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Summary Seven patients who had a diagnostic problem were presented at the British Society for Haematology, Annual Scientific Meeting in 2003. The likely diagnosis was discussed on the basis of a synopsis of the history and blood film and trephine biopsy features and forms the basis of this report. Diagnostic problems dealt with included lymphoproliferative and myeloproliferative disorders and haemolytic anaemia.

Keywords Diagnostic haematology, blood film

Each year, at the Annual Scientific Meeting of the British Society for Haematology there is an educational session in which two experts discuss the morphological features of blood films or bone marrow biopsy sections from patients who have presented a diagnostic problem or who are otherwise instructive. The experts are given no information beyond the brief details that are provided to all participants who review the slides before the meeting. After the discussants have given their opinions, the case contributor presents further details and gives the final diagnosis. This report follows the format of the meeting so that the reader can reach a provisional diagnosis for him or herself before the definitive diagnosis is revealed.

Case 1

A peripheral blood film (PB) was provided from a 20-year-old woman who had presented with amenorrhoea of 2 months' duration. She was found to be anaemic with minor axillary lymphadenopathy. Her full blood count (FBC) was: WBC 2.45×10^9/l, Hb 8.4 g/dl, MCV 85,fl and platelet count 826×10^9/l. (case contributed by Dr F. Brito-Babapulle)

The first discussant (NL) found all lineages to be abnormal. The red cells showed anisocytosis, poikilocytosis (pencil cells, spherocytes), polychromasia and basophilic stippling and there was a population of hypochromic microcytes (Figure 1). There was neutropenia with 14% circulating blast cells; there was no eosinophilia or basophilia and granulocyte precursors were not present in appreciable numbers. There were giant and hypogranular platelets, megakaryocyte fragments and probable megakaryoblasts. The discussant thought the most likely diagnosis was essential thrombocythaemia with development of myelodysplastic features and probably M7 acute myeloblastic leukaemia (AML). He thought that either acquired haemoglobin H disease or sideroblastic erythropoiesis could have contributed to the red cell abnormalities.

The second discussant (JG) agreed with the findings. He had considered a diagnosis of chronic myeloid leukaemia in transformation but without leucocytosis or an increase in basophils he found this diagnosis difficult to sustain. He therefore favoured a diagnosis of acute myelofibrosis or M7 AML.

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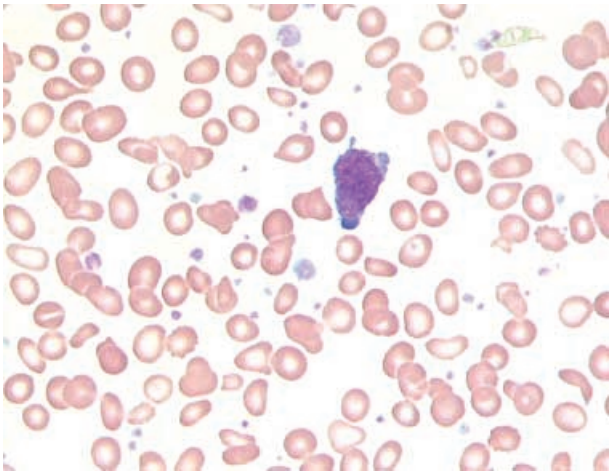

Figure 1. Peripheral blood film from case 1 showing anisocytosis, poikilocytosis including hypochromic fragments, giant platelets, agranular platelets and a blast cell.

Case 2

A peripheral blood film was provided from a 73-year-old Caucasian man who presented with a bright red macular rash. A skin biopsy had been performed and corticosteroids had been prescribed. Three weeks later his rash had disappeared but he was gravely ill. His FBC was: WBC 96×10^9/l, Hb 11.4 g/dl, MCV 90 fl and platelet count 46×10^9/l. (case contributed by Dr M. Bhavnani)

The first discussant (JG) described lymphocytosis with cleft and vacuolated lymphocytes (Figure 2). There was poikilocytosis. Despite the vacuolation of the lymphocytes, he did not think the features were those of Burkitt's lymphoma or L3 acute lymphoblastic leukaemia. He favoured a T-cell lymphoma and wondered if the patient might be HTLV-I positive.

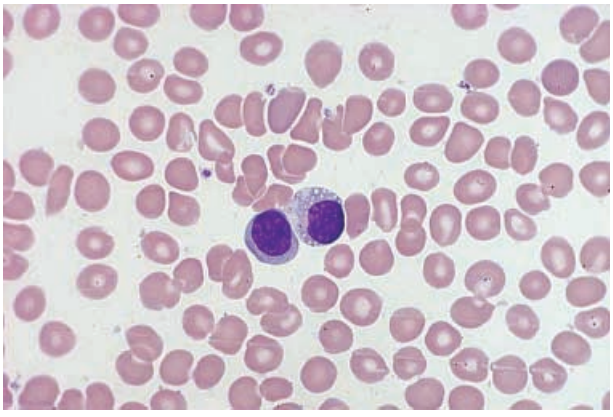

Figure 2. Peripheral blood film from case 2 showing two lymphocytes with grooved nuclei, one of which has numerous cytoplasmic vacuoles.

The second discussant (NL) also noted vacuolated lymphocytes but, in addition, detected immunoblasts, plasmacytoid lymphocytes and haemophagocytosis. There were red cell features suggesting dyserythropoiesis and there was neutrophilia with vacuolation and toxic granulation. He thought the most likely diagnosis was a haemophagocytic syndrome, possibly triggered by a herpesvirus infection with the neutrophil changes possibly resulting from the corticosteroid administration. He wondered if there was an underlying autoimmune or lymphoproliferative disorder.

Case 3

A peripheral blood film was provided from an 82-year-old Caucasian man. He suffered from chronic renal failure, attributed to hypertension; he was receiving erythropoietin therapy, which had led to a 5 g/dl improvement in his Hb. He had generalized erythroderma with dry scaly skin. FBC was: WBC 14×10^9/l, Hb 15.3 g/dl, MCV 93 fl and platelet count 157×10^9/l. (case contributed by Dr F. Britobabapulle)

The first discussant (NL) found there to be a lymphocytosis with 13% atypical lymphocytes and a total lymphocyte count of 6.3×10^9/l. Some of the atypical lymphocytes resembled Sézary cells and some were vacuolated (Figure 3). He thought the most likely diagnosis was Sézary syndrome but commented that the transient appearance of similar cells has been reported, e.g. following primadone therapy.

The second discussant (JG) agreed that this was likely to be a T-lineage lymphoproliferative disorder, possibly Sézary syndrome.

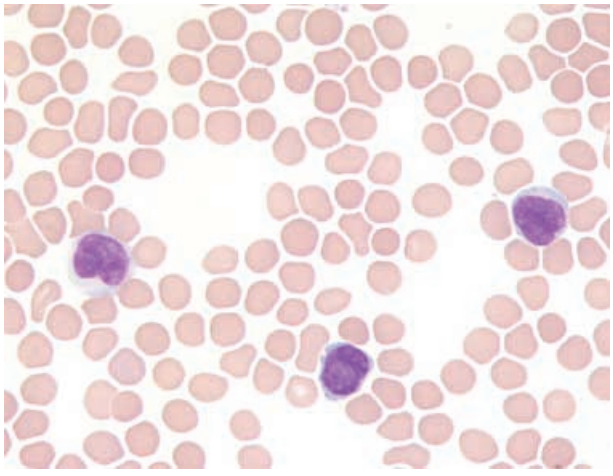

Figure 3. Peripheral blood film from case 3 showing three abnormal lymphocytes with irregular lobulated nuclei; one lymphocyte has a vacuole.

Case 4

A peripheral blood film was provided from a 31-year-old man (Figure 4) who had first developed peri-orbital oedema and an itchy skin rash at the age of 15 years. He was asthmatic with an allergy to house dust and mites. A biopsy of the peri-orbital area had shown infiltration by mast cells, eosinophils and immunoglobulin E-secreting plasma cells. Over the next 9 years his eosinophil count fluctuated between 0.53 and 2.39×10^9/l. He also developed reactive lymphadenopathy and tonsillar enlargement. His disease was controlled by corticosteroids. At the time this blood film was made, he was taking 15 mg per day of prednisolone and his FBC was: WBC 9.8×10^9/l, Hb 15.2 g/dl and platelet count 295×10^9/l. (case contributed by Dr M. Pocock)

The first discussant (JG) commented an eosinophilia and the presence of some mononuclear cells of uncertain lineage. There were also some heavily granulated cells and he was not sure whether these were basophils of mast cells (Figure 5). He thought that the differential diagnosis lay between an 'idiopathic hypereosinophilic syndrome' and a chronic myeloproliferative disorder (MPD). He thought it was probably not the MPD that has been described with t(5;12)(q33;p13) but systemic mastocytosis would be a possibility. If this were an example of the 'idiopathic' hypereosinophilic syndrome, then it should be noted that a significant proportion of such cases have recently been found to represent a clonal, neoplastic disorder with formation of a *FIP1L1-PDGFRA* fusion gene (Cools *et al.*, 2003).

The second discussant (NL) had made very similar morphological observations and noted, in addition, hypogranular neutrophils and neutrophils with nuclear projections. He thought the most likely diagnosis was systemic mastocytosis with associated myelodysplasia.

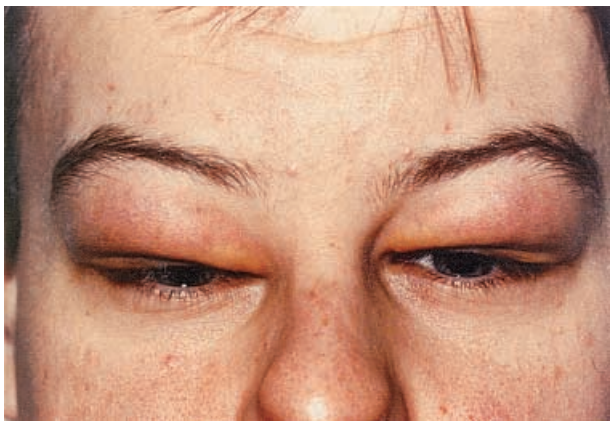

Figure 4. Facial appearance of the patient showing periorbital oedema.

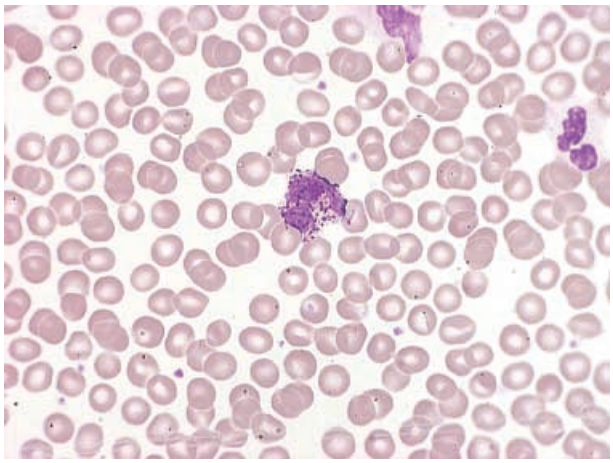

Figure 5. Peripheral blood film of case 4 showing one of the heavily granulated cells of uncertain lineage.

At this stage the chairman (BB) informed the discussants that the patient had been reported to have a *BCR-ABL* fusion gene detectable by reverse transcriptase-polymerase chain reaction (RT-PCR) in peripheral blood on one occasion and in bone marrow (BM) on another occasion. NL reported himself 'surprised' and JG 'amazed'. The latter commented that *BCR-ABL* can be detected in some normal people if a very sensitive technique is used but that a strong band would be hard to believe.

Case 5a

A trephine biopsy section was provided (to some participants only) from an 80-year-old Caucasian man having a staging biopsy for a diagnosis of probable blastic transformation of mantle cell lymphoma. His FBC was: WBC 10.1×10^9/l, Hb 7.9 g/dl and platelet count 30×10^9/l. (case contributed by Dr A Kubie and Dr R Jan-Mohamed, with thanks to Dr M. Williamson for providing the trephine biopsy sections)

The first discussant (JG) noted that there was a lymphoid infiltration in the bone marrow (Figure 6) but in addition the bone structure was very abnormal and there was an increase of osteoclasts (Figure 7) and of vessels. He thought there was a bone disease that was unrelated to the lymphoma, specifically Paget's disease.

The second discussant observed thickened bone trabeculae, an increase of large osteoclasts some located in Howship's lacunae, a lesser increase in osteoblasts and vascular connective tissue. He wondered if there were Dutcher bodies in the lymphocytes and did not think that

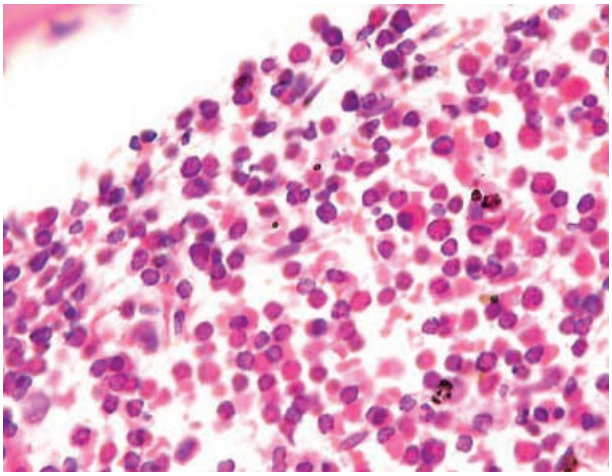

Figure 6. Bone marrow trephine biopsy section in case 5a showing a diffuse lymphoid infiltrate.

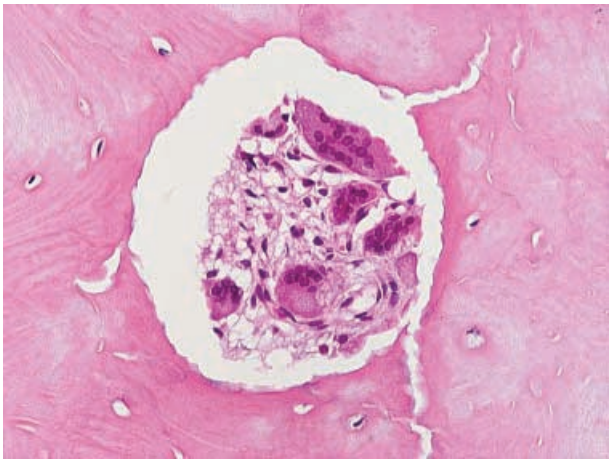

Figure 7. Bone marrow trephine biopsy section in case 5a showing thickened bone and numerous abnormal osteoclasts.

he could make a diagnosis of blastoid mantle cell lymphoma from the section provided.

Case 5b

A peripheral blood film was provided from a 52-year-old man who had presented 5 years earlier following the incidental discovery of a platelet count of 1157×10^9/l. At that time he had been found to have an Hb of 12.2 g/dl, a normal MCV, slightly increased WBC and neutrophil count, a neutrophil alkaline phosphatase score of 206, 32% ring sideroblasts in the bone marrow and a 16-cm spleen on ultrasonography. Hydroxyurea caused the Hb to drop to 7 g/dl and thereafter the thrombocytosis was controlled with anagrelide. At the time this blood film was made, the FBC was: WBC 61.2×10^9/l, Hb 11.6 g/dl and

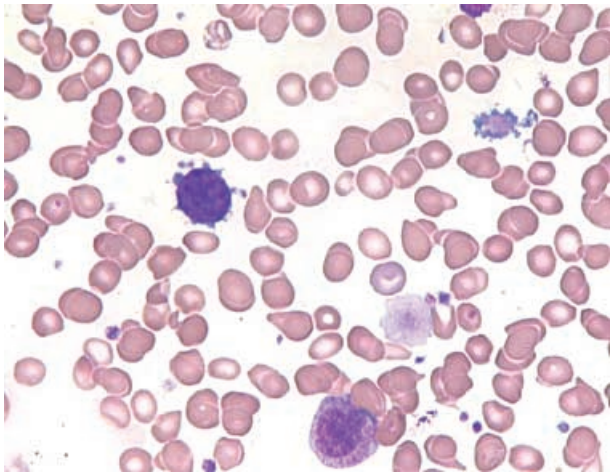

Figure 8. Peripheral blood film from case 5b showing anisocytosis, poikilocytosis, a granulocyte precursor and a giant hypogranular platelet.

platelet count 361×10^9/l. (case contributed by Dr B. Bain)

The first discussant (JG) noted abnormal red cells, white cell and platelets. Myelocytes and some blast cells were present and there were giant and hypogranular platelets. He thought that there were features of both a myeloproliferative disorder and of myelodysplasia and suggested that this patient fitted into the WHO category of myeloproliferative/myelodysplastic disorder, unclassified (Bain *et al.*, 2001). He did not think that the features were those of the 5q– syndrome.

The second discussant (NL) noted a leucoerythroblastic blood film, teardrop poikilocytes, basophils at all stages of differentiation, giant platelets and megakaryoblasts (Figure 8). He postulated that the initial diagnosis was essential thrombocythaemia with evolution to myelofibrosis and subsequently to either basophilic leukaemia or to M7 AML with myelodysplasia. He wondered if the patient might be Ph-positive.

Case 6

A peripheral blood film was provided from a 7-year-old boy of Northern European descent who had presented with neonatal jaundice from the first day of life. Subsequently he had transfusion-dependent anaemia and neurodevelopmental delay. Following splenectomy at the age of 3 years, he did not require transfusion though he suffered an episode of pigmenturia accompanied by erythroblastosis following a respiratory infection. For the last 2 years he had required chelation therapy for iron overload. FBC was: Hb 9 g/dl, MCV 118 fl, MCH 35.4 pg,

MCHC 30 g/dl and reticulocyte count 961×10^9/l. (case contributed by Dr M. Layton)

The first discussant (NL) noted post splenectomy changes (including spherocytes and Pappenheimer bodies) and, in addition, stomatocytes, echinocytes, basophilic stippling and nucleated red blood cells (Figure 9). The combination of a haemolytic anaemia and neurodevelopmental delay suggested the possibility of phosphofructokinase deficiency, phosphoglycerate kinase deficiency, glucose phosphate isomerase deficiency or triosephosphate isomerase deficiency. The range of other diagnostic possibilities was quite wide and included severe hereditary spherocytosis, hereditary spherocytosis plus a glycolytic enzyme deficiency or hereditary stomatocytosis.

The second discussant (JG) was in broad general agreement.

Discussion and final diagnoses

Case 1

The case contributor (FB-B) said that bone marrow aspiration had been difficult but showed an increase of blast cells, some with cytoplasmic blebs. No ring sideroblasts were detected. Trephine biopsy showed osteomyelofibrosis. Immunophenotyping of peripheral blood cells (gating on the blast cell area) showed: CD13 77%, CD33 83%, CD117 20%, CD34 82%, myeloperoxidase 10%, CD42 74% and glycophorin 17%. There was no expression of B-cell markers, T-cell markers, CD15, lysozyme or terminal deoxynucleotidyl transferase. The patient was found to have t(9;22)(q34;q11) and a *BCR-ABL* fusion gene. The final diagnosis was accelerated phase of Ph-positive essential thrombocythaemia with blast cells being mainly of megakaryocyte lineage but with some granulocytic and erythroid differentiation. The patient is responding well to imatinib therapy. The possibility of acquired haemoglobin H disease had not been explored.

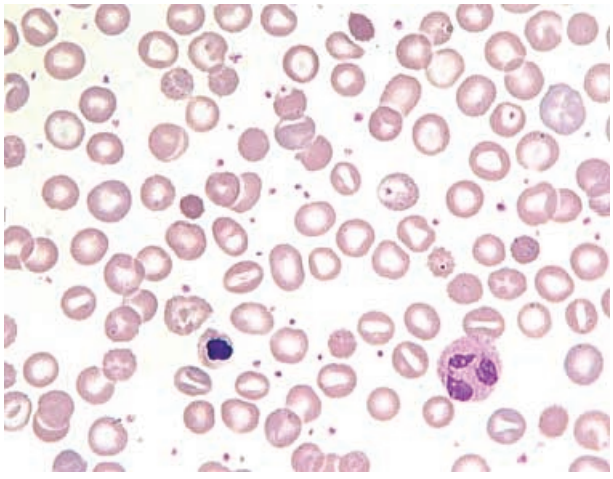

Figure 9. Peripheral blood film from case 6 showing a nucleated red blood cell, stomatocytes, small dark cells (probable spherocytes) and polychromasia.

Case 2

The chairman (BB) reported, on behalf of the case contributor (MB) that the patient had a T-cell lymphoma. In some cells the vacuoles (which were positive for periodic acid-Schiff) were in a ring around the nucleus, the 'rosary bead' appearance sometimes seen in cutaneous T-cell lymphoma. The skin biopsy was considered to show non-specific changes, specifically oedema and a mild perivascular chronic inflammatory infiltrate without any epidermal infiltration. The cells expressed CD3, CD4, CD5, T-cell receptor (TCR)αβ and HLA-DR. They did not express CD8, CD25 or TCRγδ. No antibodies to HTLV-I or II were detected. The reference laboratory did not perform the requested analysis for TCR gene rearrangement. The disease was rapidly progressive with the white cell count rising from 68 to 96×10^9/l in less than 24 h. Despite urgent reduced-dose combination chemotherapy the patient died 10 days after starting treatment. The final diagnosis was cutaneous T-cell lymphoma.

Case 3

The case contributor (FB) reported that a skin biopsy had shown lymphocytes in the dermis but no intraepithelial accumulations. The cells expressed CD2 and CD3 but not CD4, CD7 or CD8. Electron microscopy had shown occasional cells resembling Sézary cells (Figure 10). The immunophenotype (CD4-negative CD8-negative) was not that most often found in Sézary syndrome; such 'double negativity' is, however, found in 10% of cases. The final diagnosis was Sézary syndrome.

Case 4

The case contributor (MP) reported that, following the unexpected detection of *BCR-ABL* fusion, conventional cytogenetic analysis and fluorescence *in situ* hybridization (FISH) had both failed to demonstrate t(9;22)(q34;q11). Subsequently RT-PCR in a different laboratory had been negative.

Professor N. Cross was invited to comment on how different laboratories could get different answers for RT-PCR analysis. He commented that contamination

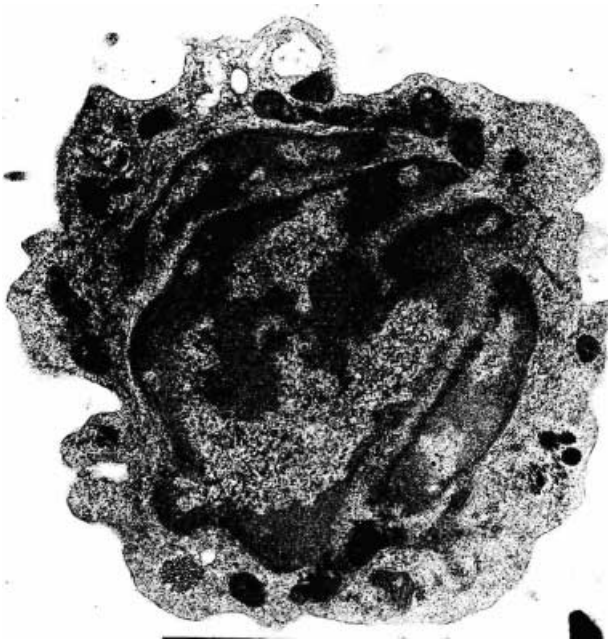

Figure 10. Electron microscopy of a lymphocyte from case 3 showing a complex nucleus (with thanks to Prof. D. Catovsky).

leading to false positive results was a well recognized phenomenon and it was concerning that as yet there is no National External Quality Assurance Scheme (NEQAS) for such molecular analysis in the United Kingdom.

Subsequent to the BSH meeting analysis for *FIP1L1-PDGFRA* was carried out by Professor Cross; the fusion gene was not detected. The final diagnosis is therefore idiopathic hypereosinophilic syndrome.

Case 5a

The chairman (BB) reported, on behalf of the case contributors (AK and RJ-M), that a year previously the patient had had a platelet count of 100×10^9/l. On this presentation there was minimal left inguinal lymphadenopathy and the spleen tip was palpable. There were lymphoma cells in the peripheral blood and some of these appeared blastic. Immunophenotyping on blood and bone marrow cells showed the lymphoma cells to express CD5, CD19, CD20, CD25, cytoplasmic CD79a, CD79b, FMC7, cytoplasmic mu chain and strong surface membrane kappa light chain. CD22 and CD23 were expressed on only a minority of cells. The immunophenotype of large and small cells was the same. The patient had an elevated creatinine concentration and alkaline phosphatase was 212–425 IU/l.

Cyclin D1 staining of the trephine biopsy sections was negative. Cytogenetic analysis failed and FISH analysis was not performed.

The final diagnosis was non-Hodgkin's lymphoma, probably blastoid mantle cell lymphoma, and coincidental Paget's disease.

Case 5b

The case contributor (BB) reported that the patient had a mixed myeloproliferative/myelodysplastic disorder, unclassified (Gupta, Abdalla & Bain, 1999). Cytogenetic analysis had been normal on two occasions.

Case 6

The chairman (BB) presented information on behalf of the case contributor (ML). He thought the particular features to consider were the pigmenturia, the response to splenectomy and the (non-progressive) neurodevelopmental delay. The red cell enzymes deficiencies that are linked to neurodevelopmental delay and whether or not the haemolytic anaemia is responsive to splenectomy is summarized in Table 1. However, these deficiencies were excluded by assay of red cell enzyme activities and assays

Table 1. Red cell enzyme deficiencies that have been linked to neurodevelopmental delay and whether or not the haemolytic anaemia is responsive to splenectomy

Pathway	Enzyme	Anaemia responsive to splenectomy
Glycolytic	Glucosephosphate isomerase	Yes
	Phosphoglycerate kinase	Yes
	Aldolase*	No
	Triosephosphate isomerase	No
Glutathione synthesis	Glutathione synthetase	Yes
	γ-glutamylcysteine synthetase	No
Nucleotide metabolism	Adenylate kinase	Yes
	Pyrimidine 5′-nucleotidease*	No

*Link to neurodevelopmental delay unproven.

of glutathione and glycolytic intermediates. Multiple other abnormalities were, however, detected including Heinz bodies in 25% of cells, an increased cation leak and an abnormal nucleotide profile. A heat test for an unstable haemoglobin was negative but an isopropanol instability test was positive on one of two occasions. There was a deficit of α chain synthesis with a β : α ratio of 1.23. The α globin genotype was $-\alpha^{3.7}/\alpha\alpha$. The findings are compatible with unstable α globin variant. **BUT** none was detected by mass spectrometry. The final diagnosis remains uncertain but possibilities include interaction between α^{+} thalassaemia and an unstable α chain variant or an undefined metabolic defect with combined haematological and neurological manifestations.

The chairman thanked the contributors and the expert discussants and closed the meeting.

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