimension >one-half but ≤1 of the diameter of the reference normotopic bone** in any projection
5	Single contiguous HO with longest dimension >1 but ≤2 of the diameter of reference normotopic bone* in any projection
6	Single contiguous HO with longest dimension >2 diameters of reference normotopic bone** in any projection

HO, heterotopic ossification.

Ankle, tibial shaft; back or chest, height of cervical vertebral body nearest to HO midpoint; Distal lower extremity, tibial shaft; Distal upper extremity, radial shaft; Foot, metatarsal; Hip or proximal lower extremity, width of femoral neck; Jaw or chin, width of hyoid; Knee, femoral shaft; Neck, height of cervical vertebral body nearest to HO midpoint (lateral projection); Proximal upper extremity, humeral shaft; TMJ, height of cervical vertebral body nearest to HO midpoint.

* Score is assigned based on highest grade feature.

** Reference normotopic bone for the indicated location of HO is defined as follows.