Correspondence

Bones, groans, moans . . . and salivary stones?

A 46 year old man was referred to hospital by his general practitioner with abnormal bone biochemistry. He had presented with poor appetite, fatigue, myalgia, and backache. Serum calcium, corrected for albumin, was 2.63 mmol/litre (reference range, 2.12–2.62); serum phosphate was 0.85 mmol/litre (normal range, 0.7–1.4); and alkaline phosphatase was 367 IU/litre (normal range, 80–280). There was no history of previous fractures or of renal calcification. The parathyroid hormone concentration was raised at 19 pmol/litre (normal range, 0.375–0.65). Bone densitometry provided evidence of osteoporosis. The calcium to creatinine ratio was 0.375 (normal range, 0.085–0.65). Bone densitometry provided evidence of osteoporosis. The calcium to creatinine ratio was 0.375 (normal range, 0.085–0.65). Bone densitometry provided evidence of osteoporosis. The calcium to creatinine ratio was 0.375 (normal range, 0.085–0.65).

It is possible to estimate accurately the true extent of any putative link between hyperparathyroidism and sialolithiasis, precisely because such a link has not been widely recognised. Certainly, most patients with salivary stones are not investigated for abnormal bone biochemistry. The time honoured mnemonic has it that hyperparathyroidism and other hypercalcaemic states are classically associated with “bones, stones, abdomino-mal pain, and psychic groans”. Although this full blown clinical presentation is rarely seen today, we suggest that it may include salivary stones as well. The following case demonstrates a rare association between a hyperparathyroid patient and sialolithiasis.

A 62 year old male non-smoker had passed a stone from a salivary gland. He had attended hospital as an emergency two months previously and had been diagnosed as having sialadenitis of the left submandibular gland with a small calculus present in the duct subsequently. In retrospect, he had been exasperated with the pain and manually forced the calculus out of the duct. There was no history of chronic infection or of other pathology to explain the presence of the calculus. The calculus weighed 2 mg and consisted of calcium phosphate (59%) and calcium oxalate (41%).

Sialolithiasis has been reported in hyperparathyroidism secondary to chronic renal failure but not previously in primary hyperparathyroidism. Salivary stone formation may be promoted by the combined effects of hypercalcaemia and secretory stimulation; the mechanism involves excessive calcium release into the acinar lumina resulting in calcium phosphate aggregates. Such calcium phosphate intermediates may transform into more stable hard deposits. Their saturation in solution varies widely, partly because of the variation in pH that occurs in saliva. As a result, some of these calcium phosphate aggregates may precipitate.

Why do salivary stones occur so much less frequently than urinary stones in hyperparathyroidism? Some ions in saliva and urine, such as citrate, inhibit the growth of precipitated crystals, whereas others, like calcium and phosphate, accelerate growth. The balance of these and other molecules might favour stone formation in urine but not in saliva. Certainly, mechanisms involved to explain urolithiasis in hyperparathyroidism include hypercalciuria, hyperphosphaturia, and hypocitraturia. However, salivary concentrations of calcium and phosphate are also raised in primary hyperparathyroidism; the comparative rarity of salivary stones in hyperparathyroidism probably owes more to specific salivary proteins that control mineralisation, such as statherin and proline rich protein. It is possible that when salivary stones develop in hyperparathyroidism, they arise via the mechanism outlined above, with alterations in the concentrations of calcium and phosphate playing a primary pathogenetic role. Such a putative similarity in the pathogenesis of hyperparathyroidism and sialolithiasis might be consistent with the observed association between the two conditions. In one large series, six of 56 patients with sialolithiasis were reported to suffer from nephrolithiasis as well.

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A case of purple urine bag syndrome associated with Providencia rettgeri

We would like to report this interesting case of an elderly lady (85 years old) who has been passing violet coloured urine over the past four weeks. She is living in a nursing home and has a long term urinary catheter. There were no other symptoms but her general practitioner was worried about urine discoloration and sent three urine samples to the biochemistry department on three separate occasions to identify the cause of the violet colour. There was no history of intake of medication, food colouring or anything else that may alter the urine colour. The urine sample was alkaline (pH 8.5) with a strong smell of ammonia. It was centrifuged and a precipitate of fine blue crystals was identified in the sedi-ment. The supernatant was clear and purple coloured, and was negative for haemoglobin, myoglobin, and porphyrins. At this stage, the purple urine bag syndrome (PUBS) was suspected and an aliquot was sent to microbiology for culture and sensitivity. There was heavy growth of a coliform species identified as Providencia rettgeri, an ammonia producing bacterium, adding support to the diagnosis of PUBS. This interesting phenomenon in which the urinary catheter of some elderly patients develops intense purple colouration is thought to be caused by indirubin formation. Various observers stated that indigo producing bacteria, which possess indoxyl sulphatase activity, usually bring about the decomposition of urinary indoxyl sulphate to indigo and indirubin. Several bacterial species have been reported in association with PUBS including Escherichia coli, Proteus mirabilis, Morganella morganii, Klebsiella pneumoniae, and Providencia stuartii. Providencia rettgeri was isolated from one of our patients; to our knowledge this organism has not been reported previously in PUBS cases. Awareness and prompt identification of this syndrome by biochemistry and microbiology departments should avoid the undertaking unnecessary tests on such urine samples.

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Fetal legionella pneumonia after fludarabine treatment in chronic lymphocytic leukaemia

Treatment of chronic lymphocytic leukaemia (CLL) with nucleoside analogues may cause T cell dysfunction, thereby predisposing to opportunistic infections in addition to bacterial infections as a result of neutropenia and humoral immune dysfunction. The following case provides an example of fatal legionella pneumonia arising in these circumstances. A 62 year old male non-smoker had obtained a good partial response after completing four courses of fludarabine treatment for relapse of stage B CLL. He had been treated at age 2½ years ago with chlorambucil and purinibucin but had never received corticosteroids. His general health had been good and he had continued in full time employment throughout. He developed “flu-like” symptoms just before returning to the UK from holiday in Spain and was prescribed co-amoxiclav by his general prac-titioner immediately on arrival. The next day he was admitted to hospital after a general medical team with lobar pneumonia and commenced treatment with ceftazidime. Clarithromycin, ciperoxacin, and rifampicin were added soon after Legionella pneumophila was suspected (and later confirmed) to be the usual causal organism, but he died two days later.

There are approximately 200 cases of legionnaire’s disease notified each year to the National Surveillance Scheme in England and Wales, of which half are associated with short overseas travel, mainly to Spain or Greece. Immunosuppression, usually from corticosteroids or human immunodeficiency virus (HIV) infection, is known to predispose to infection and to increase mortality, as is

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chronic pulmonary disease. Of the haematological diseases, the risk of leukaemia seems highest in hairy cell leukaemia, possibly because of impairment of monocyte function, and has been seen after treatment with 2-chloro-2-deoxyadenosine. Opportunistic infections after treatment with fludarabine are usually seen with advanced Rai stage, severe neutropenia, impaired renal function, or concomitant prednisolone treatment. Leukaemia is uncommon in CLL, although it has been described after treatment with fludarabine.

Treatment with co-trimoxazole is recommended for prophylaxis against pneumocystis in patients receiving nucleoside analogues but is not clear from its use in HIV infected patients whether this decreases the risk of leukaemia infection. With the increasing use of fludarabine as a first line treatment, the number of treated patients with CLL who are fit enough to consider travelling abroad will probably increase. Because patients may present for medical help to those unfamiliar with immunosuppression after treatment with nucleoside analogues, the carrying of an alert card specifying infective and transfusion risks seems warranted.

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Is it useful to test for antibodies to extractable nuclear antigens in the presence of a negative antinuclear antibody on Hep-2 cells?

Antinuclear antibody (ANA) negative lupus has long been recognised as a distinct entity affecting a small number of patients with systemic lupus erythematosus (SLE). Initial estimates of the prevalence of this entity (5% of patients with lupus) were based upon studies using rodent tissues as substrate for antinuclear antibody testing. The increasing use of human epithelial cell lines (Hep-2 cells), which have greater sensitivity for extractable nuclear antigens (ENA), has meant that new patients with true ANA negative lupus are now rarely encountered. Many immunology laboratories are faced with a substantial number of requests for antibodies to ENA and double stranded DNA, even in patients with negative ANA, on the grounds that patients with ANA negative lupus might go undetected. Using Hep-2 cells, we have attempted to define the prevalence of ANA negative, anti-ENA positive disease in a series of consecutive, unselected serum samples.

Over a 12 month period, all laboratory requests for antibodies to ENA (antibodies to Sm, Ro, La, and ribonuclear protein) were scrutinised to determine the number of samples that had antibodies to ENA despite a negative ANA on Hep-2 cells. The notes of patients who were ANA negative, anti-ENA positive were examined to verify the clinical diagnosis.

During the 12 month study period, 7077 Hep-2 ANA samples were processed and 468 patients had an anti-ENA profile performed, 3 of these had a negative ANA. Of these 468 patients, nine were identified who were ANA negative, anti-ENA positive. Review of their clinical notes indicated that six of these nine patients had previously been ANA positive and were known to have lupus but were receiving immunosuppressive treatment. Only three patients were persistently ANA negative despite positive anti-Ro antibodies before treatment. Thus, the prevalence of anti-ENA positivity combined with a negative ANA was three out of 468 (0.64%). Because ANA negative lupus characteristically presents with cutaneous disease the clinical notes of 90 of the dermatology patients were reviewed. Of the 90, seven of these patients had confirmed lupus erythematosus. Only one patient from the dermatology group had ANA negative, anti-Ro positive lupus before the commencement of immunosuppressive treatment.

Our finding of a low prevalence of anti-ENA positivity in the presence of a negative ANA on Hep-2 cells is in keeping with other studies in the literature. Manoussakis et al found that only 0.4% of 243 Hep-2 negative patients with systemic autoimmune disease had positive anti-ENA antibodies and Homburger, reporting on the experience of the Mayo Clinic immunopathology laboratory, stated that anti-ENA antibodies were unlikely to be positive in the presence of a negative ANA result on Hep-2 cells. However, neither of these studies included a clinical evaluation of the ANA negative, anti-ENA positive patients.

We recognise that our study is subject to potential sources of bias. The failure to scrutinise patients’ notes on all ANA negative samples irrespective of anti-ENA antibody status might have resulted in some patients with strong clinical evidence of connective tissue disease being overlooked. We think it unlikely that this would have greatly changed our findings given the rarity of uniformly seronegative lupus (ANA negative, anti-ENA negative, and anti-DNA negative) and the general acceptance that a repeatedly negative ANA effectively excludes systemic lupus. Second, if clinicians failed to request ENA along with ANA, it is possible that some cases of ANA negative, ENA positive disease would be missed.

Based on these findings and others in the literature we have modified our testing strategy for antibodies to ENA. All requests for anti-ENA antibodies are “gated” by performing an initial ANA screen on Hep-2 cells. Samples that are ANA negative do not proceed to further testing unless there are compelling clinical reasons to suggest lupus. In conjunction with this clinical liaison this testing strategy allows streamlining in busy clinical laboratories.

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Handling of renal biopsies: different approaches reflect a lack of evidence for what constitutes “best practice”

We read ACP Best Practice No 160 “Renal biopsy specimens” with interest. Dr Furness rightly avoids providing a list of specific procedures to follow because, as he points out, “there is a need to assess each case on its merits, rather than following rigid rules”. It is clear from an audit of handling of renal biopsies in the UK, performed in 1999, that standard operative procedures were not the norm and that many laboratories fall short of “best practice”. A probable reason for this is that there is very little hard evidence to support any specific recommendations. In the UK audit, a questionnaire was sent to all members of the UK Renal Pathology Group and returns were received from 50% of the 54 laboratories represented. It is interesting to compare current practices with Dr Furness’s guidelines.

Dr Furness recommended that all specimens should be examined in the biopsy room for adequacy, using a dissecting microscope. However, in only 15% of units this was performed as routine. Failure to confirm the presence of renal cortex in the specimen would be expected to increase dramatically the proportion of inadequate biopsies. This was not the experience in the audit, however, where in 1994, as a result of staff shortages, the practice of sending an MLSO to attend every biopsy procedure was stopped. In Oxford, the histopathology laboratory is on a different site to the renal and transplant units; neither an MLSO nor a pathologist attends biopsies, as was once the case. Furthermore, what constitutes an adequate specimen is difficult to define and to some extent depends on the nature of the pathology. More tissue is required to detect focal than diffuse lesions. This has been demonstrated in renal allograft biopsies; in the validation study of the CCTT classification of allograft pathology, those biopsies showing acute vascular rejection contained the diagnostic arteritic lesion in only one of two cores taken in 82% of cases. In the UK audit, it was found that the proportion of renal tissue routinely taken varied from one to four in different centres. Dr Furness recommends that division of the specimen should be done within minutes of the biopsy being taken, to avoid artefactual ultrastructural changes. Although subtle subcellular changes do develop if fixation is delayed, for routine diagnostic electron microscopy (EM) rapidity of fixation is much less crucial. Formaldehyde fixation alone may produce...
There is also variation in the immunohistochemical techniques used when handling native renal biopsies. A frozen sample for immunofluorescence (IF) is taken routinely in 81% of laboratories; the remaining 19% rely entirely on immunoperoxidase (IP) stains performed on paraffin wax embedded sections. This, in part, reflects varying success in achieving reliable results with IP for immunoglobulins and complement. In the case of early transplant biopsies, only 30% of laboratories routinely take frozen tissue for IF. In those that do, it is often taken for research purposes rather than for patient management. Similarly, most laboratories (88%) routinely take tissue for EM from native renal biopsies. Because some of the most common renal diseases, such as thin membrane nephropathy, can only be diagnosed ultrastructurally, those laboratories that do not take tissue for EM are certainly falling short of “minimum adequate practice”. Although it may be “best practice” to perform EM in all cases, it is probably sufficient to store this tissue as a resin block and only perform EM if the light microscopy is non-diagnostic. In many instances, EM will not influence patient management and the “minimum adequate practice” would, therefore, be to consider each case on its own merits and perform further investigations only if necessary. At present, EM does not have a clearly defined role in the assessment of early transplant biopsies and the UK audit found that only 38% of laboratories routinely take tissue for EM from these specimens.

The choice of which special investigations are performed should, at least in part, be determined by our clinical colleagues. Nephrologists differ widely in how aggressive they are in investigating patients with asymptomatic renal disease, such as those presenting with microscopic haematuria detected at a routine health check. In some centres a biopsy will only be performed if it is likely to affect management of that patient, in others, biopsy practice is partly driven by research interests. Equally, the information required from the pathologist will depend on its potential clinical value. For example, providing a measure of the severity of chronic tubulointerstitial injury in a patient with membranous nephropathy is of far more value to the nephrologist than knowing the glomerular disease stage, as defined by ultrastructural appearances.

In the UK audit, the number of paraffin wax sections routinely cut for native renal biopsies varied greatly—from two sections on two slides to 80 sections on 10 slides—again reflecting a lack of evidence base. In his article, Dr Furness indicated that the number of sections that should be cut and examined depends on the question. A renal biopsy standard operative procedure should, however, include examination of sufficient sections to enable the diagnosis of conditions in which the pathology is usually focal. In the case of primary focal segmental glomerulosclerosis, this is considerably in excess of two. For renal transplant biopsies, the Banff classification recommends that at least three haematoxylin and eosin (H&E) and three periodic acid Schiff or methenamine silver stained sections should be examined. The rationale behind this is that the diagnostic lesions of acute rejection—tubulitis and arteritis—are often focal. A recent review of transplant biopsies in Manchester concluded that one third of diagnoses of acute vascular rejection would be missed if only one, rather than three, H&E sections were examined (GP McCarthy, ISD Roberts, 2007, unpublished data).

All laboratories that handle renal biopsies should review their standard procedures, particularly if they do not conform to Dr Furness’s guidelines or “usual practice”, as indicated by the UK Renal Pathology Group audit. As the diagnostic questions asked by nephrologists change and new techniques emerge, procedures will inevitably require updating, but we will need to provide the evidence that any changes introduced are of demonstrable benefit to patient management.

2 Roberts ISD, Tarpey P. Use of a transport gel for antigen preservation in unfixed renal biopsies. J Pathol 1996;179:31A.

In reply

I am grateful for the opportunity to respond to the letter of Drs Roberts and Davies on the ACP Best Practice article “Renal biopsy specimens”1, although they say very little with which I disagree. Most of their points of difference relate to “current practice” or “minimum adequate practice” rather than “best practice”. For example, the observation that electron microscopy (EM) can provide useful information even if fixation is delayed for a day or more is interesting and useful information. It supplements my observation that tissue from the paraffin wax block can be reprocessed for EM, but it does not alter the fact that best practice is to get the tissue fixed quickly!

The UK audit that they describe is a welcome update of a similar study that we performed in 1995,2 albeit which influenced the development of the ACP guidelines. There is one small point where I think that Roberts and Davies misrepresent my suggestions. In their discussion of identifying and dividing the sample under a dissecting microscope, they imply that this has to be done by a pathologist or an MISO. We have found that nephrologists and radiologists can identify renal cortex and divide the biopsy appropriately with only minimal training. Again, rapid division is best practice; taking a bit longer is probably quite adequate in most circumstances, but (for example) in the future a delay will probably invalidate studies of gene expression.

Apart from these rather trivial quibbles I welcome Roberts and Davies’s contribution to the discussion.

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To my knowledge, this is the first comprehensive textbook dealing exclusively with the histological interpretation of needle core biopsy samples. To date, there have been only one or two books edited by radiologists on needle core biopsy, which include chapters on histological interpretation. These by their nature have been devoted to basic principles.

The author of this book is of course widely known to pathologists involved in breast disease reporting. He has numerous widely cited publications in peer review and in recent years has produced a major textbook on breast pathology based on his personal experience. Personally, I am a great admirer of his achievement, enthusiasm, and dedication to the field of breast pathology. For this reason, reading this book has been a pleasure.

First, I would point out that this book although dealing principally with needle core biopsy interpretation is also a distilled version of Rosen's textbook of breast pathology. Diagnostic entities are described in succinct detail and are well referenced.

The book includes 31 chapters, the first seven dealing with normal anatomy and benign conditions, including one chapter on myoepithelial neoplasms, which form a diagnostic entity that appears to be gaining prominence, particularly in the American literature.

Rosen recognises that most adenomyoepitheliomas are variants of intraduct papilloma and closely related to ductal adenoma and pleomorphic adenoma.

There is a substantial chapter on ductal hyperplasia and intraduct carcinoma, which cover in detail the difficulties of distinguishing the microfocal changes present in core biopsy. In this chapter the author recognises that there are some challenging forms of atypical ductal proliferation that exhibit prominent architectural and architectural changes, which form a diagnostic group that appears to be gaining prominence, particularly in the American literature.

Rosen recognises that most adenomyoepitheliomas are variants of intraduct papilloma and closely related to ductal adenoma and pleomorphic adenoma.


In this book, an impressive amount of different molecular techniques that can be used in vascular research are described in great detail.

In summary, methods of molecular biology are described related to gene isolation, characterisation, expression, and transfer, and (of course) cell death.

In each chapter, the principle of the technique is first elucidated (of course a basic knowledge of molecular biology is necessary). Subsequent materials and methods sections are described stepwise. Each chapter ends with notes that give extra clues for doing the experiments, and also functions as a trouble-shooter. Also helpful are the illustrations of the outcome of the described experiments, when successful. In the last chapter, gene transfer protocols are described, according to recent developments in the field.

Although all these methods can be used in pathological specimens, for general histopathology it is probably less suitable. Nevertheless it is a very interesting book and I recommend it strongly for researchers, including pathologists, who are doing research in the field of vascular disease.

H W M NIJESSEN


I thoroughly enjoyed reading this compact, stimulating, and refreshingly thought provoking book. It really puts cancer into an evolutionary context. It was pitched just right for me; as for most of other doctors, even those involved with cancers and leukaemias, my knowledge of evolution, history, epidemiology, and molecular biology is very focused on and tends to be limited to what affects my daily practice. So essentially, most of us are laymen. It is the sort of book that will be enjoyed by scientists, doctors, and many of those whose primary interests lie in the arts and the humanities, not to mention pathologists too.

This book, with its almost conversational tone, allows us all to follow the arguments in what are potentially impenetrable arenas with surprising ease. Some of that ease is probably an illusion, but a welcome one. One's confidence in Mel Greaves to lead us through the jungle of cancer is probably as important as truly understanding the implications and fine detail of the paths and surrounding countryside through which he takes us. Gradually one can end up at a conclusion really believing one understands how one got there, only on reflection to realise that one might need to read the argument all over again. Perhaps I should replace all the "ones" with "I". It's a seductive story, and well told too—that's what carried me along, rather than my own intrinsic abilities to understand. But I do confess I got almost as much pleasure rediscovering how I got to some of his destinations as I had when I first arrived.

M M REID

Calendar of events

Full details of events to be included should be sent to Maggie Butter, Technical Editor, JCP, The Cedars, 36 Queen Street, Castle Hedingham, Essex CO9 3HA, UK; email: maggiebutler@pilotree.prestel.co.uk

International Consultation on the Diagnosis of Noninvasive Urothelial Neoplasm
11–12 May 2001, University of Ancona School of Medicine, Torrette, Ancona, Italy
Further details: R Montironi, Ancona Italy (email: rmontironi@unipd.it), M Bassi, Padua, Italy (email bassip@student.unipd.it), M Droller, New York, USA (email: m_droller@emplink.com), or D Waters, Seattle, WA, USA (email: waters@vet.vet.purdue.edu)

Human Adverse Drug Reactions
30 May 2001, Royal College of Pathologists, London, UK
Further details: Michelle Casey, Academic Activities Coordinator, 2 Castle Road, London SW1Y 5AF, UK. (Tel +44 020
Correction


In table 1 the time of the first sample should have been at −11, −7, and −4 days in patients 1, 2, and 3, respectively; similarly, in table 2 the time of the first sample should have been at −12, −6, −4, and −1 days in patients 1, 2, 3, and 4, respectively. The authors apologise for this oversight.
Cancer. The Evolutionary Legacy.: Greaves M.
(£27.50.) Oxford University Press, 2000. ISBN 0
19 262835 6.

M M Reid

*J Clin Pathol* 2001 54: 415
doi: 10.1136/jcp.54.5.415-b

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