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Report on Slide Session, British Society for Haematology, 44th Annual Scientific Meeting, Cardiff, 2004

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Each year at the Annual Scientific Meeting of the British Society for Haematology Annual Scientific Meeting, there is a morphology session in which two experts discuss the diagnosis, on the basis of a blood film or bone marrow trephine biopsy section provided from six patients. The same slides are provided in advance to other participants in the meeting. The experts receive no information other than that provided to all participants. After a provisional or definitive diagnosis has been made, the case contributors present further details and discuss the final diagnosis. The slide session presented here is in the same order as at the meeting so that readers can arrive at their own conclusions about the diagnosis.

Case 1

From Dr Sahra Ali Hull Royal Infirmary

A blood film of a 85-year-old man who presented with occasional tiredness and mild anaemia was provided. He was found to have a palpable spleen and raised bilirubin (50 μmol/l) and lactate dehydrogenase (LDH) (588 U/l). On ultrasound examination, the spleen measured 15 cm and the gallbladder was full of calculi. His FBC was: WBC 5.4×10^9/l, Hb 9.9 g/dl, MCV 96 fl, platelet count 86×10^9/l.

The first discussant (V.C.) noted the presence of spherocytes, polychromatic cells and unusual pincer- or mushroom-shaped poikilocytes (Figure 1). She thought that the unusual poikilocytes were not 'bite cells' from which a Heinz body had been removed but were likely to represent the poikilocytes that have been described in the variant of hereditary spherocytosis associated with a band 3 deficiency. This was first described by Dacie *et al.* (1953) with the molecular defect subsequently being elucidated (Palek & Jarolim, 1993). The second discussant (A.Th.) agreed entirely. In addition, she noted some hypersegmented neutrophil nuclei (suggesting a possible folic acid deficiency) and occasional Howell–Jolly bodies (compatible with active haemolysis).

Case 2

From Dr Vivienne Andrews Medway Maritime Hospital

A blood film was provided from a 67-year old man who presented with anorexia and fatigue and was found to have hypercalcaemia and renal failure. His FBC was: WBC 13.3×10^9/l, Hb 9.8 g/dl, platelet count 73×10^9 l, neutrophil count 7.86×10^9/l and MCV 92.5 fl.

The first discussant (V.C.) noted that the blood film was leucoerythroblastic and there was a population of medium-sized to large abnormal, pleomorphic cells. Many of these had strongly basophilic cytoplasm and some had very lobulated nuclei (Figure 2). In some, the chromatin pattern was quite primitive. Some had cytoplasmic blebs. She thought that they might be either very abnormal plasma cells or megakaryoblasts and that an immunophenotype was essential for diagnosis. The second discussant (A.Th.) agreed and, in addition, commented on marked rouleaux formation. She thought that the cells

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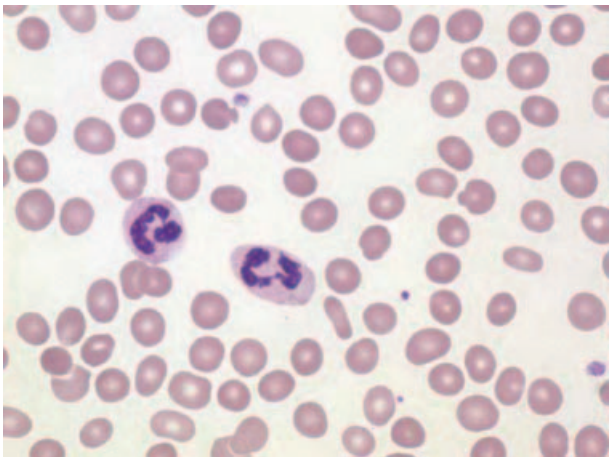

Figure 1. Peripheral blood film of case 1.

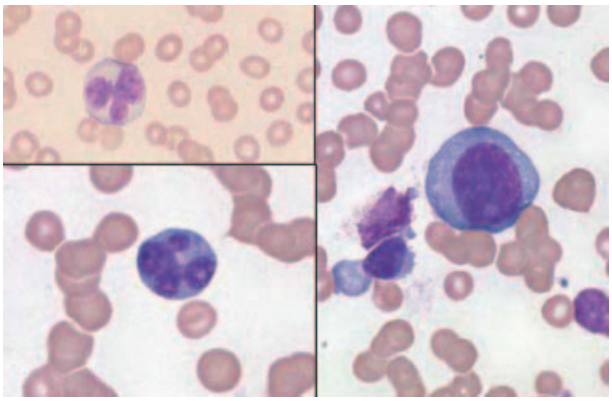

Figure 2. Three fields from peripheral blood film of case 2.

were plasmablasts and that the diagnosis was plasma cell leukaemia.

Case 3

From Dr Mark Layton Hammersmith Hospital

A blood film was provided from a 9-year-old girl, who had presented with a mild bruising tendency and thrombocytopenia at the age of 2 years, when she had also been noted to show speech and language delay. Several relatives have developed leukaemia. FBC was: WBC 3.8×10^9/l, Hb 11.5 g/dl, platelet count 52×10^9/l.

The first discussant (A.Th.) noted that the film confirmed the thrombocytopenia and showed the platelets to be small (Figure 3). Otherwise, apart from some hypersegmented eosinophils, it was unremarkable. The only condition the discussant was aware of that was characterized by small platelets was the Wiskott-Aldrich syndrome but that did not seem to fit with the features of the present patient. She suspected an autosomal dominant syndrome first described by Dowton *et al.* (1985); the kindred of 22 persons had shown mild to moderate thrombocytopenia with six individuals developing leukaemia/lymphoma. Developmental delay had not been noted. Subsequently, other kindreds were described and the syndrome was linked to a locus at 21q22 and was designated 'familial platelet disorder with a predisposition to acute myelogenous leukaemia (FPD/AML)'. The second discussant (V.C.) had also noted small platelets and thought that eosinophils were prominent.

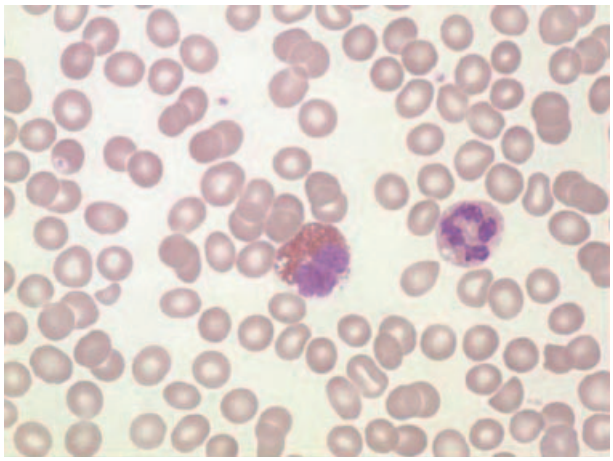

Figure 3. Peripheral blood film of case 3.

Case 4

From Dr Chi Wong and Dr David Webb Hospital for Sick Children Great Ormond Street, London

A blood film was provided from a 3-year-old Caucasian girl with a 3-week history of fever and malaise; she had cervical lymphadenopathy and hepatosplenomegaly. Her WBC count rose from 21 to 257×10^9/l over 8 days. FBC: WBC 257×10^9/l, Hb 6.8 g/dl, platelet count 110×10^9/l.

The first discussant (A.Th.) noted that the blood film was leucoerythroblastic with a population of highly abnormal cells (Figure 4). Some of these appeared blastic, often very large and sometimes with very basophilic vacuolated cytoplasm. Others were small or medium sized and appeared to be lymphoid. She thought the findings were not typical of leukaemia or of a myeloproliferative disorder and wondered about Burkitt's lymphoma and a leukaemoid reaction. The second discussant (V.C.) made similar observations and suspected Burkitt's lymphoma or a transformation of a myeloproliferative disorder. The organiser (B.B.) asked whether the basophilic vacuolated cells might not be too large to be Burkitt's lymphoma cells

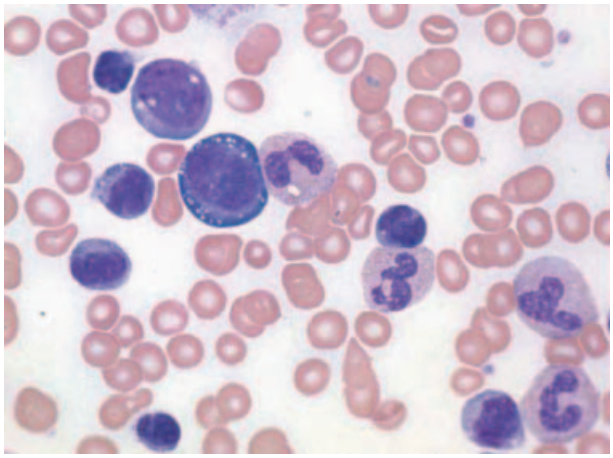

Figure 4. Peripheral blood film of case 4.

and the discussants agreed that that might well be so. A member of the audience raised the possibility of a non-haematological malignancy, specifically – in view of the cytoplasmic vacuolation – rhabdomyosarcoma. A.Th. commented that the vacuoles were too small for those of rhabdomyosarcoma cells and did not show the characteristic coalescence into 'lakes'. Neither was there any phagocytosis by the malignant cells as may be seen in this tumour.

Case 5

From Dr Adel Tawil Royal Berkshire Hospital, Reading, UK

A blood film was provided from a 57-year-old man who presented with tiredness, weight loss, subconjunctival haemorrhage and melaena. His FBC was: WBC 58×10^9/l, Hb 3.6 g/dl, platelet count 21×10^9/l.

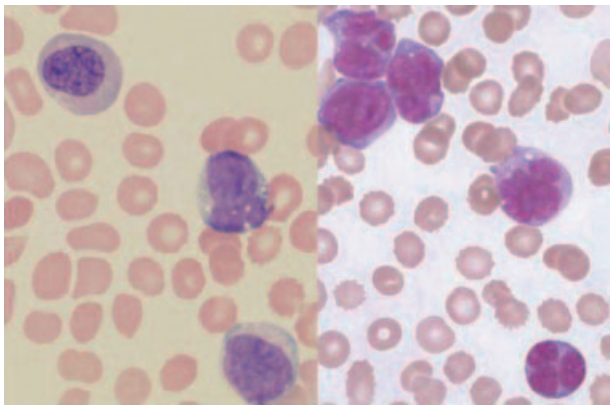

Figure 5. Two fields from peripheral blood film of case 5.

The first discussant (V.C.) demonstrated anisocytosis, poikilocytosis, red cell fragments, basophilic stippling and crenation. There were circulating megaloblasts and other dysplastic erythroblasts (Figure 5). There were blast cells, some of which were lobulated. The platelets count was reduced with there being some bizarre forms. She thought there was evidence of trilineage dysplasia and although she wondered about the variant form of promyelocytic leukaemia, on account of the lobulated nuclei, she favoured a diagnosis of M6 AML. She predicted that there would be an abnormal complex karyotype. The second discussant (A.Th.) demonstrated, in addition, polychromatic macrocytes and occasional hypochromic cells. She found 91% of cells to be blast cells; some blast cells had inconspicuous azurophilic granules. Because of the striking abnormalities in the erythroid lineage she also favoured a diagnosis of M6 AML.

Case 6

From Dr Firiad Hiwaizi Warrington Hospital

A trephine biopsy section was provided from an 87-year-old man who presented with bruising for 1 month. On examination, had been purpura but no lymphadenopathy, hepatomegaly or splenomegaly. He was found to have a mild anaemia and severe thrombocytopenia that responded to corticosteroids.

The first discussant (A.Th.) said that, as a paediatric haematologist, she felt that the patient was somewhat out of her age range. However, she thought that the trephine biopsy sections were grossly abnormal. There were numerous large pink 'blobs', some of which appeared to have elongated nuclei stretched over them (Figure 6). In addition there was an interstitial infiltrate (lymphocytes, plasmacy-

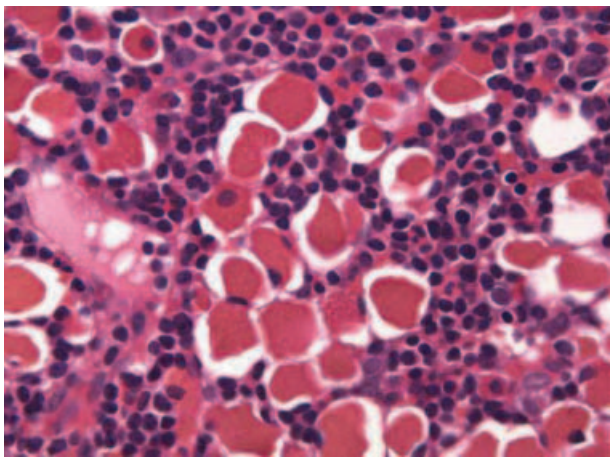

Figure 6. Trephine biopsy section of case 6.

toid lymphocytes and plasma cells) with some of these cells containing Dutcher bodies. There was also some amorphous interstitial material and she wondered about amyloid, paraprotein deposition or gelatinous transformation. The morphology of the 'blobs' suggested plasma cells containing Russell bodies and in view of the other features she favored a diagnosis of lymphoplasmacytic lymphoma. The second discussant (V.C.) had observed the same features and thought that there would be an IgM paraprotein.

Further discussion and final diagnosis

Case 1

The organiser reported on behalf of S.A., that further analysis of the red cells had been carried out at the International Blood Group Reference Laboratory, Bristol, by Dr May-Jean King. Flow cytometric analysis of red cells labelled with eosin-5-maleimide gave the results shown in Table 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of red cell membranes showed a reduction in the proportion of band 3 and a slight reduction of band 4.2. These results are compatible with a mutation in the *EPB3* or *SLC4A1* gene, which encodes the band 3 protein of the red cell membrane. This mutation is responsible for 15–20% of cases of hereditary spherocytosis but, despite this, neither the discussants nor any member of the audience had seen such a striking example of 'pincer cells'.

The final diagnosis was hereditary spherocytosis resulting from band 3 deficiency with a mutation of the *SLC4A1* gene being likely.

Case 2

B.B. reported, on behalf of V.A., that immunophenotyping (gating on large mononuclear cells) had shown weak positivity for CD45 (34%) and positivity for CD56 (60%), CD38 (99%), surface membrane kappa light chain (35%) and cytoplasmic kappa light chain (82%). Other B-cell markers, T-cell markers, myeloid markers and terminal deoxynucleotidyl transferase had been negative. Cytogenetic analysis (Barbara Czepulkowski) showed a highly complex near-triploid karyotype with structural as well as numeric abnormalities. The final diagnosis was plasma cell leukaemia.

Table 1. Flow cytometric analysis of red cells labelled with eosin-5-maleimide

	Mean channel fluorescence
Patient (case 1)	43 units
Reference ranges	
Normal adults	53.9 ± 3.2 units (n = 180)
Hereditary spherocytosis with spectrin deficiency	36.9 ± 4.2 units (n = 41)
Hereditary spherocytosis with band 3 reduction	36.0 ± 5.0 units (n = 19)
Hereditary spherocytosis with protein 4.2 reduction	39.2 ± 5.0 units (n = 36)

Case 3

B.B. reported on behalf of M.L. that the child did have 'familial platelet disorder with a predisposition to acute myelogenous leukaemia'. FPD/AML results from a germline mutation (frame-shift, nonsense or mis-sense) in the *AML1* (*RUNX1*) gene at 21q22 (Song *et al.*, 1999; Michaud *et al.*, 2002; Ganly, Walker & Morris, 2004). There have now been 11 kindreds described. The likelihood of leukaemia differs between families. Predisposition to leukaemia may result from haplo-insufficiency but with some mutations there may also be a dominant negative effect. In the family of the propositus, the mutation (R201Q) is predicted to impair binding of RUNX1 protein to target gene sequences and to inhibit the wild-type protein. In this family, 50% of affected individuals have developed a myelodysplastic syndrome or acute myeloid leukaemia. The case contributor had found a single report of a family with a syndrome resembling FPD/AML with

Table 2. Flow cytometry immunophenotyping in case 4, gating on lymphoid cells

B-cell markers		
CD10	6%	
CD19	2%	
SmIg	14%	
Kappa	13%	
Lambda	11%	
Mu	9%	
T-cell markers		
CD7	81%	Weak
CD1a	11%	
CD3	34%	Weak
CD4	31%	Weak
Myeloid markers		
CD13	87%	
CD33	7%	
Cytoplasmic myeloperoxidase	2	
Other		
CD30	72%	Weak
CD34	3%	
CD45	99%	Moderate
Terminal deoxynucleotidyl transferase	3%	

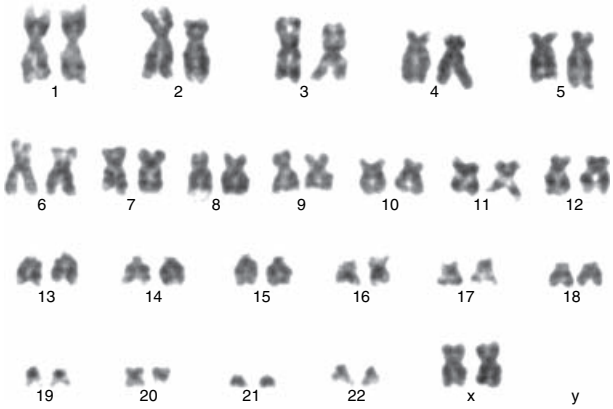

Figure 7. Karyogram of case 4 (with thanks to Mr Steve Chatters).

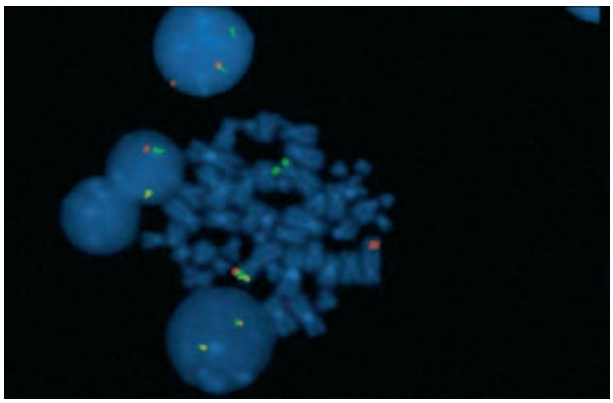

Figure 8. FISH analysis in case 4, whole chromosome painting using a red probe for chromosome 5 and a green probe for chromosome 2 (with thanks to Dr Helena Kempski).

developmental delay but whether the two features are related or unrelated in this child is unclear.

The final diagnosis was familial platelet disorder with a predisposition to acute myelogenous leukaemia as a result of mutation in the *AML1/RUNX1* gene.

Case 4

B.B. reported, on behalf of C.W. and D.W., that the diagnosis had been difficult. Rhabdomyosarcoma had indeed been considered. Immunophenotyping (Table 2) showed expression of CD30 and weak expression of T-cell antigens, suggesting a diagnosis of anaplastic large cell lymphoma. Aberrant expression of CD13 has been reported previously in this condition (Popnikolov *et al.*, 2000) and has been linked to a poor prognosis (Onciu *et al.*, 2003). The diagnosis was confirmed by cytogenetic and fluorescence *in situ* hybridization (FISH) analysis which showed a t(2;5)(p23;q35) (Figures 7 and 8) and rearrangement of the *ALK* gene (Figure 9).

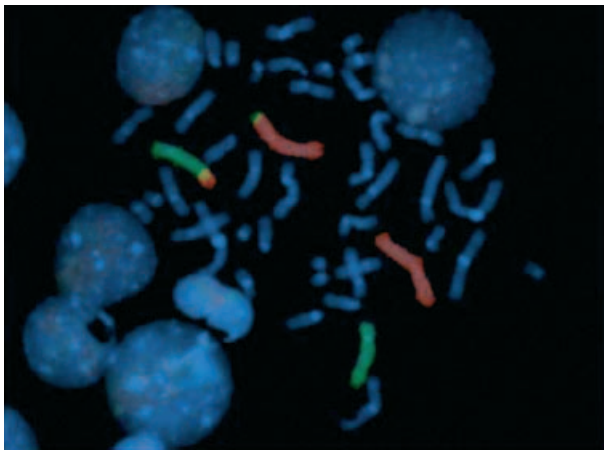

Figure 9. Dual-colour break-apart FISH analysis in case 4, using a probe for the *ALK* gene (with thanks to Dr Helena Kempski).

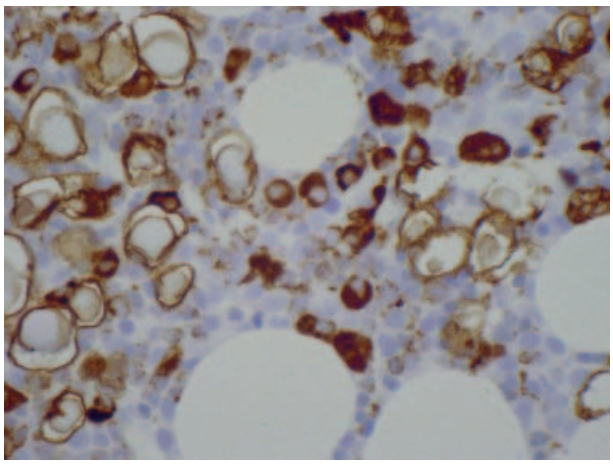

Figure 10. Immunohistochemistry with a monoclonal antibody directed at p63.

The final diagnosis was peripheral blood involvement by anaplastic large cell lymphoma.

Case 5

B.B. reported on behalf of A.Ta., that the bone marrow had shown trilineage dysplasia with particularly striking erythroid dysplasia and many giant bizarre megaloblasts. Erythroid cells were more than 50% of nucleated cells and blast cells were increased, permitting a diagnosis of M6 AML. Cytochemical reactions were negative for Sudan black B, chloroacetate esterase and alpha naphthyl acetate esterase. Immunophenotyping of bone marrow cells showed the blasts to express CD13, CD33, CD64, CD117 (weak) and CD34. There was no expression of cytoplasmic

myeloperoxidase, glycophorin, CD41 or terminal deoxynucleotidyl transferase. There was an abnormal hypodiploid karyotype with 43 chromosomes and many structural abnormalities.

The final diagnosis was M6 AML with the blast cells being myeloblasts that were Sudan black B-negative and failed to express myeloperoxidase.

Case 6

B.B. reported on behalf of F.H. that the patient had two distinct conditions. He had autoimmune thrombocytopenic purpura, which responded well to corticosteroids. In addition, the pink 'blobs' were indeed Russell bodies, but their shape was quite unusual. Russell bodies are notable for a perfectly circular outline whereas many of these bodies were somewhat irregular. The inclusions and associated occasional thin rim of cytoplasm were negative for kappa and lambda and a Congo red stain for amyloid was likewise negative. However an immunohistochemical stain for p63, an antigen expressed by plasma cells, was performed by Dr Bridget Wilkins, Newcastle Royal Infirmary, and showed a thin rim of positively staining cytoplasm related to some of the bodies (Figure 10). The patient was found to have an IgM lambda paraprotein in a concentration of 9.5 g/dl and there were free lambda light chains in the urine. A computed tomography (CT) scan did not show any lymphadenopathy.

The final diagnosis was thus lymphoplasmacytic lymphoma with Russell body formation.

Acknowledgements

Dr C. Manson, Consultant Histopathologist, Warrington General Hospital, kindly provided the histological sections from case 6. Dr Alexandra Rice, St Mary's Hospital, London, performed some of the immunohistochemical stains on case 6.

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