to surgery, is controversial[4 5] and may merit reappraisal.

The disease has a dismal prognosis, with an average survival of 13·4 months after treatment, and a five year survival of 4·2%.[1] When metastases are present, survival averages only one to five months.

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Secondary tumour deposits in needle biopsy tracks: an underestimated risk?

We report a case of adenocarcinoma metastatic to a computed tomography guided needle biopsy tract. The actual incidence of such events is higher than is often claimed.[1-4]

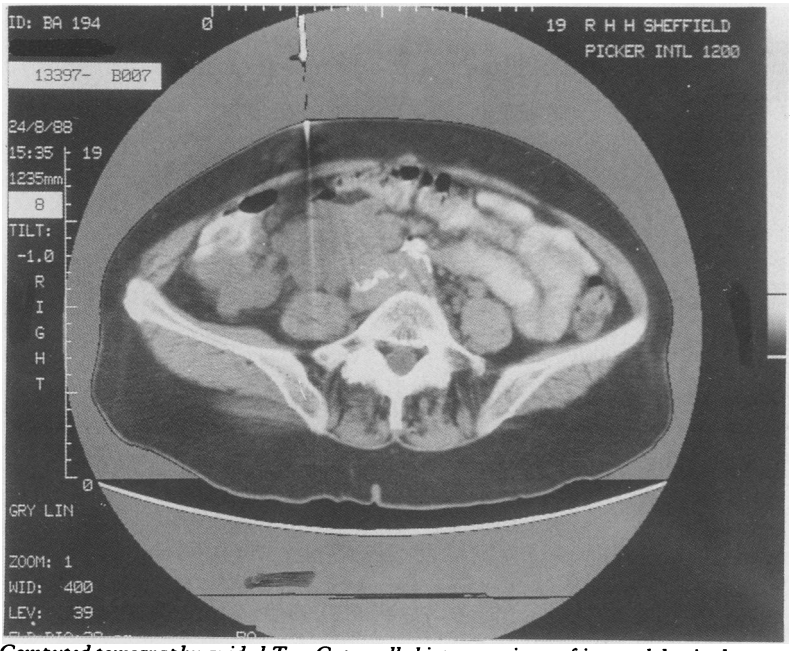
There has been a remarkable increase in various forms of needle biopsy in recent years. This has been partly due to the development of very accurate systems for guiding the procedure, and partly due to cost effectiveness. The result is that radiologists and pathologists frequently cooperate closely in the performance and interpretation of these procedures. The incidence of needle track metastases is fortunately rare, but rather more common than is sometimes stated.

The commonsense view is that although there is a real risk from large needle biopsies, fine needle aspiration biopsy is much safer. Kline, writing about fine needle aspiration in the introduction to a text on aspiration cytology, states: "Seeding of tumour cells . . . is almost a myth."[5] The commonest well established tumour that regularly seeds in needle tracks is malignant mesothelioma, but the reports on the seeding of other tumours is not inconsiderable.[1-5] We report here a further case of metastasis to a computed tomography guided needle biopsy track from an abdominal mass.

A 67 year old woman presented with vague abdominal symptoms; examination showed a large mass in the right iliac fossa. Computed tomography and ultrasound examinations were unhelpful in establishing the nature of the mass and computed tomography guided biopsy was therefore performed (figure). Two passes were made with a Tru-cut needle and at subsequent microscopy the cores of tissue contained poorly differentiated adenocarcinoma.

Six weeks later the patient returned with a skin lesion which was thought clinically to be a pyogenic granuloma. It was 7 mm in diameter and was located at the site of the biopsy. This was removed under local anaesthetic and subsequent histology showed poorly differentiated adenocarcinoma identical with that in the original Tru-cut biopsy specimen.

As the clinical and economic arguments for percutaneous biopsy, as opposed to open biopsy, seem to be irresistible, progressively more of these samples must be expected in the laboratory. Consequently the risks of such procedures must be constantly assessed. The assumption that there is less risk from fine needle aspiration than from larger needles has been challenged, at least in the case of prostatic cancer.[5] Consequently one cannot automatically assume that there is no risk from fine needle aspiration or that such risks should be neglected. There are numerous examples, such as the one reported here of metastases to large needle tracks from renal[2] and prostatic carcinomas,[5] and from fine needle aspiration of pancreatic,[4] lung, and pleural primaries.[3] Clearly the risk, though small, is real and continued recording of such cases is needed if we are to establish the true incidence for an event which may have clinical and legal implications.



Computed tomography guided Tru-Cut needle biopsy specimen of intra-abdominal mass in the right iliac fossa.

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Simplified method of preparing neutrophil slides to examine antibodies to cytoplasmic antigens

Several authors have described the specificity of indirect immunofluorescence tests for detecting antibodies to neutrophil cytoplasmic antigens (ANCA, ACPA) in diagnosing and managing patients with active Wegener's granulomatosis, microscopic polyarteritis, systemic vasculitis and necrotising glomerulonephritis.[1-3] We found the following method of slide preparation simple and less time-consuming than dextran or methylcellulose sedimentation with Ficoll-Hypaque neutrophil separation.[3] We also did not observe neutrophil clumping, sometimes associated with exposure to density sedimentation with the Ficoll-Hypaque technique. This method is adapted from that used to affix neutrophils to glass slides for the nitro-blue tetrazolium (NBT) reduction assay.[4]

One to two drops of fresh whole human blood without anticoagulant are allowed to clot for 30 minutes on warm glass slides coated with 4% bovine serum albumin in a 37°C humidified chamber. The clot is gently removed and the area briefly rinsed indirectly with phosphate buffered saline. The wet slide is immediately cytocentrifuged at 500 rpm for five minutes, air dried, and fixed with 99% ethanol for five minutes at 4°C (or acetone/formalin), as previously described.[1-3] The prepared slides are stable at −20°C for at least one month. We have found the cellular morphology to be excellent in addition to which there is low background fluorescence and reproducibility. This method can be adapted for phorbol myristate acetate neutrophil activation and subsequent immunofluorescence staining.

In view of rapidly increasing interest in ANCA/ACPA testing, and the necessity for preservation of neutrophil morphology, we feel that this technique may prove more time saving and cost effective than previously described methods.[5 6] Other methods which do not use indirect immunofluorescence are currently under evaluation[7 8] and seem to

offer similar sensitivity and specificity to that established by immunofluorescence techniques.

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MATTERS ARISING

Diagnostic importance of cytokeratin expression in linings of odontogenic cysts

Recent studies on keratin expression by odontogenic cyst epithelium suggest that while expression of keratin 19, and possibly keratin 5, is a characteristic feature of human odontogenic epithelium, there are no clear differences in keratin expression between cyst types that might be diagnostically useful.[1-3] Our own studies[2] and the report of Morgan *et al*,[4] however, suggest that the odontogenic keratocyst may be unusual in coexpressing keratins 19 and 10. Unfortunately this observation cannot form the basis of a routine diagnostic test as the epitopes recognised by the monoclonal antibodies specific to keratins 19 and 10 are destroyed by formalin fixation and paraffin wax processing. A further complication to the potential use of the "odontogenic keratocyst specific" keratin phenotype (keratin 19^+10^+) is that it may also be detected in other types of odontogenic cyst that have undergone metaplastic keratinisation.[2]

The recent paper by MacDonald and Fletcher, however, suggests that dentigerous cysts and odontogenic keratocysts may be differentiated by the pattern of staining obtained using a commercially available monoclonal antibody to intermediate molecular weight keratins (LP34; CK1, Dako) and an indirect immunoperoxidase technique on trypsin treated, formalin fixed, paraffin wax sections.[5] While dentigerous cyst linings were darkly stained throughout their whole thickness, those of odontogenic keratocysts showed no or weak staining of basal and suprabasal cell layers with stronger surface staining. This report prompted us to review the paraffin wax sections in our files which had been stained with the two monoclonal antibodies (LP34, Dako; CAM5.2, Becton Dickinson) used by MacDonald and Fletcher.[5]

Stained sections from 18 dental (periapical) cysts, 12 dentigerous cysts, and 15 odontogenic keratocysts were examined. All the specimens had been formalin fixed and decalcified in 10% formic acid for between four and 24 hours before being embedded in paraffin wax. Trypsin treated (20–30 minutes, room temperature), 5 μm sections were stained using a conventional indirect immunoperoxidase technique. Sections were sequentially treated with 0·3% hydrogen peroxide in methanol (20 minutes), 50 mg/ml ovalbumin solution (10 minutes), monoclonal antibody (60 minutes; LP34, 1/40–1/100; CAM5·2, 1/20), peroxidase conjugated rabbit anti-mouse Ig (Dako; 1/100 dilution in buffer containing 10% normal human serum) and DAB reagent. Sections were washed between all steps after the primary antibody had been applied. All washes and reagent dilutions were performed using 0·05M TRIS-HCl (pH 7·6) containing 0·1M sodium chloride).

All cysts showed moderate to strong reactivity to strong reactivity throughout their epithelial linings with LP34 (figure). Dental and dentigerous cyst linings exhibited even, strong reactivity; those of odontogenic keratocysts occasionally exhibited more intense staining of the basal cells (figure). This latter staining pattern did not seem to correlate with any local differences within the connective tissue capsule (such as extent of inflammatory cell infiltrate). Reactivity with CAM 5·2 was restricted to some surface cells of the epithelial linings of three dental cysts and eight dentigerous cysts (figure). Staining of dental cysts was clearly associated with areas of mucous metaplasia with mucus secreting cells being CAM $5{\cdot}2^+$, $LP34^-$. The reactivity of dentigerous cysts for CAM 5·2 often seemed to be within isolated single or groups of surface cells.

Our present observations, together with those obtained using frozen sections,[2] suggest that it is not possible to differentiate between odontogenic cyst types on the basis of LP34 immunoreactivity. Although epithelial reactivity of odontogenic keratocyst linings with LP34 was subjectively less intense than that of dental and dentigerous cyst linings, the considerable differences between our results and those of MacDonald and Fletcher[5] presumably indicate that LP34 reactivity in paraffin wax sections of odontogenic keratocysts is particularly laboratory or technique dependent. Indeed, the Dako data sheet concerning LP34 states that this antibody may not give "satisfactory" results on formalin fixed, paraffin wax sections unless large amounts of keratin proteins are present.

Finally, it is generally agreed that the greatest diagnostic difficulty in distinguishing between odontogenic cyst types occurs when the typical histological appearance of the epithelial lining is masked by inflammatory changes or metaplastic keratinisation. As both events are known to affect keratin expression[3 4] it is unlikely that keratin profiles will aid the histological diagnosis of odontogenic cysts.

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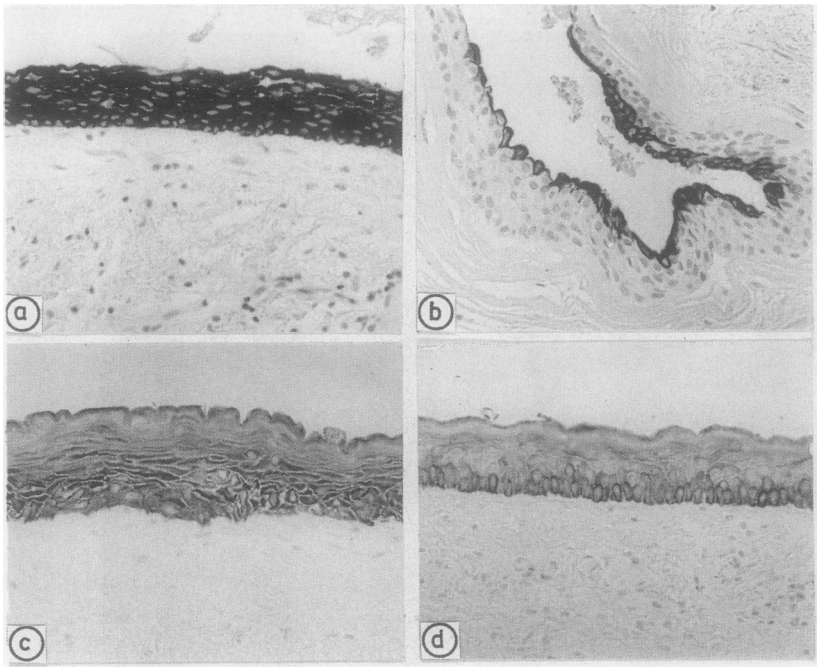

Staining patterns of epithelial linings of dentigerous cysts (a and b) and keratocysts (c and d) with LP34 (a, c, and d) and CAM 5·2(b).