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A preliminary study of ultrasound aspiration of bone erosion in early rheumatoid arthritis

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Abstract

Objective. To develop a new technique to assess the primary lesion in early rheumatoid arthritis (RA).

Methods. Ten patients with early RA and radiographically or MRI confirmed erosions had a needle introduced into the base of the erosion under sonographic guidance. Material was then aspirated from this site.

Results. The procedure was well tolerated with no complications. Small samples of necrotic bone and tissue were obtained in five out of 10 cases. In one case, a distinctive population of pleomorphic CD34+ cells with characteristics of bone marrow progenitors was isolated. Tissue invading bone with a characteristic appearance of pannus was not seen.

Conclusion. A new method of sampling the earliest lesion in RA is described. The findings raise questions about the nature of bone damage in early RA.

KEY WORDS: Ultrasound-guided biopsy, Erosion, Early rheumatoid arthritis, CD34+ cells.

Rheumatoid arthritis (RA) is a chronic erosive polyarthropathy of unknown aetiology [1]. The defining pathological feature of RA is periarticular bone erosion, which is important in both the diagnosis and prognosis of disease [2]. Although synovial tissue is well characterized, as yet there is no means of obtaining representative tissue from the site of bone erosion in early RA. Previous evaluation of areas of erosion from arthroplasty specimens from chronic RA has identified a tumour-like tissue termed pannus which seems to be associated with both bone and cartilage destruction [3]. However, pannus is not unique to RA and is seen in other arthropathies, and has features which have been likened to a scar [4], which is suggestive of a non-specific healing response. Furthermore, it has been demonstrated in the SCID mouse that synovium invades cartilage, but not bone [5], and recently distinctive cells termed pannocytes have been isolated from areas of erosion in long-standing RA [6], but the significance of these is unknown. To explore further the nature of bone damage in early RA, we describe a novel sonographic-based technique for obtaining material from sites of bone erosion.

Patients and methods

Approval for the study was obtained from the local hospital ethics committee. Ten patients fulfilling ACR criteria for RA with evidence of erosion radiographically (five cases) or on MRI (five cases) were selected for aspiration of erosions. Eight patients had metacarpophalangeal erosion aspiration and two patients had proximal interphalangeal joint erosion aspiration. The mean duration of RA was 6 months (range 3–18 months). All patients had synovitis with tenderness and swelling of the joint selected for aspiration. Only one patient was already on disease modifying drug therapy at the time of assessment.

Ultrasound-guided aspiration of bone erosions

Defects in the bone in proximity to the articular margin corresponding to radiographic or MRI evident erosions were selected for aspiration. An ATL 3000 HDI ultrasound scanner employing a 10–5 MHz linear array hockeystick transducer was used to localize erosions (Fig. 1a). An erosion was confirmed on longitudinal and transverse planes with erosion diameters ranging between 2 and 4 mm.

The skin overlying the erosion was marked and disinfected using standard methods. Infiltration of the skin with local anaesthetic using a 24 gauge needle was performed before infiltration of the base of erosion and surrounding periosteum under direct ultrasound visualization. An 18–20 g spinal paediatric needle was then manipulated into the base of the erosion under direct visualization before withdrawal of the stiletto (Fig. 1b). At this point, the needle was gently advanced into the bone for 1–2 mm. In a manner similar to bone biopsy,

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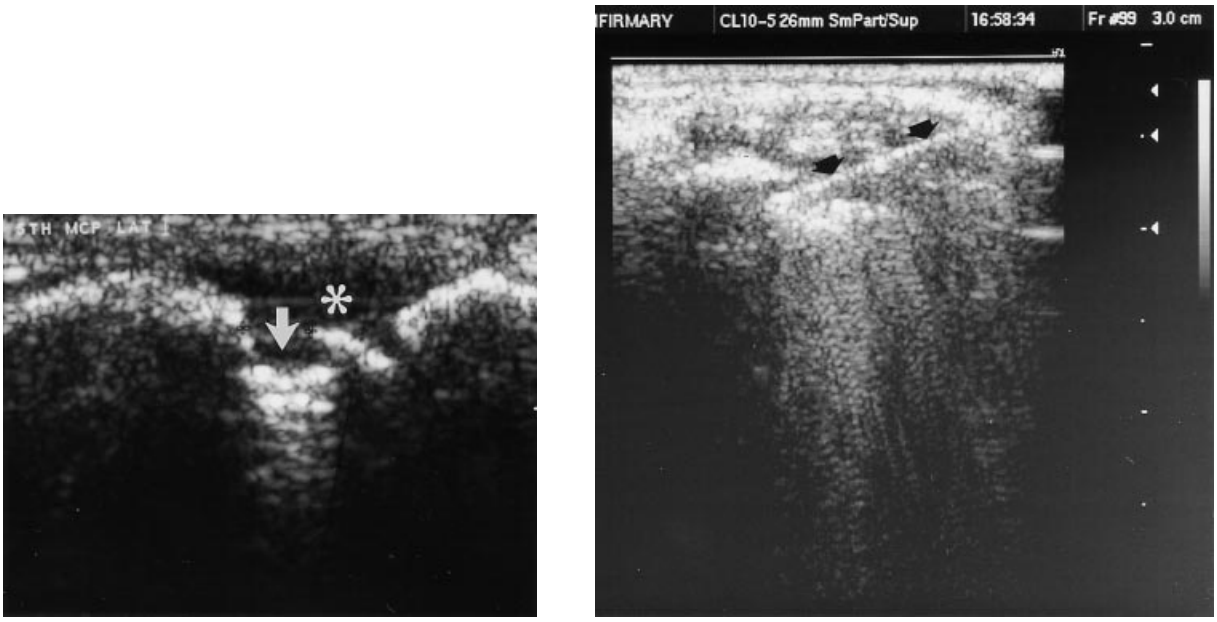

(a) (b)

FIG. 1. (a) Longitudinal ultrasound image of the lateral aspect of the fifth metacarpal head demonstrating a small cortical erosion (arrow) and overlying synovial thickening (*). (b) Same as (a), but now a needle has been passed so that its tip lies within the base of the erosion.

the needle was gently moved from side to side to ensure that a core of bone was obtained. Finally, the needle was withdrawn, employing gentle suction with a 5 ml syringe to facilitate removal of tissue. Two passes of the needle were usually performed.

The specimens were embedded in methylmethacrylate resin, which, unlike paraffin, does not require decalcification prior to histological analysis, so preserving morphology of bone [7]. Histological assessment of all samples was performed and immunohistochemistry was subsequently performed on four cellular samples following antigen retrieval using the ABC method as previously described [7]. The following antibodies were used for immunostaining: anti-CD3, CD8, CD20, CD34, CD68, vimentin and Factor VIII associated antigen (all from Dako, Denmark) and MIB-1 (Immunotech, France), directed against Ki-67 in one sample containing a distinctive population of cells. Appropriate positive and negative control tissue was used.

Results

Aspiration of erosion was well tolerated and no complications were noted at 6 months follow-up.

Aspirates containing bone

Five samples contained bone, three associated with cellular material and two bone alone, but tissue with features of pannus was not seen (Table 1). A pleomorphic population of blast cells associated with necrotic bone was isolated from the patient with a disease duration of 18 months (Fig. 2). The CD34 antigen was strongly expressed in this population of cells (Fig. 2).

TABLE 1. Findings of erosion aspiration

Patient no.	Findings
1	Necrotic bone. Pleomorphic population of blast cells (CD34 + , Factor VIII antigen–)
2	Necrotic bone. Occasional macrophages (CD68 +) and fibroblasts
3	Necrotic bone. Occasional macrophages (CD68 +) and fibroblasts
4	Necrotic bone. Acellular
5	Necrotic bone
6	No bone. Sample of synovial membrane. Macrophages (CD68 +) and fibroblasts. Synovial sample
7	Fibrin/cellular debris
8	Fibrin/cellular debris
9	Failed to isolate material
10	Failed to isolate material

These were further immunophenotyped as follows: vimentin negative, CD3 negative, CD8 negative, CD20 negative, CD68 negative and Factor VIII associated antigen negative. Ki-67 immunostaining was also negative, suggesting a slow rate of cell division. Two samples contained occasional myeloid cells and fibroblasts adjacent to necrotic bone. With the exception of occasional CD68-positive cells in these two samples, the remaining markers were negative. In two cases, fragments of necrotic bone alone and tissue debris were isolated.

Aspirates without bone

In two patients, fibrin and cellular debris, but no synovial tissue or bone, was obtained from erosions. In one case, synovial membrane showing CD68 immunoreactiv-

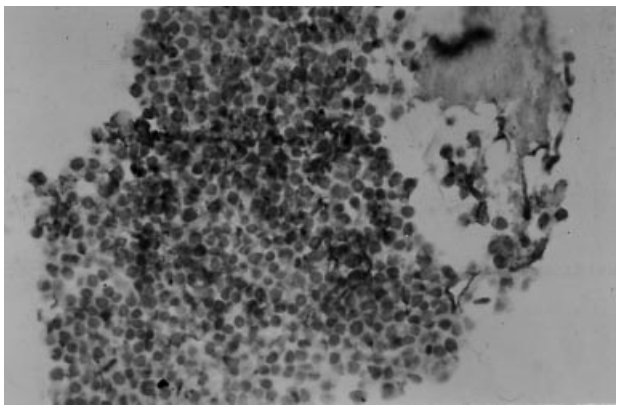

FIG. 2. A distinctive population of pleomorphic blast cells from an erosion adjacent to a fragment of necrotic bone. These cells show CD34 immunostaining, but negative staining for other markers (see the text). The morphology and CD34-positive staining are typical of haematopoietic progenitors.

ity was isolated and in two remaining cases tissue was not obtained.

Discussion

This study attempts to determine the nature of bone damage in early RA using the novel technique of ultrasound aspiration of erosions. The procedure was well tolerated and no complications were noted. Although the samples were small, these preliminary findings demonstrate necrotic bone or cellular debris from sites of erosion in most cases and a distinctive population of cells in one case. Failure to identify patients with tissue with the characteristics of pannus may be related to the small size of samples obtained. An alternative explanation to a primary invasive pannus destroying bone in RA is that pannus development is secondary to underlying bone damage. We have reported synovitis and associated bone oedema in early arthritis, as determined by MRI, without associated disruption of the bone cortex on sonographic assessment, which supports this assertion [9]. These findings need to be confirmed using larger biopsy specimens, but if confirmed, could challenge the paradigm that pannus is directly responsible for bone damage.

Cells with morphological features and immunostaining typical of haematopoietic progenitor cells (CD34 positive) were isolated in a single case [8]. Recently, circulating CD34+ cells, which may be important in inflammation, have been reported in the peripheral blood of normal individuals [10]. Another possibility is that these cells are related to poorly characterized spherical cells which have been described at the synovial–cartilage junction in the normal knee [11]. Confirmation of these findings in other subjects with early RA may have important implications for the pathogenesis of RA.

In summary, we describe a method of obtaining tissue from erosions—the characteristic feature of RA. Our preliminary findings raise questions about the nature of bone erosion in early RA, and further studies utilizing a larger and specifically adapted biopsy needle could address these issues.

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