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Letters to the Editor

ANTIBODIES TO NEUTROPHIL CYTOPLASMIC ANTIGEN IN SYSTEMIC VASCULITIS

SIR,—Dr Lockwood and his colleagues (June 20, p 1389) report antibodies to a neutrophil cytoplasmic antigen (ANCA) in patients with Wegener's granulomatosis (WG) and microsopic polyarteritis (MPA). We can confirm the diagnostic value of ANCA.

To detect ANCA we used an indirect immunofluorescence assay (IFA), a modification of the technique of van der Woude et al.[1] Fresh human neutrophils are separated by dextran sedimentation and washed and resuspended in RPMI medium with 10% fetal bovine serum. Cytocentrifuge slides are incubated with test or control serum diluted 1/10 and 1/40 in phosphate buffered saline (PBS) at room temperature for half an hour. The slides are washed for half an hour in PBS and bound antibody is detected by fluorescence microscopy after incubation with fluoresceinisothiocyanate-conjugated rabbit antibody against human IgG (Dako) diluted 1/30. In the study reported here sera were reported as positive, by one of us (A. G. B.) without knowledge of the diagnosis, when most of the neutrophils showed diffuse bright granular cytoplasmic fluorescence with nuclear sparing at a dilution of 1/40.

The assay was validated (with samples from 4 patients with active WG and 40 control sera) and then offered as a laboratory service to physicians in the Northern region. A prospective study was undertaken of its role in the diagnosis of patients with suspected vasculitis, infiltrative lung disease, or nephritis.

Sera from 101 patients were assayed for ANCA. To establish the diagnoses two of us (M. C. V. and S. A.) and a colleague in South Cleveland (Dr Marion Stevens) reviewed the case notes of all the patients with suspected WG, MPA, Churg-Strauss syndrome, polyarteritis nodosa, or unspecified "vasculitis". The case notes were reviewed for all patients in whose sera ANCA was detected and for any other patient about whom doubt was expressed about the diagnosis following direct communication with the physician who had requested the assay. In all cases the physician responsible accepted the diagnosis. WG or MPA were classified as in remission when the disease was considered inactive by the physician responsible and by the reviewers (M. C. V. and S. A.), using established criteria.[2-4]

ANCA were detected in 21 of 101 patients, with the following diagnosis:

Diagnosis	*No*
WG	14
MPA	4
Vasculitis not typical of MPA	1*
Carcinoma of lung	1*
Viral enteritis	1*

*False positives.

The diagnoses in the 80 ANCA negative patients were:

Diagnosis	*No*	*Diagnosis*	*No*
WG in remission	4	Vasculitis, other	1
MPA in remission	1	Connective tissue disorders	21
MPA, active	1*	Glomerulonephritis, other	11
Polyarteritis nodosa	3	Renal diseases, other	12
Churg-Strauss syndrome	1	Lung diseases, other	5
Henoch-Schönlein purpura	1	Others	17
Temporal arteritis	2		

*False negative.

The 3 false positives were: (A) a patient with bilateral cavitating squamous carcinoma of the lung; (B) a patient with fever, enteritis, carditis, and anaemia considered to be a viral illness with a 4-fold rise in respiratory syncytial virus titres; and (C) a patient with microscopic haematuria, renal impairment, pleuritis, and hepatomegaly with deranged liver enzymes, considered to have pneumonia with disseminated intravascular coagulation. The possibility of MPA might still be entertained in patients B and C.

Subsequent to this analysis 4 more positive sera have been detected (1 active MPA, 3 active WG). Thus in the first 25 positives the specificity of ANCA for WG or MPA has been 88%. In the first 101 patients studied the sensitivity for active WG has been 100% and for active MPA 80%.

Lockwood et al used a solid phase RIA and raised the specificity from 80% to 96% by combining this with a specific inhibition assay and an IFA similar to ours. Lockwood et al state that the RIA has the advantage of speed but in our hands the IFA does not require 2 days, as claimed, but can be done on stored slides within 2 h. Fresh slides can be stored under ethanol at −20°C for at least 2 weeks without significant deterioration in cytoplasmic antigen. The IFA is thus suitable for more widespread use, although experience is required in interpretation because of antinuclear antibodies.

In experienced hands an indirect IFA for ANCA is a highly specific and sensitive diagnostic test for Wegener's granulomatosis and microscopic polyarteritis. In some cases a positive result in the absence of histological confirmation may encourage the clinician to initiate or continue with treatment directed at WG or MPA.

We thank Dr Marion Stevens for help in reviewing some of the case notes and the many physicians in the Northern region for their cooperation.

Department of Medicine,
Royal Victoria Infirmary,
and Regional Immunology Laboratory,
Newcastle General Hospital,
Newcastle upon Tyne NE1 4LP

MICHAEL C. VENNING
SHAHAB ARFEEN
ANGUS GRAHAM BIRD

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NEW TREATMENT FOR INFERTILITY DUE TO CONGENITAL ABSENCE OF VAS DEFERENS

SIR,—We report here the achievement of conception in two couples in whom the only cause of infertility was congenital absence of the vas deferens.

In the wives induction of follicular development was achieved by intramuscular leuprolide acetate 1 mg daily, human follicle stimulating hormone 150 IU/daily, and human menopausal gonadotropins 150 IU/daily, from day 1 of the menstrual cycle until vaginal ultrasonic scans and serum oestradiols indicated several preovulatory follicles. Human chorionic gonadotropin (hCG) 10 000 IU was administered intravenously and 35 h later ultrasound-guided follicular aspiration was carried out transvaginally. Oocytes were cultured at 37°C in 5% CO_2 in air in tubes containing 1 ml Menezo's B2 medium supplemented with 15% heat inactivated human fetal cord serum.

The husbands underwent scrotal exploration immediately after oocyte aspiration. Under 10–40 × magnification with an operating microscope, an incision was made with microscissors into the epididymal tunic to expose the tubules in the most distal portion of the congenitally blind-ending epididymis. Sperm were aspirated with a no 22 'Medicut' on a tuberculin syringe directly from the opening in the epididymal tubule. Great care was taken not to contaminate the specimen with blood, and careful haemostasis was achieved with microbipolar forceps. The specimens were immediately diluted in "hepes" buffered medium, and a tiny portion was examined for motility and quality of progression. If sperm motility was absent, another aspiration was made 0·5 cm more proximally. Sperm were obtained from successively more and more proximal regions until progressive motility was found. In both cases sperm were not obtained until the most proximal portion of the caput epididymis was reached. Once the area of motile sperm was found, epididymal fluid was aspirated over 10–15 min.

Sperm were washed in hepes-buffered medium and centrifuged, and the pellet was resuspended in 100 µl of medium and incubated at 37°C for 1 h. At the time of recovery, the total number of sperm was approximately 20 million, the motility was less than 5%, and the normal forms were only 20%. After washing and incubation

motility improved to 20%. Sperm remained motile in culture for 72 h after recovery from the epididymis. 10–25 µl of the sperm resuspension was added to the culture tubes containing the oocytes and incubated for 12–15 h.

In patient 1, 28 oocytes were recovered at aspiration, resulting in 15 embryos, 5 of which were transferred to the fallopian tubes and the remainder were frozen. 6 embryos were generated from 24 oocytes recovered from patient 2, 5 of which were transferred to the fallopian tubes. The tubal embryo transfers were both done 54 h after follicular aspiration.

After embryo transfer, patients were given progesterone in oil 25 mg daily intramuscularly. Both patients conceived, indicated by increasing serum levels of the β-subunit of hCG 14, 19, and 24 days after tubal embryo transfer.

The fact that the first two couples on whom this new treatment was tried have conceived suggests that we now have the means to help couples with otherwise poor prospects of having a family—namely, those where infertility is due to congenital absence of the vas. This success proves that sperm from the most proximal caput epididymis can fertilise oocytes in vitro and casts doubt on the view that sperm must pass through the epididymis to achieve maturation and capacitation in man.

St Luke's Hospital,
St Louis, Missouri USA

S. SILBER

T. ORD
C. BORRERO
J. BALMACEDA
R. ASCH

Department of Obstetrics and Gynecology,
University of California, Irvine,
Orange, California 92668, USA

BARRETT'S OESOPHAGUS

SIR,—Barrett's oesophagus is associated with oesophageal adenocarcinoma[1] and Sontag et al[2] have suggested that Barrett's oesophagus is also associated with colonic tumours. We have done colonoscopy in 25 patients with Barrett's oesophagus to assess the prevalence of colonic tumours.

Between October, 1985, and November, 1986, all patients under review at the Royal Naval Hospitals Haslar and Stonehouse were invited to undergo 'Haemoccult' stool testing, colonoscopy, and biopsy. No patient was investigated for symptoms suggestive of large bowel disease. Barrett's oesophagus was defined as 3 cm or more of circumferential gastric epithelium extending above the lower oesophageal sphincter. Biopsy specimens were taken from at least two areas of the metaplastic epithelium and categorised as either gastric or intestinal type epithelium. Most of the patients had had more than one endoscopic examination and were being followed up in a screening programme for oesophageal dysplasia and carcinoma. All patients gave written informed consent to the study, which was approved by the medical research ethics committee.

25 patients with known Barrett's oesophagus were studied (mean age 60, range 29–80). In 2 patients colonoscopy was possible only to the hepatic flexure and double-contrast barium enema was then done to visualise the right colon. No patient had colonic symptoms but this was not an exclusion to the study.

No malignant lesions were found. 3 patients had a polyp removed at colonoscopy; two of these were found to be tubular adenomas (one of the sigmoid and one of the transverse colon) and one to be a metaplastic polyp (of the rectum). 9 patients were noted to have diverticular disease.

17 patients had haemoccult testing before colonoscopy. Of the 2 patients with tubular adenomas, 1 was positive in all three samples tested and 1 was negative. The patient with the metaplastic polyp was not tested. All other patients were negative. Histology of the Barrett's epithelium revealed intestinal type epithelium in 9 and gastric type epithelium in 16. Of the patients with tubular adenomas, 1 had gastric and 1 intestinal epithelium.

During the trial, 2 patients had colonoscopy and endoscopy soon after presentation with anaemia and were found to have malignant colonic tumours in association with Barrett's oesophagus. These patients were not included in the analysis.

Adenocarcinoma of the oesophagus subsequently developed in 1 of the patients under surveillance for Barrett's oesophagus who had had colonoscopy as part of the study. No colonic tumour was found in this patient. 1 of the patients with a tubular adenoma of the colon also had a polyp of the oesophagus which was removed at endoscopy and which was an adenocarcinoma on histology. This case will be reported elsewhere.

We have found a prevalence of adenomas of the colon in our population with Barrett's oesophagus of 2/25 (8%). Other necropsy series suggest prevalences of 46·9%, 33·2%, or 34·7%.[3–5] Sontag et al described similar figures for a population with positive occult bloods, and a lower prevalence (13·2%) was found for patients who had colonoscopy for various clinical indications.[2] Even if the metaplastic polyp (not included in previous series) is included in our analysis, the incidence of benign tumours of the colon in our patients is certainly not higher than that of the general population. The number of adenomas found prospectively by Sontag et al was 16/58 (28%) which, as expected, is rather lower than the necropsy figures and the positive occult blood group, but higher than the prevalence in our patients.

No malignant tumours were found in our population. The prevalence of malignant tumours in Sontag's series was 5%, although more cases were identified, either as "index" cases or as patients who had had previous tumours. This prevalence was compared with a conservative guess at the prevalence based on incidence figures, and was significantly increased. There may obviously be inaccuracies in this calculation since the true prevalence in an age/sex-matched population is unknown.

If there were a significant increase in the prevalence of benign or malignant tumours in Barrett's oesophagus, we would have expected to have found more than the 8% adenomas and 0% carcinomas in our series. A small increase in colonic tumours in our population may have been missed by chance. The question must also be asked whether it makes biological sense that colonic tumours should be associated with Barrett's oesophagus? The answer must be "no" if Barrett's oesophagus is indeed a lesion caused by long-standing oesophageal reflux.[6]

The purpose of these studies is to detect a population that is at high risk of developing treatable lesions in the colon such that screening would be worthwhile. We are confident that the risk, if present, is not great enough for screening our population with Barrett's oesophagus. In addition the findings that only one of the two adenomas would have been picked up by haemoccult screening and that no false positives occurred in patients with a normal colon but with inflammation in the oesophagus are of interest.

We found no significant increase in the prevalence of colonic tumours in our patients with Barrett's oesophagus and colonic investigation is not being done as part of our routine screening of these patients.

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Department of Gastroenterology,
Royal Naval Hospital,
Gosport, Hants PO12 2AA

J. K. RAMAGE
J. HALL
J. G. WILLIAMS

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NEBULISED BUDESONIDE IN SEVERE INFANTILE ASTHMA

SIR,—Severe asthma requiring maintenance corticosteriods in children can usually be managed by use of a metered dose inhaler. In young children who are unable to master these inhalers oral corticosteroids have been used, the drug being given every other morning. However, the control of symptoms on the non-steroid day is far from perfect. There has also been interest in the use of a