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, 0.74–1.40), and alkaline phosphatase was 367 IU/litre (normal range, 25–130). Bone densitometry provided evidence of osteoporosis (T score, −3.05). Ultrasound of his neck revealed a solid lesion of low echodensity at the lower pole of the right lobe of the thyroid gland, typical of a parathyroid adenoma. At surgery the lower right parathyroid gland was excised, and confirmed by histology to be an adenoma.

At outpatient one week before elective parathyroidectomy, the patient reported that he 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, he became exaerated 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 saliva is promoted by the combined effects of factors which accelerate growth. The balance of these and other molecules might be determined by the pH in that an increase in the pH of the urine from 6.0 to 8.0 can inhibit the formation of calcium phosphate stones. 

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 been in hospital under a general practitioner with abnormal bone biochemistry. She has been found to have osteoporosis (T score, −3.05). Ultrasound of her neck revealed a solid lesion of low echodensity at the lower pole of the right lobe of the thyroid gland, typical of a parathyroid adenoma. At surgery the lower right parathyroid gland was excised, and confirmed by histology to be an adenoma.

At outpatient one week before elective parathyroidectomy, the patient reported that he 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, he became exaerated 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 saliva is promoted by the combined effects of factors which accelerate growth. The balance of these and other molecules might be determined by the pH in that an increase in the pH of the urine from 6.0 to 8.0 can inhibit the formation of calcium phosphate stones.

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 any food items that might 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 sediment. 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 investigation. 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 morgani, Klebsiella pneumoniae, and Providencia stuartii. Providencia rettgeri was isolated from our patient; 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 patient undergoing unnecessary tests on such urine samples.
chronic pulmonary disease.1 Of the haematological diseases, the risk of legiossia seems highest in hairy cell leukemia, possibly because of impairment of monocyte function, and has been seen after treatment with 2-chloro-2-deoxyadenosine.2 Opportunistic infections after treatment with fludarabine are usually seen with advanced Rai stage, severe neutropenia, impaired renal function, or concomitant prednisolone therapy.3 Legiossia is uncommon in CLL,4 although it has been described after treatment with fludarabine.1

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 legiossia infection.1 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).1 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 antibodies (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 during the lifetime of 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 lupus antibodies before treatment. Thus, the prevalence of anti-ENA positivity combined with a negative ANA was three out of 468 (0.6%). Because ANA negative lupus characteristically presents with cutaneous disease the clinical notes of 90 of the dermatology patients were reviewed. Twenty-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.1,3,5 Manousakis et al found that only 0.4% of 243 Hep-2 negative patients with systemic autoimmune disease had positive anti-ENA antibodies6 and Homburger,7 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 literature8 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 clinical liaison this testing strategy allows streamlining in busy clinical laboratories.

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M J D GODFREY
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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.1 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 adopted 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 carried out by a member 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 of 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.2 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.3 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.4 In the UK audit it was found that the least adequate 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...
excellent ultrastructural detail and is the fac-
tive of choice for EM in some laboratories. Oc-
casionally, we have received specimens that have been stored unfixed in transport gel for two days, and found preservation to be ade-
quate for the purposes of diagnostic EM.

There is also variation in the immunohisto-
chemical 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 per-
formed on paraffin wax embedded sections.
This, in part, reflects varying success in
achieving reliable results with IF for
immunoglobulins and complement. In the
case of early transplant biopsies, only 30% of
laboratories routinely take 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 diag-
nosed ultrastructurally, those laboratories
that do not take tissue for EM are certainly
falling short of “minimum adequate prac-
tice”. Although it may be “best practice”
to perform EM in all cases, it is probably suf-
cient 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, there-
fore, be to consider each case on its own mer-
its 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. Ne-
phologists differ widely in how aggressive they
are in investigating patients with asymptom-
atic 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 know-
ing 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
slides to 10 sections on 10 slides—again
reflecting a lack of evidence base. In his ar-
ticle, Dr Furness indicated that the number of
sections that should be cut and examined
stands on the question. A
renail biopsy standard operative procedure
should, however, include examination of suf-
ficient 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 classification1 recommends that at
least three haematoxylin and eosin (H&E)
and three periodic acid Schiff or methen-
amine silver stained sections should be ex-
and performed in 1995,
which is very little with
which I disagree. Most of their points of dif-
ference relate to “current practice” or “mini-
mum adequate practice” rather than “best
practice”. For example, the observation that
electron microscopy (EM) cannot 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 and which influenced the
development of the ACP guidelines.
There is one small point where I think that
Roberts and Davies misrepresent my sugges-
tions. 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
detect 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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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”, although they say very little with
which I disagree. Most of their points of dif-
fference relate to “current practice” or “mini-
mum adequate practice” rather than “best
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to the discussion.

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1 Furness PN. Renal biopsy specimens. J Clin
2 Furness PN, Boyd S. Electron microscopy and
immunocytochemistry in the assessment of
renal biopsy specimens: actual and optimal
Book reviews


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 one or two books edited by radiologists on needle core biopsy, which include chapters on histological interpretation. These by their nature have been restricted to basic principles.

The author of this book is of course well 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 major complications, 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.

There is a substantial chapter on ductal hyperplasia and intraduct carcinoma, which covers 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 pronounced architectural and architectural atypia, which are described related to gene isolation and (of course) cell death.

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 troubleshooting guide. Also helpful are the illustrations of the outcome of the described experiments, which are valuable chapters that I personally feel would have been better placed at the beginning rather than the end of the book.

The book on pathological examination is succinct but I would give a critical analysis of the coverage of strategy for reporting core biopsies. Although specific diagnostic problems are covered in the various chapters on diagnostic entities, an overview on strategy and handling diagnostic problems, with guidance on reporting and avoidance of pitfalls would have been useful.

All of the chapters are well illustrated in colour, although the colour balance could have been improved. Until relatively recently there has been few textbooks on breast pathology. This position has changed and we have several major textbooks from authorities. Does this book merit purchase for your reporting room? In my view there are two good reasons for considering the purchase of this book. First, the widespread option of needle core biopsy for diagnostic sampling of breast lesions is the door to a textbook, such as this, which considers many of the diagnostic problems that are now being encountered. Second, it serves as an updated and concise version of Rosen’s major textbook. Those of you reporting breast disease who have not purchased this textbook could “bell the cat with one stone” by acquiring a copy of this book. I will be placing my copy in our reporting room and suspect that it will spend more of its time open on the bench top rather than gathering dust on our library shelf.


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.

International Consultation on the Diagnosis of Noninvasive Urothelial Neoplasms

11–12 May 2001, University of Ancona School of Medicine, Torrette, Ancona, Italy

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Human Adverse Drug Reactions

30 May 2001, Royal College of Pathologists, London, UK

Further details: Michelle Casey, Academic Activities Coordinator, 2 Queen’s Gate, London SW1Y 5AF, UK. (Tel +44 020
Recent Advances in Genetics
5 July 2001, Royal College of Pathologists, London, UK
Further details: Michelle Casey, Academic Activities Coordinator, 2 Carlton House Terrace, London SW1Y 5AF, UK. (Tel +44 020 7451 6700; fax +44 020 7451 6701; www.rcpath.org)

BSCC Annual Scientific Meeting
9–11 September 2001, Majestic Hotel, Harrogate, UK
Further details: BSCC Office, PO Box 352, Uxbridge UB10 9TX, UK. (Tel +44 01895 274020; fax +44 01895 274080; email lesley.couch@psilink.co.uk)

41st St Andrew’s Day Festival
Symposium on Therapeutics
6–7 December 2001, Royal College of Physicians, Edinburgh, UK
Further details: Eileen Strawn, Symposium Coordinator. (Tel +44 0131 225 7324; fax +44 0131 220 4393; email 2.strawn@rcpe.ac.uk; website www.rcpe.ac.uk)

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.

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Fatal legionella pneumonia after fludarabine treatment in chronic lymphocytic leukaemia

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*J Clin Pathol* 2001 54: 412-413
doi: 10.1136/jcp.54.5.412-b