Correspondence

Multifactorial audit of invasive cervical cancer

We read with interest the article by Dr Slater,[1] in which he makes recommendations for the Cervical Screening Programme based on 20 cases of cervical cancer occurring in the Rotherham district.

We have several comments. The cases were selected from cytology records and this method is bound to underestimate the number of patients who develop cervical carcinoma and who have never had a smear (six patients in this study). These patients are very hard to identify. Possible methods include Cancer Registry records and mortality data from the Office of Population Census and Surveys (OPCS), as well as histology records. In our experience none of these sources successfully identifies all cases.

Another factor discussed is the issuing of an inappropriate laboratory report. The table quotes this as occurring in 16 of 20 cases (30%) which appears to be mathematically incorrect. It is worth noting that of these (presumably six) cases only four involved missed dyskaryosis and this was of the "easily missed" type. As stated, there is as yet no definition of an acceptable false negative rate in the Cervical Screening Programme and we look forward to forthcoming guidelines on this important matter.

Dr Slater also comments on the lack of failsafe procedures for inadequate smears. While it is true that the national guidelines[2] refer "only to follow up of women with abnormal smears", there is no reason why laboratories or FHSAs should not also include follow up of inadequate smears in their failsafe systems. Indeed, the Avon Cervical Screening Programme has incorporated such a mechanism.

A further point of interest is Dr Slater's suggestion that opportunistic smears should be performed during hospital visits. We contend that this is impractical and potentially dangerous. Most hospital wards and non-gynaecological outpatient departments do not have the equipment or trained personnel to perform cervical smears and the resulting specimens are likely to be of poor quality, which may well lead to a false sense of security, or inadequacy, leading to increased workload and patient anxiety because the smears need repeating.

Finally, we would like to point out how small the numbers in this audit are. Expressing the results in terms of percentages seems rather meaningless and no values for statistical significance are included. While this audit makes interesting anecdotal reading, we feel that the results derived are of limited value in assessing the effectiveness and quality of the National Screening Programme.

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1 Salter DN. Multifactorial audit of invasive cervical cancer: key lessons for the National Screening Programme. *J Clin Pathol* 1995;**48**: 405–7.
2 Pike C, Chamberlain J. *Guidelines on Fail-Safe Actions.* Oxford: National Co-ordinating Network Publication, 1992:1–12.

Dr Salter comments:
I thank Drs Denton and Brett for their interest in my recent article.

I wholeheartedly agree that there are numerous sources from which to obtain such patient information. My study merely highlighted that derived from the Rotherham Hospital records. In fact, as they suggested, the results were derived from both the cytopathology and histopathology records. The identification of all cases of cervical cancer, along the lines recommended by the authors, will be an important aspect of the work of the proposed "Regional" Quality Assurance Teams (QATs).[1]

I apologise for any confusion conveyed with the mathematics in my report. The figure in brackets represented the percentage number of times the factor occurred in the patients. The reason for the apparent discrepancy is that some factors occurred more than once in one specific patient. Retrospectively, this should have been emphasised by a gap between the two columns.

I am pleased to hear that the Avon Cervical Screening Programme has incorporated inadequate smears into their failsafe procedures. Unfortunately, the same cannot be said for most of the remainder of the UK. Sadly, funding for such failsafe procedures will not be made available until this aspect is specifically incorporated into national guidelines.

I fully acknowledge that it is usually inappropriate for cervical smears to be undertaken during "non-gynaecological" hospital attendances. As highlighted in my discussion, my proposal was to incorporate cervical smear history into the routine past medical history. Appropriate advice and referral could then be given.

I am appreciative that the numbers in my audit were small. The reason for the article was merely to generate national discussion, as evidenced by the current correspondence. I was also hoping to highlight factors that could be used regionally on a unified basis in the new QATs. It would appear highly desirable that all QATs approach this important area of audit in a similar way so that there can be national amalgamation and comparison of data.

1 NHS Cervical Screening Programme. Assuring the Quality and Measuring the Effectiveness of Cervical Screening. National Co-ordinating Network. Hall The Printer, 25.

I was puzzled by Dr Slater's assertion that failsafe systems for following up abnormal smears should also include follow up of inadequate smears.[1] He is suggesting that the National Guidelines on failsafe should be changed, but the only evidence given is a single case of cancer occurring when an inadequate smear had not been repeated for two years.

It is worth remembering that the main responsibility for follow up is with the smear taker. Failsafe mechanisms for abnormal smears is worthwhile because dyskaryosis has a strong association with cervical intraepithelial neoplasia and cancer. Failsafe mechanisms are especially important in cases where women have been suspended from FHSA recall. To justify failsafe of inadequate smears requires evidence that there was an association between inadequacy and disease of a similar order to that between dyskaryosis and disease.

When I have looked at cervical cancers presenting at Watford General, I have found several occurring in women whose last smear was taken more than five years before and was normal. If we followed Dr Slater's argument, we would also have to institute failsafe procedures for all normal smears!

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1 Slater DN. Multifactorial audit of invasive cervical cancer: key lesions for the National Screening Programme. *J Clin Pathol* 1995;**48**: 405–7.

Dr Slater comments:
I am appreciative to Dr Rubin for his interest in my article. Although not specifically itemised, my previous audit of deaths from cervical cancer also identified occasional cases where inadequate smears had not been repeated.[1] Furthermore, I hope that my article will encourage larger regional audits that will more accurately ascertain the size of the problem. To date, however, inadequate smears have been undoubtedly the "poor relative" of cytopathology reports. For example, there is still no national recommendation with regard to the time within which an inadequate smear should be repeated. Similarly, the potential clinical importance of inadequate smears misreported as negative remains poorly emphasised. Indeed, there are even proposals, in my opinion unreasonably, to exclude inadequate smears from the national proficiency testing scheme. I agree wholeheartedly that failsafe mechanisms were instigated for the follow up of abnormal smears and that the primary responsibility for follow up still remains with the smear taker. In these days of laboratory computerisation, however, it would not appear totally unreasonable that there are secondary checks to ensure inadequate smears have indeed been repeated within, say, three months.

1 Slater DN, Milner PC, Radley H. Audit of deaths from cervical cancer: proposal for an essential component of the National Screening Programme. *J Clin Pathol* 1994;47:27–8.

Detection of autoantibodies to neutrophil cytoplasmic antigens

ACP Broadsheet No. 143 has recently been distributed to Australian pathologists.[1] It states that indirect immunofluorescence (IIF) is the technique of choice in testing serum samples for antineutrophil cytoplasmic antibodies (ANCA), but that all positive samples should be confirmed using formalin fixed neutrophils and that antibody levels determined by titration of fluorescence. Most laboratories would use IIF to screen for ANCA, but would confirm positive serum samples, determine antigen specificity and antibody titre using enzyme linked immunosorbent assays (ELISAs) for proteinase 3 and myeloperoxidase antibodies, rather than the techniques described in the Broadsheet.

Antigen specific ELISAs have a number of advantages over the other techniques. These ELISAs will confirm the presence of ANCA that have been demonstrated by IIF: nonspecific binding can occur with IIF, but is unlikely to occur with both methods. In addition, ELISAs will confirm the presence of ANCA in serum samples with a coincidental antinuclear antibody (ANA). An ANA may obscure perinuclear fluorescence, and ANA occur in up to 40% of some series of patients with Wegener's granulomatosis or microscopic polyarteritis.[2]

The most important advantage, however, is that the ELISAs will determine antigen

specificity in an ANCA positive specimen, and hence whether a diagnosis of Wegener's granulomatosis or microscopic polyarteritis is more likely. Serum samples associated with cytoplasmic fluorescence (cANCA) and antiproteinase 3 antibodies are found in nearly 90% of all patients with active generalised Wegener's granulomatosis.[3] Perinuclear fluorescence (pANCA) with antimyeloperoxidase antibody activity is present in about 80% of patients with active microscopic polyarteritis. These specificities occur in almost no other disease. However, pANCA and other neutrophil cytoplasmic fluorescence patterns ("atypical" ANCA) with activity against elastase, lactoferrin, lysozyme, cathepsin G, and some unidentified antigens (reports of β-glucuronidase have not been confirmed) have been described in rheumatoid arthritis, systemic lupus erythematosis, ulcerative colitis, primary sclerosing cholangitis, and occasionally in other conditions. These antibodies do not indicate the presence of a vasculitis and do not correlate with disease activity. They have no clinical significance.

Finally, ELISAs indicate the level of ANCA antibody activity better than titration with IIF. Antibody levels correlate well with disease activity in both Wegener's granulomatosis and microscopic polyarteritis. In our experience, all patients presenting for the first time with these conditions have high titres of the corresponding antibodies. Levels usually fall with treatment, and can be used to monitor the response to treatment.

It is possible for a laboratory to make its own ELISA plates for antiproteinase 3 and antimyeloperoxidase antibodies. Each antigen is available commercially in a purified form. We coat plastic microtitre plates with these antigens at a concentration of about 0·5 mcg/ml in phosphate buffered saline at 4°C for 18 hours, and then block the plates with 1% human serum albumin (HSA) (Sigma) for one hour. It is possible to coat a second round of plates with the same antigen preparation after it has been removed from the wells. Serum samples are tested at a 1 in 50 dilution, and the binding is determined by subtracting that seen on a plate coated with just HSA. This technique overcomes the non-specific binding that occurs with some infections. The inclusion of standard borderline and positive serum samples allows values to be compared between assays. Control serum samples are available commercially, but otherwise there are no international units or any way of comparing binding. We report results as negative, borderline, low, medium, or high titres, because these are easier to interpret intuitively than numerical values. There is a need for a quality control programme to standardise results between laboratories. Kits for assays for antiproteinase 3 and antimyeloperoxidase antibodies are also available commercially.

There are several additional comments that I would like to make about testing for ANCA as described in the Broadsheet.

Serum samples can be screened on ethanol fixed, normal peripheral blood smears rather than using purified neutrophil preparations. ANCA were first described using peripheral blood films, and these are cheap and convenient. However, it is usually only possible to examine two samples per slide and there may be some background fluorescence. Commercial slides with six or 12 wells per slide are also available but are expensive.

The Broadsheet describes ANCA in classic polyarteritis but this is not correct. pANCA are not found in classic polyarteritis unless microscopic polyarteritis is also present. Classic polyarteritis, or polyarteritis nodosa involves medium vessels only, is characterised by "aneurysms" of the coronary, coeliac, mesenteric, and renal arteries, and ischaemia in these organs. It is an uncommon condition, and it not associated with ANCA. However, probably half of all patients with microscopic polyarteritis have features of classic polyarteritis too. It is these patients who have pANCA with antimyeloperoxidase specificity.

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1 Lock RJ. ACP Broadsheet No. 143. Detection of autoantibodies to neutrophil cytoplasmic antigens. *J Clin Pathol* 1994;47:4–8.
2 Savage COS, Winearls CG, Evans DJ, Rees AJ, Lockwood CM. Microscopic polyarteritis: presentation, pathology and prognosis. *Q J Med* 1985;56:467–83.
3 Jennette JC. Antineutrophil cytoplasmic autoantibody-associated diseases: a pathologist's perspective. *Am J Kidney Dis* 1991;XVIII:164–70.

Note: I am submitting a similar letter to *Pathology*, the journal of Australian pathologists.

Dr Lock comments:

I read with interest the comments of Dr Savige regarding the ACP Broadsheet on the detection of ANCA. Several points were raised and I should like to address them in the order they occurred. Dr Savige contends that "most laboratories ... confirm positive serum samples ... using ELISAs". While this may be true in mainland Europe and, presumably in Australia, in the UK only one third of the laboratories registered with UK NEQAS use ELISAs. A slightly higher proportion, just over 40%, use formalin fixation. That said, however, it is our practice to further subtype all fluorescence positive results by ELISA for proteinase 3 and myeloperoxidase antibodies.

I would agree that proteinase 3 antibodies have a high specificity for a diagnosis of Wegener's and myeloperoxidase antibodies a high specificity for microscopic polyarteritis. However, I feel that the statement that these specificities "occur in almost no other disease" is very broad. False positive results do occur. For example, myeloperoxidase antibodies may be found in systemic lupus erythromatosis and Henoch Schönlein purpura[1] and in rheumatoid arthritis.[2] Furthermore, proteinase 3 and myeloperoxidase antibodies are the major reactivities in necrotising vasculitis, but are not the only reactivities. Anti-elastase and anti h-lamp-2 have been described.

To dismiss atypical patterns as being of no clinical significance is perhaps ungenerous. I agree that they have poor specificity, but are supportive data in the right clinical background—for example, in ulcerative colitis. Furthermore, we have found that the titre of IgA class ANCA does correlate with disease activity in childhood Henoch Schönlein purpura.

Whether ELISAs are better indicators of disease activity remains to be confirmed. Certainly fluorescence titres have not been a universal success, but a recent case study suggests similar problems will be found with ELISA.[3] I strongly support the sentiments of Dr Savige with respect to the need for international standards for these assays.

We have no experience in the use of peripheral blood smears as a source of antigen. However, we have examined a number of commercial slides and the preparations are very variable. In many cases the cell morphology is not well preserved and defining the patterns of ANCA is difficult.

1 Küster S, Apenberg S, Andrassy K, Ritz E. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. In: *Systemic lupus erythematosus: renal vasculitis.* Sessa A, Meroni M, Battina G, eds. Vol 99. Basel: Karger, 1992: 94–8.
2 Cambridge G, Williams M, Leaker B, Corbett M, Smith CR. Anti-myeloperoxidase antibodies in patients with rheumatoid arthritis: prevalence, clinical correlates, and IgG subclass. *Ann Rheum Dis* 1994;53:24–9.
3 Kaname S, Uchida S, Minoda S, Arimura Y, Nagasawa T, Asano S, *et al.* A rise in antineutrophil cytoplasmic antibody in a patient with systemic vasculitis in remission. *Nephron* 1994;68:380–4.

Physical state of human papillomavirus using non-isotopic in situ hybridisation

It was interesting to note the application of non-isotopic in situ hybridisation (NISH) for the detection of the physical state of human papillomavirus (HPV) in cervical intraepithelial neoplasia (CIN) in the study by Pöllänen *et al.*[1] However, there appears to be a discrepancy in the interpretation of the NISH signal patterns in the results section of the abstract. This was rather surprising as the correct interpretation is in fact quoted in the text of the last paragraph of the methods section (although the reference number is incorrect). NISH signal patterns may either be diffuse (episomal, type 1), punctate (integrated, type 2), or punctate and diffuse, representing both episomal and integrated virus in the same cell/tumour (type 3).[2]

In addition, there is also a discrepancy between the abstract and text regarding the number of cases demonstrating a type 3 NISH signal. Nevertheless, it is far less than that reported previously, where half of the archival lesions defined morphologically as CIN III with morphological evidence of HPV in patients from the United Kingdom and South Africa contained either a type 2 or type 3 NISH signal.[3] This discrepancy could be attributed to a smaller number of cases in the Finnish study where six of eight cases with CIN III (and morphological evidence of HPV infection) harboured HPV with a type 1 pattern (episomal).[1] The alternative explanation for not detecting integrated HPV using NISH may be technical; nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate as a substrate in the detection system tends to diffuse within the nucleus and not impart a crisp clear punctate signal (type 2) as is demonstrated with peroxidase/aminoethylcarbazole. (Cooper, Herrington and McGee, personal observations). This is crucial if NISH is to be used as a method to detect the physical state of HPV, as caution is required regarding both technique and interpretation.

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1 Pöllänen R, Vuopala S, Lehto V-P. Detection of HPV infection by non-isotopic in situ hybridisation in condylomatous and CIN lesions. *J Clin Pathol* 1993;46:936–9.
2 Cooper K, Herrington CS, Strickland JE, Evans MF, McGee JO'D. Episomal and integrated HPV in cervical neoplasia shown by non-isotopic in situ hybridisation. *J Clin Pathol* 1991; 44:990–6.
3 Cooper K, Herrington CS, Graham AK, Evans MF, McGee JO'D. In situ HPV genotyping of CIN in South African and UK patients: evidence for putative HPV integration in vivo. *J Clin Pathol* 1991;44:400–5.