Letters

M2-AMA do not directly produce ANCA indirect immunofluorescence patterns

The importance of distinguishing atypical cytoplasmic indirect immunofluorescence patterns from the “classic,” centrally accentuated cytoplasmic immunofluorescence pattern on ethanol fixed human neutrophils has recently been re-emphasized. Autoantibodies to other cytoplasmic autoantigens such as antimitochondrial antibodies (AMA), antismooth muscle antibodies, and antiribosomal-P antibodies have also recently been reported to produce atypical cytoplasmic immunofluorescence patterns on ethanol fixed human neutrophils. However, an alternative explanation is that the atypical cytoplasmic immunofluorescence patterns might be produced by concomitant antineutrophil cytoplasmic antibodies (ANCA) in these sera, especially in cases of autoimmune liver disease. Therefore, we investigated: (1) whether sera containing AMA with confirmed M2 specificity produced positive indirect immunofluorescence patterns on ethanol fixed human neutrophils. However, an alternative explanation is that the atypical cytoplasmic immunofluorescence patterns might be produced by concomitant antineutrophil cytoplasmic antibodies (ANCA) in these sera, especially in cases of autoimmune liver disease. Therefore, we investigated: (1) whether sera containing AMA with confirmed M2 specificity produced positive indirect immunofluorescence patterns on ethanol fixed human neutrophils. However, an alternative explanation is that the atypical cytoplasmic immunofluorescence patterns might be produced by concomitant antineutrophil cytoplasmic antibodies (ANCA) in these sera, especially in cases of autoimmune liver disease. Therefore, we investigated: (1) whether sera containing AMA with confirmed M2 specificity produced positive indirect immunofluorescence patterns on ethanol fixed human neutrophils.

How-
polypoid lesion showed poorly differentiated adenocarcinoma and gastric adenoma with high grade dysplasia, respectively (fig 1). Four weeks after the first symptoms had arisen the liver edge was palpable 3 cm below the costal margin. Computed tomographic scan of the abdomen demonstrated several hypodense lesions in the liver and aortic adenopathy compatible with metastases. The following results were found: haematocrit, 0.24; white blood cell count, 12.7 × 10⁹/litre; platelets, 460 × 10⁹/litre; calcium, 2.54 mmol/litre; albumin, 31 g/litre; international normalised ratio, 1.2; alkaline phosphatase, 1075 units/litre, alanine amino transferase, 16 units/litre; total bilirubin, 10.26 µmol/litre; lactate dehydrogenase, 1765 units/litre; carcinoembryonic antigen, 327 µg/litre; ferritin, 302 µg/litre; iron, < 2 µmol/litre; and total iron binding capacity, < 21 µmol/litre. Chest x ray was negative. Two weeks later the patient developed mental confusion and dehydration. Serum calcium was 2.8 mmol/litre, phosphorus 0.5 mmol/litre, and albumin 26 g/litre. With intravenous hydration and pamidronate 90 mg, serum calcium rapidly became normal and the patient's mental status returned to baseline. Neither radionucleide bone scan nor magnetic resonance scan of the brain suggested metastases. The intact PTH (IRMA; Nichols Institute) was 6 ng/litre (normal, 10–65). However, PTHrP (1-40 IRMA; Nichols Institute) was 6 ng/litre (normal, 1–25). The patient died of progressive liver failure three weeks later.

Hypercalcaemia is rarely associated with gastric cancer. In the literature, two cases of gastric adenocarcinoma and gastric adenoma with hypercalcaemia have been reported in which the tumour cells expressed PTHrP. In both cases, bone metastases were present and plasma PTHrP values were not reported. Alipov et al found that PTHrP was expressed on tumour cells in 71 of 92 patients with gastric adenocarcinoma, none of whom had humoral hypercalcaemia. PTHrP expression in the tumour tissue was strongly associated with poorly differentiated cancers (54% of 54) as opposed to well differentiated ones (10 of 23). Normal gastric mucosa and adenomas did not express PTHrP. Our case is consistent with the findings of Alipov et al because cancer cells expressed PTHrP, whereas adenoma did not (fig 1). Our case is notable for rapid clinical deterioration coupled with raised tissue and plasma PTHrP. These results suggest that PTHrP expression is associated with poor prognosis in gastric cancer. Whether PTHrP plays a direct role in cancer progression or is a byproduct of oncogene activation (for example, ras and src) remains to be determined.

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ELISA is the superior method for detecting antineutrophil cytoplasmic antibodies in the diagnosis of systemic necrotising vasculitis.

Dr Harris's results showed that indirect immunofluorescence is a more sensitive technique than antigen specific enzyme linked immunosorbent assay (ELISA) for the diagnosis of systemic necrotising vasculitis (70% vs 50%) but that ELISAs have a higher positive predictive value (87% vs 76%). It was the greater sensitivity of indirect immunofluorescence that prompted the “Inter-

national consensus statement on testing and reporting antineutrophil cytoplasmic antibodies (ANCA)” to require all laboratories to screen for ANCA by indirect immunofluorescence, but to confirm the specificity of fluorescent sera by ELISA. In our hands, adherence to the minimum number of cases required for the consensus statement results in a higher positive predictive value than either indirect immunofluorescence or ELISA alone (62% compared with 44% and 50%, respectively). Screening by indirect immunofluorescence has the additional advantages of being a quicker and cheaper technique than using the two commercial antigen specific ELISAs that are usually required and, furthermore, indirect immunofluorescence might demonstrate coincidental but unsuspected autoantibodies such as antinuclear antibodies. We believe that the use of ELISAs alone to diagnose a systemic necrotising vasculitis is analogous to testing for systemic lupus erythematosus with anti-doubled stranded DNA antibodies rather than initially screening for antinuclear antibodies by indirect immunofluorescence.

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The authors reply

Our study was carried out following the preliminary observation that in our laboratory a positive immunofluorescence antineutrophil cytoplasmic antibody (ANCA) result was less frequently associated with a diagnosis of systemic necrotising vasculitis than a positive enzyme linked immunosorbent assay result (PR3 or myeloperoxidase). Our clinicians frequently did not alter management based on the immunofluorescence ANCA result. In fact, our renal physicians invariably proceeded with renal biopsy, regardless of immunofluorescence results, to confirm or refute the diagnosis of systemic necrotising vasculitis. The ELISA result frequently came back too late to alter clinical management, the ELISA test being done when immunofluorescence results were positive. Our study has shown that for active systemic necrotising vasculitis, ELISA has a superior positive predictive value and specificity, and comparable sensitivity to the immunofluorescence technique.

Savage et al quoted our sensitivity results with respect to “all cases” of systemic necrotising vasculitis, active and inactive combined, which are largely irrelevant parameters in clinical practice because the need is to identify active cases. The principal result of the study was the finding that in active, biopsy confirmed cases of systemic necrotising vasculitis, ELISA ANCA is just as sensitive (85% vs 88%; p = 0.056) yet has a signifi-
cantly better specificity (97% ± 90%; p = 0.0006) and positive predictive value (73% ± 50%; p = 0.0013) compared with immunofluorescence ANCA.

The fact that the sensitivity of ELISA ANCA falls as inactive cases are added to active cases implies that ELISA more quickly becomes negative as active disease settles, whereas immunofluorescence remains positive. This observation suggests that ELISA is also a better tool following disease activity after diagnosis and initiation of treatment.

Savage et al reported their finding of a higher positive predictive value if results of ELISA and immunofluorescence ANCA are combined. This was not found in our study. In fact, combining immunofluorescence and ELISA ANCA resulted in a lower positive predictive value than ELISA ANCA alone. It is also a better test in following disease activity after diagnosis and initiation of treatment.

Finally, in addition to the eccetocervical and endocervical edges we routinely comment upon the presence of CIN (cervical intraepithelial neoplasia) at the deep lateral edge. This is the edge that runs between the superior, endocervical edge of the specimen to the lateral, ectocervical edge of the specimen. Although this is composed of cervical stroma with variable degrees of cautery artefact, we regard this involvement as being important because there is the potential of residual disease being covered in the re-epithelialisation process, so that it will not be detected on colposcopic or cytological surveillance. Residual disease, if undetected, has been suggested as a cause of late invasive cervical carcinoma in patients treated for CIN.

Based on our results, we conclude that ELISA ANCA is the principal serological test for the diagnosis of systemic necrotising vasculitis. Immunofluorescence ANCA should be avoided because it has inferior specificity and poor positive predictive value open the way to incorrect or delayed diagnosis and treatment. We would like to restate the importance of recognising the different clinical syndromes caused by systemic necrotising vasculitis and of appropriate histological testing even with a positive or negative ELISA ANCA result.

We have not investigated the value of ANCA with respect to the diagnosis of other conditions, such as inflammatory bowel disease, and so we do not recommend which tests should be used in a particular laboratory. We have only compared ELISA and immunofluorescence ANCA in a particular disease (systemic necrotising vasculitis) and found ELISA to be superior by all criteria.

Our results indicate that the “International consensus statement on the testing and reporting of ANCA” should be revisited.

I should again like to congratulate Dr Al-Nafussi and her colleagues for providing us with an interesting and stimuating paper and to take the opportunity to add some comment of my own. Following earlier correspondence in the journal,1,3 I have sought to develop a system of standardised phrases that are used in reporting the features listed in the paper by Reid et al.1 Secretarial or medical staff can enter a short code of up to 35 letters, which is expanded electronically to produce a phrase or sentence in coherent English. In this department, we use the Telepath system, which is available commercially and is used widely for the assessment of antiretroviral treatments. The kit is called the Amplicor1 HIV-1 monitor test kit version 1.0 from Roche Diagnostics (Tokyo, Japan). However, this system is not sensitive enough for the accurate measurement of genetic subtypes A and E, and it gives falsely low titres for these virus subtypes.1,3

To surmount this problem, additional gag primers (AG primers) have been provided by Roche for research use (Ver. 1.0 plus). Furthermore, a new improved version (Ver. 1.5) was developed recently, which is said to yield accurate results not only on subtype B but on subtypes A and E. With the Ver. 1.0 plus kit, adding the AG primer set from the Ver. 1.5 to the PCR master mixture containing the Ver. 1.0 primer set makes it possible to amplify even subtype A and E viruses. In the Ver. 1.5 kit, the downstream primer is 12 bases downstream from the Ver. 1.0 primer position, whereas the upstream primer position is unchanged but the primer has two base substitutions. In our laboratory, we have examined over 1500 samples (148 cases) using the Ver. 1.0 kit, and among them, 150 samples (65 cases) were also measured with the Ver. 1.0 plus kit. As expected, most cases with the HIV-1 subtypes A and E, which could not be measured with the Ver. 1.0 kit, could be measured with the Ver. 1.0 plus kit. However, we were particularly interested in a few cases that gave unexpected results. Clinically and epidemiologically, these patients are not different from our other patients infected with HIV-1 subtypes A or E. As shown in table 1, case 1 gave equivocal results with all of the kits but in cases 2 to 5 higher results were obtained with the Ver. 1.0 plus kit than with the Ver. 1.0 kit. We measured these specimens with the Ver. 1.5 kit (kindly provided by Roche Diagnostics, Japan). In these five cases, all but case 2 revealed equivalent values with both the Ver. 1.0 plus and Ver. 1.5 kits. Unexpectedly, in case 2, only the Ver. 1.0 plus kit yielded the actual viral load.

On the other hand, in case 6, a higher result was obtained with the Ver. 1.0 kit but not with the Ver. 1.0 plus or Ver. 1.5 kits. An additional two cases showed a similar trend, although the degree of discrepancy was less severe (data not shown).

### Table 1 Amplicor1 HIV-1 monitor kit

<table>
<thead>
<tr>
<th>Case</th>
<th>Ver. 1.0</th>
<th>Ver. 1.0 (+)</th>
<th>Ver. 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.6 × 10^4</td>
<td>2.7 × 10^4</td>
<td>2.0 × 10^4</td>
</tr>
<tr>
<td>2</td>
<td>&lt;0.4 × 10^4</td>
<td>3.7 × 10^4</td>
<td>&lt;0.4 × 10^4</td>
</tr>
<tr>
<td>3</td>
<td>4.2 × 10^4</td>
<td>1.2 × 10^4</td>
<td>9.2 × 10^4</td>
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<tr>
<td>4</td>
<td>1.7 × 10^4</td>
<td>5.0 × 10^4</td>
<td>3.5 × 10^4</td>
</tr>
<tr>
<td>5</td>
<td>1.6 × 10^4</td>
<td>6.1 × 10^4</td>
<td>3.5 × 10^4</td>
</tr>
<tr>
<td>6</td>
<td>4.0 × 10^4</td>
<td>&lt;0.4 × 10^4</td>
<td>0.5 × 10^4</td>
</tr>
<tr>
<td>7</td>
<td>3.1 × 10^4</td>
<td>&lt;0.4 × 10^4</td>
<td></td>
</tr>
</tbody>
</table>

Test results of each case are summarised. Measurements with the Amplicor HIV-1 monitor test kits Ver. 1.0, 1.0 plus, and 1.5 were performed according to the manufacturer’s protocol. The correlation coefficient of variation on the same samples tested with the Amplicor HIV-1 monitor test kits Ver. 1.0 and 1.0 plus in our laboratory were 19.9% (n = 17) and 31.9% (n = 12), respectively. In cases 2 and 6 the viral load was confirmed with another independent measurement (second result).
To clarify the cause of these discrepant results, sequence analysis of the amplified regions of these cases was performed. The nucleotide sequences of these cases were found to be homologous to subtype A or E virus when they were aligned with the consensus sequences of HIV-1 subtypes B, and E obtained from the HIV Sequence Database WWW home page (Sequences. Online. http://hiv-web.uthscsa.edu/). Surprisingly, the sequences of the primer regions of all cases were completely identical (fig 1). In conclusion, cases 2 and 6 are measurable by one of these kits, Ver. 1.0 plus and Ver. 1.0, respectively; and case 1 is measurable by all kits despite having the same nucleotide sequence in the primer regions as the other cases. The results of sequence analysis of the primer regions suggests that the minor differences in sequence between the virus and the primers does not always affect amplification efficiency in these kits. Although such cases might form a minor population among HIV-1 infections, these results indicate that some cases could not be measured by a single kit. As far as we have experienced, even if one kit fails to measure virus, the other will yield the expected viral load, as judged by disease history, CD4 count, treatment, and so on, suggesting that these kits are mutually complementary. If other methods, such as nucleic acid sequence based amplification or branched DNA (bDNA) systems, are available, it would be useful to test with them. We have found some cases of subtype A or E that have shown a higher viral load with bDNA (Chiron Quantiplex™ HIV RNA 2.0 assay Chiron Corporation, Emeryville, California, USA) than the Amplicor HIV-1 monitor test kit (data not shown). In patients infected with subtype B virus, the correlation between the Amplicor HIV-1 monitor test kit and the bDNA method was excellent ($r = 0.904; n = 21$). In conclusion, because the minor difference in the three versions of the Amplicor HIV-1 monitor test kit is the primer set, we emphasise that for versions of the Amplicor HIV-1 monitor test because the major difference is the primer set, we emphasise that for versions of the Amplicor HIV-1 monitor test, we believe of some benefit to the next of kin.


Next of kin clinics

We read with interest the considered views of Professor Vanezis and Dr Leadbeattor regarding the role of forensic pathologists dealing with the next of kin of those people whose deaths are subject to medicolegal investigation. It might be of interest to your readers to know that the issues raised by the authors have been dealt with at the department of forensic medicine, at Westmead in Sydney for the past decade.

The department offers a specialist grief counselling service, targeting the needs of families attempting to come to terms with the complications of a coronial investigation at a time of crisis and great distress. The counsellors routinely contact the next of kin in all cases, discussing with them forensic and coronal procedures and outlining the counselling service, which includes individual sessions, group debriefing, and court support. The service operates with the full support of the New South Wales State Coroner, but is funded solely by the department of forensic medicine.

Professor Vanezis and Dr Leadbeattor propose the provision of “next of kin clinics”, conducted by the pathologist, to discuss postmortem findings. Such a service is regularly provided at Westmead as part of the counselling unit’s brief. Although thoroughly endorsing the authors’ remarks on the need for accurate and timely information, we have found that several alterations need to be made to the model broadly outlined by the authors for the families to gain maximum benefit from the information sessions. These include:

- Ongoing contact between the family and the counsellor from the time of necropsy to the receipt of its results, to ensure that the family is confident that forensic staff will be both frank and reliable in the delivery of information and support.
- A preliminary meeting between the family and the counsellor, to ensure that all the family’s concerns are identified, thereby ensuring that the meeting with the pathologist is as comprehensive as possible.
- The presence of the counsellor at the meeting as mediator and support person for the family. Such a mediation role involves ensuring that all the family’s issues are adequately considered, that the tendency some pathologists have to use jargon is kept under control, and that clarification is sought where necessary.

We have found that these alterations are necessary to ensure that the emotional needs of the family and the occasionally unavoidable power imbalance between grieving relatives and a medical specialist are properly dealt with. This latter concern can most clearly be seen in the reluctance of non-medically trained relatives to seek clarification and to admit to doubts and concerns while speaking with a representative of “the system”, however well intentioned.

We have been pleased with the success of the service over many years, and self reported feedback from clients suggests both a sense of confidence in the forensic system and in coronal findings as a result. We are delighted that our colleagues in the Northern Hemisphere are finally becoming aware of the value of such a system.

The authors reply

Drs Drayton and Eddy are to be congratulated on setting up what appears to be a splendid service for relatives, and we note with interest their comments, particularly on how we could modify our work practice in the UK.

We have always been conscious of the fact that when running such clinics we have had to tread very carefully, bearing in mind the sensitivities of relatives, as well as our medicolegal obligations to the investigating authorities. The system we have adopted is designed to provide information to the next of kin regarding the necropsy performed on their loved one, and to answer any concerns they might have regarding our findings. It is not designed to be a specialist grief counselling service. We have intentionally not attempted to take this approach because as pathologists we are not grief counsellors. Nevertheless, it would be extremely useful for all doctors to have some formal training in dealing with the bereaved. As you can appreciate, we have given some thought to the structure we have adopted, and what our role would be in such clinics in relation to our own situation in the west of Scotland, and obviously we would like to see the service that we provide extended to other areas in the UK. As we have stated in our paper, the service we offer at the present time, albeit limited when compared with the one offered in Australia, is nevertheless very welcome and we believe of some benefit to the next of kin.

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Correction

Photodynamic treatment of pooled coumarin plasma for external quality assessment of the prothrombin time.

Van den Besselaar AMHP, Moor ACE.


Because of an error in the publishing process, the wrong figure was published as fig 6 in this paper. The following figure is the correct one.

The journal apologises for this error.
Raised plasma parathyroid hormone related peptide in gastric adenocarcinoma

Georg Engelich, Niall Swan and Kevan L Hartshorn

doi: 10.1136/jcp.53.8.643-a

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