References

- ¹ Thomas GDH, Dixon MF, Smeeton NC, Williams NS. Observer variation in the histological grading of rectal carcinoma. *J Clin Pathol* 1983;**36**:385-91.
- ² Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. *Psychol Bull* 1968;**70**:213-20.

Dr Stenkivist replies as follows:

In relation to Dr Paul Silcocks comments on observer variation I wish to make the following comment:

The breast cancer classification systems we analysed not only have a poor reproducibility but they also lack clinical value in that they do not take into account rate of recurrence. However, parameters whose measurement is easily reproducible, such as "tumour markers" (CEA etc), DNA distribution among tumour cell nuclei, measures of tumour size, etc, although clearly correlated with prognosis when you compare groups of patients, are of little or no value for prognosis in the individual patient unless they are combined in a reliable way into a malignancy index that can be obtained by step-wise logistic regression analysis. We have recently addressed this problem,¹ and we think it is time that pathologists and clinicians start to look for optimal combinations of parameters to create "malignancy indices" for tumour diseases in order to have practicable methods in daily clinical work.

Breast cancer patients deserve the greatest possible assurance in estimates of the severity of their disease and in the selection of their treatment, just as the clinician should receive the most complete information possible about his patient's condition. Our study indicates that it will be possible to give the clinician such complete information (superior to so-called clinical staging), provided that the significant variables are recorded in a meticulous way and combined into a risk curve. Patients with low malignancy grade as demonstrated in our study could then be confidently reassured that they will not suffer from recurrent disease, and resources for adjuvant therapy and follow-up can be saved for those patients who really need them (ie patients with a high malignancy grade).

Our study further emphasises that there is no evidence that THE factor, a single entity of absolute prognosis, exists or is likely to exist.

We regret our mistake in proof-reading the standard error of kappa of Dr Jacob Cohen's formula. The computation, how-

ever, was performed using the correct formula.

BJÖRN STENKIVIST

Department of Clinical Cytology,
University Hospital,
S-751 85 Uppsala, Sweden

Reference

- ¹ Stenkivist B, Bengtsson E, Dahlqvist B, et al. Predicting breast cancer recurrence. *Cancer* 1982;**50**:2884-93.

Antinuclear antibody-negative systemic lupus erythematosus—how common?

We read with interest the paper in the October 1982 issue of the Journal.¹ We feel that two factors require further consideration before accepting that 8.9% of patients with SLE may be ANA negative. Firstly, the authors did not test their ANA-negative sera to see whether they would react with human substrates. It is well recognised that a small number of such sera will react only with human tissues such as peripheral granulocytes.

Secondly, our own experience over several years has led us to become increasingly concerned about results obtained with radioimmunoassay kits supplied by RC Amersham. We have found positive anti-DNA results at levels of 30 U/ml or greater, not uncommonly, in sera of patients who have no reliable clinical evidence of SLE as determined by the American Rheumatism Association criteria. Reasons for this may be several and include DNA reactions with basic proteins, binding of C1q to DNA or to low density lipoproteins as well as the possibility that some preparations may contain some single stranded DNA. Binding to basic proteins is not uncommon in patients with malignant disease and may be reversed with sodium dodecyl sulphate. Resulting from our dissatisfaction with RIA kits we compared RIA results with those obtained by immunofluorescence using either *Crithidia luciliae* or human metaphase chromosomes as substrate. Our findings indicate that both immunofluorescent procedures gave a far better correlation with clinical manifestations of SLE and we have now standardised our test procedures on *C luciliae* as substrate.

KC WATSON

EJC KERR

Central Microbiological Laboratories,
Western General Hospital,
Crewe Road, Edinburgh EH4 2XU

Reference

- ¹ McHardy K, Horne CHW, Rennie J. Antinuclear antibody-negative systemic lupus erythematosus—how common? *J Clin Pathol* 1983;**35**:1118-21.

Professor Horne and Dr McHardy reply as follows:

Although we did not screen the ANA negative sera against human tissues we agree with Watson and Kerr that a small number of sera will react with such tissues. However, we must point out that we never claimed that the radioimmunoassay kits supplied by RC Amersham did not give false positive results. They most certainly do. It was for this reason that we only studied sera where a value of 40 U/ml or greater had been obtained. The main point of our argument in this paper is simply that if clinicians rely solely on the conventional immunofluorescence screening procedure for antibodies to nuclear constituents they are likely to be misled if they hold the traditional view that ANA are present in over 98% of SLE cases.

Finally, we accept that an immunofluorescence screening test based on *Crithidia luciliae* is of diagnostic value and we are currently comparing it with the RIA kits.

CHW HORNE

K McHARDY

Department of Pathology
University Medical Buildings
Forresterhill, Aberdeen AB9 2ZU

IgA pyroglobulinaemia in lymphoma

Pyroglobulins are abnormal immunoglobulins, usually IgG or IgM, which when heated to 56°C form a gel irreversible by changes of temperature, pH or dilution. They were first recognised in 1953,² and since then have been reported in a variety of conditions, mainly multiple myeloma, Waldenström's macroglobulinaemia,⁴ and lymphoma.⁵ We would like to report a patient with an unusual pyroglobulin. The patient had abnormal bleeding associated with defective ristocetin-induced platelet aggregation. This appeared to be related to the presence of the abnormal globulin. When the patient's serum was fractionated to obtain the abnormal pyroglobulin and when this fraction was added to normal plasma it produced a similar defect in platelet aggregation.

Case report

The patient was a 72-year-old woman. Six weeks prior to admission, she complained of arthralgia, involving both ankles, wrists and knees. She was admitted after the development of a purpuric rash.

On examination, she had a marked purpuric rash over both legs. There was no lymphadenopathy and no hepatomegaly but her spleen was palpable 4-5 cm below

Letters to the Editor

the costal margin. Investigations showed Hb 7.9 g/dl; MCV 94 fl; leucocytes $10.3 \times 10^9/l$; platelet count $356 \times 10^9/l$; ESR (Westergren) 150 mm. Her urea and electrolytes were normal; bilirubin 7 mmol/l (NR 2–20); AST 43 U/l (NR 2–35); alkaline phosphatase 371 U/l (NR 60–170); GGT 78 U/l (NR 5–60); prothrombin time 16.5 s (control 14.5); PTTK 42 s (control 41); thrombin time 13 s (control 14); fibrinogen 280 mg/dl (NR 200–400); FDP 20 $\mu\text{g/ml}$ (NR 0–10 $\mu\text{g/ml}$); factor V, VIII, X, XI and XII were within normal range. Platelet aggregation was abnormal with absent second phase aggregation with adrenalin and decreased aggregation with ristocetin (Fig. 2(1)). However, aggregation with collagen, thrombin, and ADP or concentrations of 0.2 $\mu\text{mol/l}$, 0.4 $\mu\text{mol/l}$, and 1.0 $\mu\text{mol/l}$ was normal.

A bone marrow aspirate and trephine biopsy was normal on two occasions. Her plasma viscosity was 2.69 (NR 1.51–1.71), total protein was 92 g/l and the globulin 61 g/l. Serum electrophoresis showed an IgA κ paraprotein which had a considerable tendency to form polymers producing multiple bands and precipitated irreversibly at 56°C. IgG was 14.33 g/l (NR 6.5–16.0); IgA 36.20 g/l (NR 1.0–4.5); IgM 0.41 g/l (NR 0.45–2.0). No Bence-Jones protein was detected and urinary electrophoresis was normal.

Her condition continued to deteriorate and splenectomy was undertaken on diagnostic and therapeutic grounds. Prior to splenectomy, her serum viscosity rose to 6.07 N mPa s and plasma exchange was performed on three occasions preoperatively. There was no purpura at this time.

At operation, the spleen was found to be adherent to the diaphragm. Microscopic examination of the 275 g spleen revealed a diffuse lymphoma with plasmacytoid differentiation.

There was a spectrum of tumour cells ranging from mature plasma cells to plasmablasts with centrally located nuclei (Fig. 1) and occasional binucleated cells. The neoplastic proliferation markedly increased the cellularity of the pulp, and the normal architecture of the spleen was largely obliterated. An immunoperoxidase preparation demonstrated in the tumour cells, a monoclonal IgA immunoglobulin with κ light chains identical to the serum paraprotein.

Postoperatively, she had a pulmonary embolus, but nevertheless made a good recovery. Later the patient developed generalised lymphadenopathy, her purpuric rash recurred and her viscosity rose

Fig. 1 Section of spleen showing spectrum of tumour cells ranging from mature plasma cells to plasmablasts. Haematoxylin and eosin $\times 700$.

to 3.63 N mPa s, and treatment was started with intermittent chlorambucil and prednisone. Since then her lymph nodes are no longer palpable. She has had no further episodes of purpura, and her platelet aggregation with ristocetin has returned to normal (Fig. 2(2)) although her aggregation with adrenalin remains abnormal. Her IgA paraprotein level peak has decreased to 3.46 and she is presently well and off cytotoxic therapy.

Material and methods

Platelet aggregation was induced in a Born Aggregometer,⁶ using adenosine-5'-diphosphate (Dade) at concentration of 0.2×10^{-4} M, collagen (Dade) at concentration of 0.2 mg/ml, epinephrine (Dade) at concentration of 0.1×10^{-4} M and ristocetin (Helena Labs) at concentration of 1.5 mg/ml.

Immunoglobulins were separated using the following method. The serum was brought to 33% saturation with respect to $(\text{NH}_4)_2\text{SO}_4$, by the addition of saturated solution of this salt at room temperature. The suspension was centrifuged and the pellet was dissolved in 0.002 M sodium phosphate buffer, pH 7.2 containing 0.15 M NaCl. This was repeated on two occasions. The combined supernatants were then brought to 50% saturation. The insoluble protein was collected by centrifugation and the pellet was dissolved in phosphate-buffered saline (PBS). Both fractions were dialysed against PBS at 4°C.

A portion of the 0–33% $(\text{NH}_4)_2\text{SO}_4$ sample was fractionated on a column of Sephacryl S300. The fractions containing IgA, determined by single radial

Fig. 2 Ristocetin-induced aggregation in (1) patient at presentation; (2) after treatment; (3) normal platelets in normal plasma; (4) normal platelets incubated with 150 μl of the patient's plasma.

immunodiffusion were pooled, concentrated by precipitation with $(\text{NH}_4)_2\text{SO}_4$ and dialysed.

The aggregation studies on the patient and the normal control were performed using 0.38 ml of citrated platelet rich plasma. The serum extract studies were performed at concentrations of 0.05 ml, 0.10 ml and 0.15 ml and added to 0.38 mls of platelet rich plasma. An aliquot of 0.38 ml of this was used.

Results

The platelet aggregation studies during the acute stages of the patient's illness showed normal aggregation with ADP and collagen. The second phase of epinephrine induced aggregation was markedly reduced and ristocetin induced aggregation depressed (Fig. 2(1)). The defect of ristocetin induced aggregation corrected after treatment whereas the defect in epinephrine induced aggregation remained. In the studies in which the patient's IgA extract was added to a normal control ADP,

epinephrine and collagen induced aggregation remained unaffected. However, ristocetin induced aggregation was reduced to a level comparable to the patient's pre-treatment findings at concentrations of 0.15 ml but not at concentrations of 0.05 ml and 0.01 ml (Fig. 2(4)).

Discussion

Only three patients have been reported with an IgA pyroglobulin.⁷⁻⁹ In contrast to our patient all had multiple myeloma, two of whom had the hyperviscosity syndrome. This is the first report of an IgA pyroglobulin in association with a lymphoma. An interesting feature of our patient was her purpura. As her coagulation screen, platelet count and factor assays were normal, it was felt this may have been due to abnormal platelet aggregation. The patient had absent second phase aggregation with adrenalin and markedly decreased aggregation with ristocetin. The abnormality of platelet aggregation induced by adrenalin may occur in normal individuals,¹⁰ and the fact that it persisted after treatment suggests that it is probably a normal variant. In contrast, the ristocetin induced aggregation was probably directly related to her illness as it was reversed with treatment. We propose that it was related to the presence of the IgA pyroglobulin. This is suggested by the studies in which her IgA fraction was isolated and added to normal platelets, producing impaired ristocetin induced aggregation.

The bleeding tendency associated with paraproteinaemia is complex. Abnormalities of the coagulation,¹¹ or fibrinolytic¹² systems may be involved but defects of platelet function,¹³⁻¹⁵ and hyperviscosity,⁸ may be more important. In particular, defects of ristocetin induced aggregation may play a significant role by coating the surface of both platelets and connective tissue

and thereby blocking their interactions.¹² Similar defects of ristocetin induced aggregation occur in rural Nigerians where it is postulated their increased IgM and other macroglobulin production caused by parasitaemia may be important.¹⁶ In addition, macromolecules such as dextran are known to inhibit ristocetin induced platelet aggregation.¹⁷ The role of hyperviscosity in this patient's bleeding disorder is difficult to assess. Although the serum viscosity at times rose to very high levels (6.06 N mPa s), bleeding did not occur. Instead, this seemed to be related to the activity of her illness, and her pyroglobulin levels. We therefore suggest that her circulating IgA κ pyroglobulin blocked the platelet vessel wall interactions in a manner similar to lack of von Willebrand factor, perhaps by coating one or both surfaces and thereby producing defective ristocetin induced platelet aggregation.

We wish to thank Dr Brian Wisdom and Messrs Michael Rocks, Fred Lloyd and John Orchin for technical assistance and Miss Diane Barker for typing the manuscript.

MICHAEL MADDEN

T CARSON M MORRIS

DOROTHY HAYES

CAROLINE A HUMPHREY

JEFFREY H ROBERTSON

Departments of Haematology and Histopathology, Belfast City Hospital, Belfast, Northern Ireland

References

- ¹ Zinneman HH. Cryoglobulins and pyroglobulins. *Pathobiol Annu* 1980;**10**:83-104.
- ² Martin WJ, Mathieson DR. Pyroglobulinaemia (heat coagulable globulin in the blood). *Proc Staff Meet Mayo Clinic* 1953;**34**:95-101.
- ³ Fisher B, Schaer LR, Messinger S. The occurrence of pyroglobulins in unsuspected myeloma. *Am J Clin Pathol* 1963;**40**:291-4.
- ⁴ Stefanini M, McDonnell EE, Andracki EG,

et al. Macropyroglobulinaemia: immunochemical studies in 3 cases. *Am J Clin Pathol* 1980;**54**:94-101.

⁵ Krauss S, Sokal JE. Paraproteinaemia in the Lymphoma. *Am J Med* 1966;**40**:400-135.

⁶ Born GVR. Aggregations of blood platelets by adenosine diphosphate and its reversal. *Nature* 1962;**194**:927-9.

⁷ Invernizzi F, Cattaneo R, Rossi DI, San Secondo V, et al. Pyroglobulinaemia. *Acta Haematol* 1973;**50**:65-74.

⁸ Sugai S. IgA Pyroglobulin, hyperviscosity syndrome and coagulation abnormality in a patient with multiple myeloma. *Blood* 1972;**39**:224-37.

⁹ Watanabe T, Oshima T, Minamizawa S, et al. Pyroglobulin developing in a case of IgA lambda type multiple myeloma presenting as plasmacytoma of the mandible. *Rinske Ketsueki* 1980;**7**:984-90.

¹⁰ ten Cate JW, Jenkins CSP. Blood coagulation and haemostasis. In: Thompson J, ed. London: Churchill Livingstone, 1980:33.

¹¹ Castaldi PA, Penny R. A macroglobulin with inhibitory activity against coagulation factor VIII. *Blood* 1970;**35**:370-6.

¹² Hardisty RM, Caen JP. Haemostasis and thrombosis. In: Bloom AL, Thomas DA, eds. London: Churchill Livingstone, 1980:301-20.

¹³ Perkins HA, McKenzie MR, Fudenberg HN. Hemostatic defects in dysproteinaemias. *Blood* 1970;**35**:695-707.

¹⁴ Vigliano EM, Horowitz HI. Bleeding syndrome in a patient with IgA myeloma: interactions of protein and connective tissue. *Blood* 1967;**29**:823-36.

¹⁵ Cohen I, Amir J, Ben-Shaul Y, et al. Plasma cell myeloma associated with an unusual myeloma protein causing impairment of fibrin aggregation and platelet function in a patient with multiple myeloma. *Am J Med* 1970;**48**:766-76.

¹⁶ Dupuy E, Fleming AF, Caen JP. Platelet functions, factor VIII, fibrinogen and fibrinolysis in Nigerians and Europeans in relation to atheroma and thrombosis. *J Clin Pathol* 1978;**31**:1094-101.

¹⁷ Aberg M, Hedner U, Holmberg L, et al. Dextran induced changes of the factor VIII molecule (Abstract). *Thrombosis et Diathesis Haemorrhagica* 1975;**34**:903.

Notices

The British Digestive Foundation (Scottish Appeal)

The British Digestive Foundation (Scottish Appeal) exists to promote research into the alimentary tract and its diseases. The Awards Advisory Group of the Scottish Appeal invites applications from workers in Scottish Institutions who wish support for research work related to any aspects of normal or disordered structure and function of the alimentary tract. A wide variety

of forms of support will be considered ranging from that required for apparatus or reagents to Fellowships. There is no requirement that applicants be medically qualified.

The Awards Advisory Group meets annually to consider applications and these should be submitted before 15th September, 1983.

An application form can be obtained from: The Secretary, The British Digestive Foundation (Scottish Appeal), 9 Queen Street, Edinburgh, EH2 1JQ.

Plasma proteins in clinical diagnosis: European meeting

The Fourth European meeting on Plasma Proteins in Clinical Diagnosis will be held on 5-7 October, 1983 at the Centro Congressi Milanofiori, Milan, Italy.

For information, please write to: The Scientific Secretary, Dott Santica Mancovina, Laboratori di Ricerca-Istituto Scientifico S Raffaele, Via Olgettina, 68-20132 Milan, Italy.