A quantitative study of eosinophil polymorphonuclear leucocytes in granulocytic sarcoma (chloroma)

Granulocytic sarcoma or chloroma may precede the haematological or clinical diagnosis of myeloid leukaemia. It is often difficult to diagnose histologically as the tumour cells are frequently undistinctive. Traditionally, the presence of eosinophil polymorphonuclear leucocytes has been a useful diagnostic pointer,^{1,2} but Whitcomb *et al* recently described the case of a tumour initially diagnosed as granulocytic sarcoma on the basis of its high eosinophil content which subsequently proved to be a T cell non-Hodgkin's lymphoma.³ The polymorphonuclear leucocyte cell content and frequent eosinophils seen in T cell non-Hodgkin's lymphoma may be a ready source of diagnostic confusion. To obtain objective data on numbers of eosinophilic polymorphonuclear leucocytes in granulocytic sarcoma we examined 10 cases of granulocytic sarcoma which had previously been fully characterised clinicopathologically and by means of granulocyte markers.⁴

Routinely processed sections 3 µm thick which had been fixed in formalin and embedded in paraffin wax were used; these were stained by the vital new red (chlorazol fast pink BK) method.⁵ This is highly selective for eosinophilic polymorphonuclear leucocytes, and where present their granules stained an intense red on a pale blue—mauve background. Counting of eosinophilic polymorphonuclear leucocytes was performed independently by the authors using an eyepiece graticule; 100 consecutive high power fields were studied at ×400 magnification. The degree of differentiation of the specimens was also assessed by virtue of their content of recognisable granulocytic cells on haematoxylin and eosin or Giemsa staining.

The numbers of eosinophilic polymorphonuclear leucocytes varied considerably from specimen to specimen, ranging from 0 per 100 high power fields to 247 per 100 high power fields (table). This was in the face of high interobserver consistency. The results show that even in a small series of cases of granulocytic sarcoma there is considerable variation in the number of eosinophilic polymorphonuclear leucocytes. It is of interest that of the two cases where there was more than one biopsy specimen, one showed great variation between the three specimens taken, whereas counts for the specimens taken in the other case were closely correlated.

In a study of 61 cases of granulocytic sar-

Table Numbers of eosinophilic polymorphonuclear leucocytes in the specimens of granulocytic sarcoma

Case	Sex	Age	Site	No of eosinophilic polymorphonuclear leucocytes/100 high power fields (counted by SM)	No of eosinophilic polymorphonuclear leucocytes/100 high power fields (counted by JC)
1	F	37	Postnasal space	4	6
			nasopharynx/tonsil	5	6
3	M	56	Breast	16	11
4	F	26	Retroperitoneum	232	251
			Inferior turbinate	37	43
			Nose/antrum	53	41
			Orbit/ethmoid	1	0
5	F	44	Uterine cervix	16	10
6	M	75	Lymph node	8	5
7	F	39	Tonsils	37	30

coma Neimann *et al* found that 49% were blastic with no evidence of eosinophilic differentiation, the remainder being "well" or "poorly" differentiated with "numerous" or "occasional" eosinophilic polymorphonuclear leucocytes, respectively.² Our results agree with his findings in that about half of our cases were devoid or virtually devoid of eosinophilic polymorphonuclear leucocytes, however, unlike him we did not find any correlation between the numbers of eosinophilic polymorphonuclear leucocytes and the degree of differentiation of the neoplasms. It has been considered that eosinophilic differentiation is the single most useful histological feature of granulocytic sarcoma; it is uncertain whether the eosinophilic polymorphonuclear leucocytes seen in granulocytic sarcoma are an intrinsic part of the neoplasms or have moved into the tumour from the blood stream. Our results, together with the case of T cell non-Hodgkin's lymphoma misdiagnosed as granulocytic sarcoma,³ suggest that distinguishing T cell non-Hodgkin's lymphoma, which may often contain many eosinophilic polymorphonuclear leucocytes,^{6,7} from granulocytic sarcoma may be a recurrent problem. We believe that contrary to previous reports the presence of many eosinophilic polymorphonuclear leucocytes is not a reliable guide to the diagnosis of granulocytic sarcoma.

SALLI MULLER

J CROCKER

P J SMITH

Department of Histopathology,
East Birmingham Hospital,
Bordesley Green East,
Birmingham B9 5ST

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Acute anaemia and aplastic crisis without haemolysis in human parvovirus infection

Human parvovirus infection (HPV) causes erythema infectiosum¹ and aplastic crisis in chronic haemolytic anaemia.² We report a case of aplastic crisis in an HPV infection without underlying haemolytic anaemia.

Case report

A child aged 12 years was brought to hospital with a six day history of abdominal pain, vomiting, and headache. On admission he had a fever of 40°C, pallor, cervical lymph nodes, and absence of erythema and splenomegaly but was not very ill. Blood count was as follows: haemoglobin concentration 4.7 g/dl, reticulocyte count less than $5 \times 10^9/l$, platelet count $30 \times 10^9/l$, white cell count $2.6 \times 10^9/l$. His serum bilirubin concentration was normal (8 µmol/l), and a direct Coombs' test was negative. Bone mar-

row showed erythroblastopenia (less than 1% of erythroblasts). The presence of serum anti-HPV IgM (radioimmunoassay) suggested a recent HPV infection. Packed red cells (600 ml) were transfused. Over the next few days the symptoms disappeared and haemoglobin concentrations remained stable. Ten days after admission reticulocyte count was $150 \times 10^9/l$. Eighteen months later, the patient was quite well and all haematological investigations yielded normal results blood count, haemoglobin electrophoresis, erythrocytic enzymes (glucose-6-phosphate-dehydrogenase, glucose-phosphogluconate-dehydrogenase, hexokinase, glucose-isomerase-phosphate, glucose-pyruvate, glutathione reductase, acetyl-cholinesterase, pyrimidine-5'-nucleotidase), osmotic resistance and autohaemolysis.

Our observation of HPV infection associated with aplastic crisis but without haemolysis differs from the transient erythroblastopenia seen in childhood, which often affects younger children (1 to 4 years) and occurs without HPV infection.³

Aplastic crisis associated with HPV infection has hitherto only been described in hereditary^{2,4-6} or acquired⁷ haemolytic anaemias. It seems that the erythroblastopenic effect of HPV is constant but goes unnoticed if the red cell life span is normal. A shortened red cell survival (haemolysis) is necessary to cause acute anaemia. Acute anaemia occurring without haemolysis due to an HPV infection is difficult to explain. In our patient only isotopic labelling of his erythrocytes could have completely excluded underlying haemolysis. Nonetheless, we wanted to record the experience to encourage doctors to ask for parvovirus serology in similar clinical circumstances.

M GUILLLOT
JJ LEFRERE
N RAVENET
E LEVEQUE
R GIROT

Service de Pédiatrie—Centre
Hospitalier de Lisieux
14107 Lisieux,
Laboratoire d'Hématologie—Hôpital
des Enfants Malades
75015 Paris, France.

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Long term freeze storage of *Campylobacter pyloridis*

The letter by Westblom *et al*¹ prompted us to review our technique for storing cultures of *Campylobacter pyloridis*.

For the past year we have been isolating *C. pyloridis* from gastric biopsy specimens by inoculating the tissue on to chocolate blood agar containing 3 µg/ml amphotericin B and 10 µg/ml vancomycin. Plates were incubated in an anaerobic jar containing 90% nitrogen and 10% carbon dioxide for seven days at 37°C. The identity of the organisms was confirmed by colonial and Gram morphology and their ability to split urea very rapidly. Initially such organisms were harvested in to tryptone soy broth containing 15% glycerol and stored in a deep freeze at -70°C. Following Westblom *et al*'s letter we retrieved some of these cultures, thawed them, and inoculated them on to chocolate agar plates as described above. Three cultures frozen seven and a half, seven and a half, and five and a half months previously yielded profuse growths and one frozen 10½ months previously still contained viable organisms although in small numbers. More recently cultures have been stored on "beads in cryopreservative fluid", supplied with the Protect Bacterial Preserver system (Technical Service Consultants Ltd PO Box 31, Bury BL9 5RA). A profuse growth was obtained from one of these which had been frozen three months previously.

Large numbers of strains will need to be stored for longer periods to confirm our observations, but in contrast to the experi-

ence of Westblom *et al*, we have not found freeze storage of *C. pyloridis* in conventional media to be a problem.

CD RIBEIRO
SJ GRAY

Public Health Laboratory,
University Hospital of Wales,
Heath Park, Cardiff CF4 4XW

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Comparative sensitivities to antimicrobial agents of *Campylobacter pylori* and the gastric campylobacter like organism from the ferret

Increasing evidence supports the association of *Campylobacter pylori* with antral gastritis and peptic disease, notably duodenal ulceration, in man.¹ Results of antimicrobial sensitivity tests on 110 isolates of *C. pylori* from Australia, the United Kingdom, and France have shown good agreement,²⁻⁴ and limited clinical trials have shown that treatment with certain antibacterial drugs clears *C. pylori* from the gastric mucosa.

The isolation of campylobacter like organisms from the gastric mucosa of ferrets was first reported from Boston, USA^{5,6}; this organism, with morphological similarities to *C. pylori*, was isolated from about half of the animals examined. Histological studies suggested a possible association between the presence of the campylobacter and gastric inflammation.

In contrast, Rathbone *et al*⁷ isolated a campylobacter like organism from the gastric tissue of all of the 17 ferrets that they examined, but the organism was associated with neither histological inflammation nor ulceration.

We have compared the sensitivities to antimicrobial and antiulcer drugs of gastric campylobacter like organisms (GCLO) isolated from 14 ferrets with those of 11 isolates of *C. pylori*. Comparative studies, including enzyme, protein, and isoprenoid quinone composition, will be reported later.

Samples of gastric mucosa from the antrum, body, and fundus of 14 mature male ferrets obtained from one supplier were taken when the animals were killed after emesis protection experiments. On macroscopical examination one of the 14 ferrets