

British Society for Haematology Slide Session, Annual Scientific Meeting, Harrogate, 2001

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Case Contributors and

Discussants:

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The 2001 British Society of Haematology (BSH) Annual Scientific Meeting took place at the Harrogate International Centre with great success. Now an established feature of this annual conference, the BSH slide session took place on the afternoon of the third day. The session aims to demonstrate the enduring importance of morphology as a pivotal skill in diagnostic haematology and to promote a sense of community between diagnostic haematology laboratories in Britain and abroad. Before the conference, slides are distributed to those who intend to be present at the meeting and who request them. These slides are examined in many haematology departments as an educational exercise. The session organizer invites two 'experts' to discuss the morphological features of six blood or marrow films, each accompanied by a short clinicopathological vignette, and to come to a diagnostic conclusion. Attendees at the session are given the opportunity to express their views, before the case contributor is asked to present further investigations and the final diagnosis, if one was reached. In previous years, the two experts were consultant haematologists, but on this occasion one consultant and one specialist registrar were invited, in order to let a trainee

rise to the challenge and to encourage participation by other trainees, who stand to benefit greatly from the session.

The cases are described below, along with the clinicopathological vignettes; relevant photographs and the discussants' opinions are presented first so that readers can make their own diagnoses. The diagnoses of the case contributors follow.

Case 1

*Contributed by Dr G. Lucas and Dr B. Pottinger,
Manchester Royal Infirmary*

Figure 1 shows the peripheral blood film of a 59-year-old man, who was admitted for elective hip replacement. He was otherwise fit and had no abnormality on physical examination. The full blood count (FBC) was: WBC $5.1 \times 10^9/l$, Hb 11.0 g/dl, MCV 96 fl and platelet count $2300 \times 10^9/l$.

The first discussant, GV, identified gross morphological abnormalities in the erythrocytes with abundant keratocytes ('moth-eaten' cells), and multiple nonstaining red cell inclusions, which were round, with irregular edges, and were similar in size to small platelets (Figure 1a). He suggested that the appearances were vaguely reminiscent of those found in dehydrated stomatocytosis. The platelet count on the smear appeared much lower than the

Accepted for publication 11 November 2002

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automated count. Platelets were of normal morphology, as were the white blood cells.

He suggested that both the red cell abnormalities and the elevated automated platelet count were artefactual, as they were incompatible with the clinical picture and blood film appearances, respectively. He thought the red cell changes were likely to have occurred *in vitro*, with the formation of small particles, which constituted the red cell inclusions and were also present in the plasma, where they were counted as platelets by the automated counter. He did not think the precipitates were cryoglobulin, as cryoglobulins normally take up the May–Grünwald Giemsa (MGG) stain and are not found in association with erythrocytes. He speculated that the particles might be Heinz bodies, which formed in the presence of an oxidant substance that had contaminated the blood sample after phlebotomy. He cited examples from the literature where this had happened (e.g. in the presence of acetylphenylhydrazine) and had led to appearances similar to this blood film (Beutler, Dern & Flanagan, 1955).

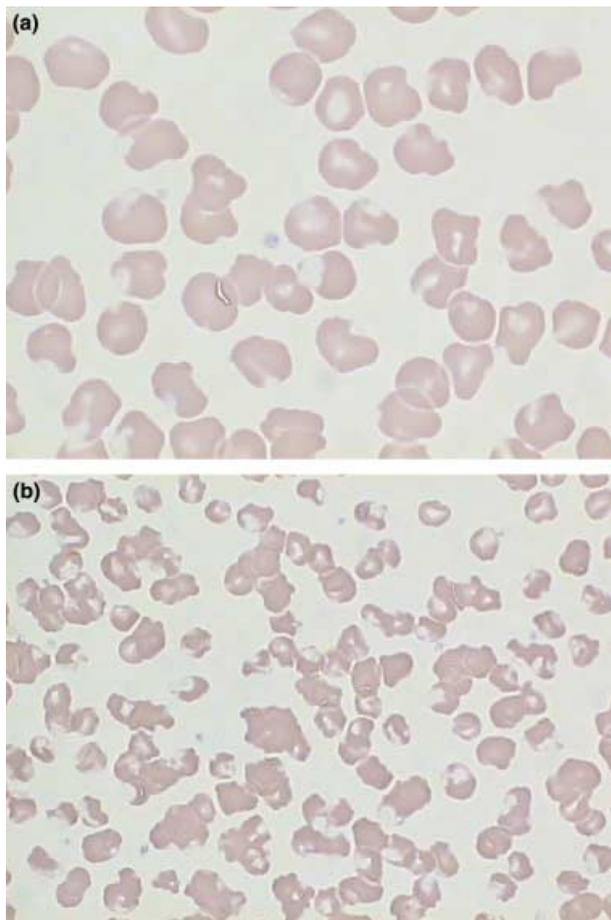


Figure 1. Peripheral blood film, case 1.

Heinz bodies can also be released from red cells *ex vivo* (Lessin, 1973).

The second discussant, RO, also commented on the red cell changes and spuriously elevated platelet count. In addition, he demonstrated the presence of red cell agglutinates (Figure 1b) and suggested that the appearances were due to the presence of a cryoglobulin. He was fortunate to have been 'tipped off' prior to the session by one of his regional colleagues, who had reported similar blood film changes in a patient with cryoglobulinaemia in the *Journal of Clinical Pathology* some 20 years ago! (Patel, Hughes & Parapia, 1987).

Case 2

Contributed by Dr Finella Brito-Babapulle, Reading

Figure 2 shows the peripheral blood film of a 49-year-old Pakistani woman, who had had no previous illnesses or

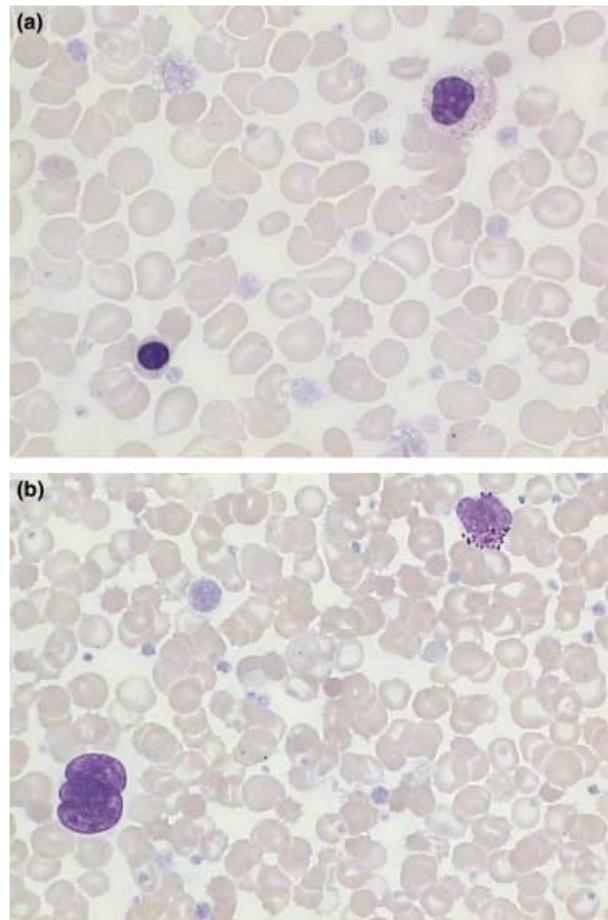


Figure 2. Peripheral blood film, case 2.

operations when, in 1994, a blood sample was sent for malaria diagnosis. The FBC showed RBC $6.1 \times 10^{12}/l$, Hb 14.4 g/dl, PCV 0.48, MCV 78 fl, MCH 23.3 pg and MCHC 29.9 g/dl. A diagnosis was made. In 1999, malaria was again suspected and FBC now showed WBC $19.9 \times 10^9/l$, Hb 13.6 g/dl and platelet count $455 \times 10^9/l$. A second diagnosis was made. By 2000, the FBC was WBC $24.8 \times 10^9/l$, Hb 13.7 g/dl and platelets $694 \times 10^9/l$. A third diagnosis was made after bone marrow examination.

RO suggested that the initial presentation in 1994 was suggestive of a thalassaemia trait and that the relatively high Hb was suggestive of α - or β -thalassaemia trait. It was commented that the supplied blood film (Figure 2a) was leucoerythroblastic, showing marked thrombocytosis with platelet anisocytosis and giant platelets, while bare megakaryocyte nuclei were also noted (Figure 2b). The red cells also showed features of hyposplenism and eosinophilia (20%) and basophilia (4%). He thought these changes were suggestive of both hyposplenism and an underlying myeloproliferative disorder and concluded that the three diagnoses were thalassaemia trait, hyposplenism and a myeloproliferative disorder. He speculated that the hyposplenism may have been due to coeliac disease, with associated iron deficiency having masked the myeloproliferative disorder which, given the red cell count at that time, could have been present in 1994.

GV agreed broadly but felt that the third diagnosis was likely to be polycythaemia rubra vera (PRV). This would have been made on the basis of bone marrow findings such as cytogenetic abnormality. He first thought that the diagnosis was thalassaemia trait or a similar disorder, probably with associated iron deficiency. Given the elevated red cell count at presentation, a diagnosis of PRV masked by the iron deficiency might have been considered even at that stage. The second diagnosis was hyposplenism, which could have been the result of splenic infarction or splenic vein thrombosis secondary to PRV. The third diagnosis would have been PRV. GV acknowledged that the eosinophilia was difficult to explain on the basis of these diagnoses and might have had an alternative aetiology.

Case 3

Contributed by Dr M. Layton, Hammersmith Hospital, London

Figure 3 shows the peripheral blood film of a 11-year-old girl of northern European descent, who had presented

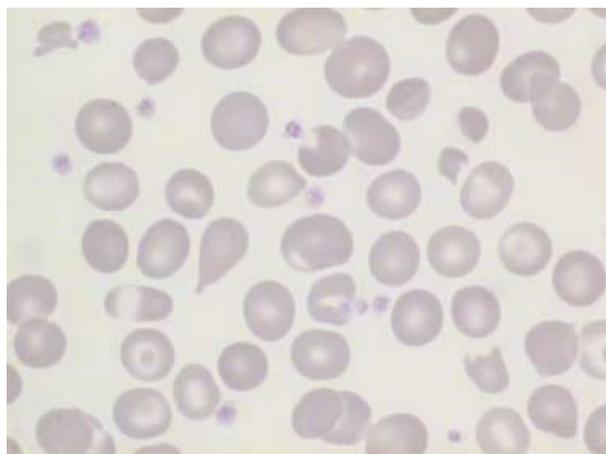


Figure 3. Peripheral blood film, case 3.

with a 1-week history of jaundice and dark urine. The FBC showed: WBC $6.23 \times 10^9/l$, Hb 11.6 g/dl, MCV 85.6 fl, MCH 29.7 pg, MCHC 34.7 g/dl, platelet count $563 \times 10^9/l$ and reticulocyte count $318 \times 10^9/l$ (8%).

GV demonstrated keratocytes, red cell 'hemighosts', basophilic stippling and increased numbers of stomatocytes and target cells. He suggested that some features were suggestive of acute oxidant-induced haemolysis but conceded that this could not explain the presence of stomatocytes and target cells. He therefore suggested that the latter features might be indicative of a separate disorder, perhaps related to the factor precipitating the haemolytic episode. Possible diagnoses included rare inherited defects of the pentose-phosphate pathway, Wilson's disease or the use of a potent oxidant such as dapsone in a patient with dermatitis herpetiformis (hyposplenism being the cause of the atypical red cell changes).

RO agreed that the blood film features were suggestive of oxidative haemolysis. He also commented that G6PD deficiency was unlikely but there may have been a defect of another red cell enzyme or possibly an unstable haemoglobin.

Case 4

Contributed by Dr G. Lucas and Dr B. Pottinger, Manchester Royal Infirmary

Figure 4 shows the peripheral blood film of a middle-aged woman who had presented 2 years earlier with lymphocytosis and splenomegaly. The cytological features showed some similarities to atypical chronic lymphocytic leukaemia (CLL) but the immunophenotype and cytogenetic analysis suggested another diagnosis. Remission was

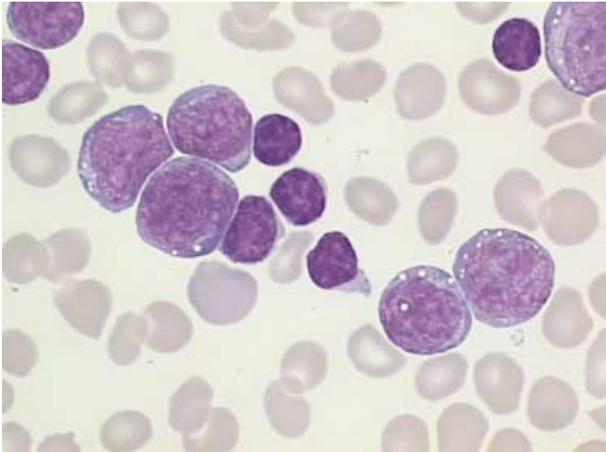


Figure 4. Peripheral blood film, case 4.

induced by combination chemotherapy followed by fludarabine but later she developed painful splenomegaly and a WBC of $380 \times 10^9/l$.

RO commented that the blood film demonstrated a marked lymphocytosis with two populations of cells evident. There was a small cell component, consisting of cells with a high nuclear:cytoplasmic ratio, some with nuclear irregularities including clefting. There was also a population of large cells with prominent nucleoli and moderate amounts of basophilic cytoplasm, some with vacuolation (Figure 4). He suggested that the features were those of an 'indolent' lymphoproliferative disorder undergoing large cell transformation. He commented that if the immunophenotype and genotype did not support a diagnosis of CLL, then the most likely diagnosis in this case was large cell transformation of mantle cell lymphoma.

GV suggested that the appearances were in keeping with a diagnosis of transformed follicular lymphoma, as suggested by the presence of a dual population of lymphoid cells. One population was made up of very small mature lymphocytes (smaller than CLL cells) with dense chromatin, angular and often cleaved nuclei and scanty cytoplasm. The other population consisted of large cells with open chromatin, a high nuclear:cytoplasmic ratio, peripheral nucleoli, frequent mitoses and abundant cytoplasmic vacuolation. However, in spite of these suggestive morphological features, a diagnosis of follicular lymphoma was not deemed likely as the clinical features were atypical. Mantle cell lymphoma was considered, but was not thought likely on morphological grounds. A diagnosis of Richter's transformation of CLL with excess prolymphocytes (CLL/PL) was put forward as the most likely, albeit tentative diagnosis.

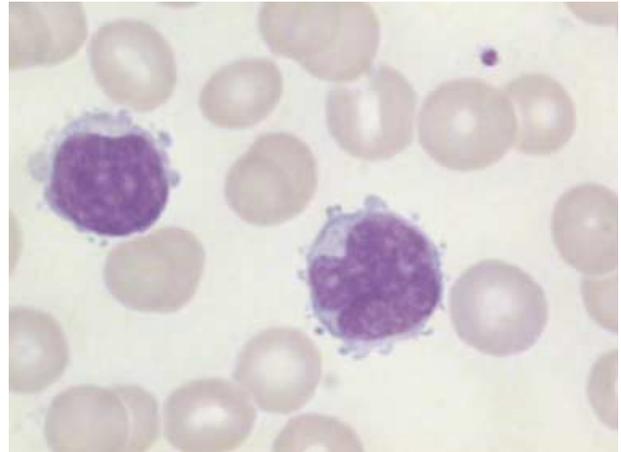


Figure 5. Peripheral blood film, case 5.

Case 5

Contributed by Dr G. Robinson, Abergavenny

Figure 5 shows the peripheral blood film of a 69-year-old woman who presented with lethargy, sore throat, cervical lymphadenopathy and peeling skin on her hands, WBC $26.2 \times 10^9/l$, Hb 12.4 g/dl and platelet count $204 \times 10^9/l$. Serology was negative for Epstein-Barr virus, cytomegalovirus and toxoplasma. There were antibodies to adenovirus at a titre of 128. Various tests were carried out, including molecular genetic analysis.

GV showed morphological evidence suggesting a reactive lymphocytosis, with large atypical lymphocytes with moderately condensed chromatin, abundant cytoplasm, often curling around red cells and many with azurophilic granules. These lymphocytes made up about 80% of nucleated cells and there was no neutropenia. The blood smear was otherwise normal. A diagnosis of atypical mononucleosis was favoured rather than a clonal disorder of mature lymphocytes. The differential diagnosis was wide, including particularly conditions that might account for the peeling skin of the hands (Coxsackie virus infection, sulphonamide hypersensitivity, secondary syphilis and adult Kawasaki's disease). The diagnosis of Kawasaki's disease was favoured, despite the age of the patient.

RO agreed that the clinical features were reminiscent of Kawasaki's disease but commented that this would have been extremely unusual in a patient of this age. He agreed that the blood film appearances were those of a reactive process, although an aggressive T-cell or NK-cell malignancy might also be considered. He also commented that T-cell receptor gene rearrangement studies might not necessarily have been helpful in this patient, as clonal rearrangements are not uncommon in reactive conditions.

Case 6

Contributed by Dr A. Thomas, Edinburgh

Figure 6 shows the peripheral blood film of a Scottish child who presented with chest infection, hepatosplenomegaly and clubbing. He was found to be pancytopenic, with a positive direct antiglobulin test, granulocyte

immunofluorescence test and platelet immunofluorescence test. The FBC was WBC $7.4 \times 10^9/l$, Hb 9.0 g/dl and platelet count $13 \times 10^9/l$. Various tests were carried out, including molecular genetic analysis.

RO showed photographs showing red cell inclusions, which, in his opinion, appeared to suggest a diagnosis of babesiosis (*Babesia divergens* infection). He commented

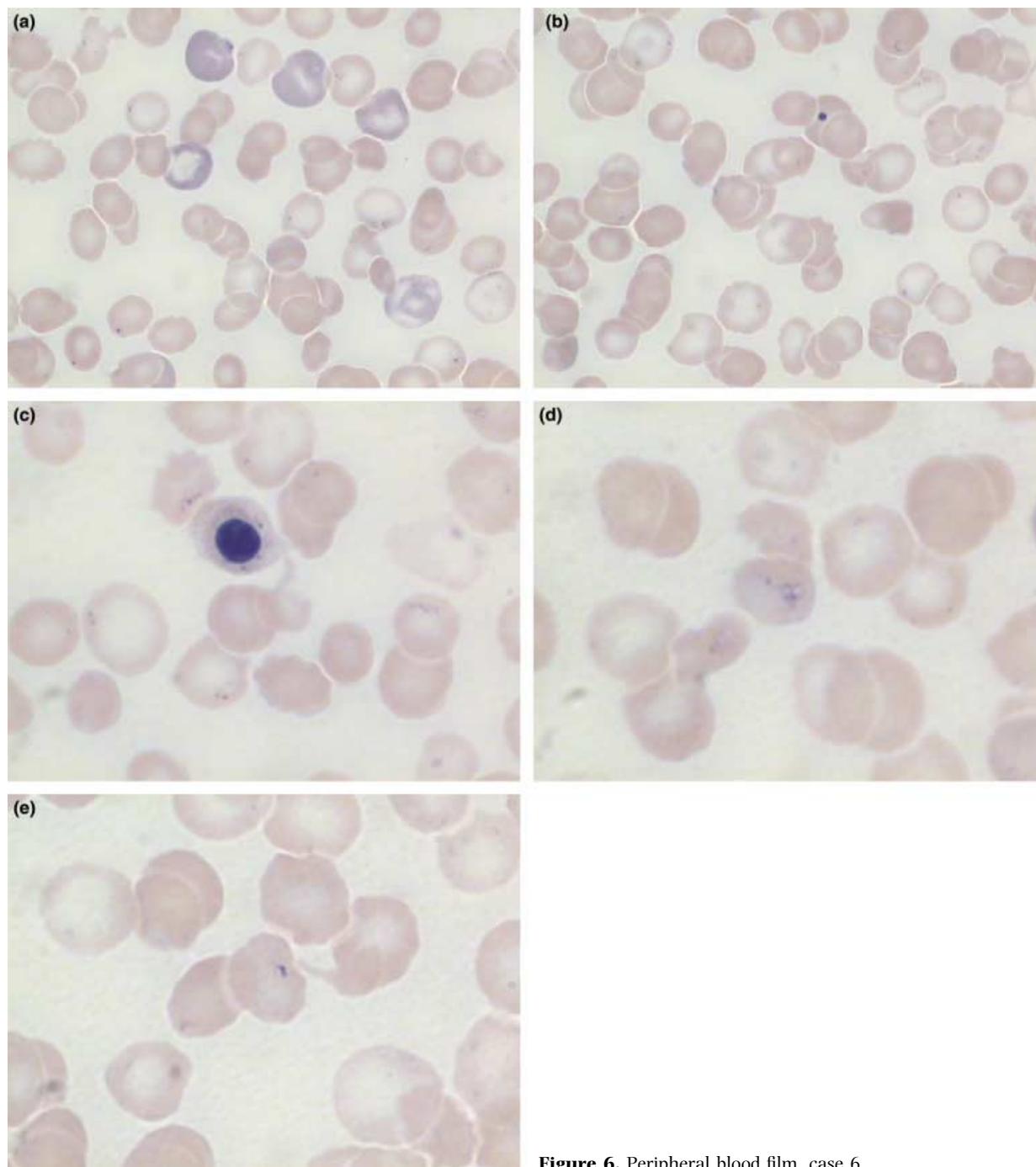


Figure 6. Peripheral blood film, case 6.

that this was the most difficult of the cases presented. He demonstrated polychromasia on the blood film and commented on the lack of spherocytes, particularly surprising given the positive Coombs (direct antiglobulin) test. He also demonstrated poikilocytosis and Howell-Jolly bodies, which he considered as representing functional hyposplenism, despite the palpable splenomegaly. There were also numerous activated lymphocytes seen but no obvious blast cells. He commented that *Babesia divergens* had been reported in Northern Europe and that it was most common in asplenic or hyposplenic individuals. It is also associated with haemolysis (often Coombs positive), leucopenia, thrombocytopenia and respiratory complications such as pneumonia and adult respiratory distress syndrome.

GV disagreed and felt that the inclusions were more likely to represent Pappenheimer bodies. He concentrated instead on the white cell morphology and the finding of hyposplenism in the presence of an enlarged spleen, which suggested splenic infiltration. He also identified what he thought to be small lymphoid-like blasts. The diagnosis of autoimmune lymphoproliferative syndrome (ALPS) was considered, given the serological findings, but was thought unlikely, given the presence of blast cells. Another possibility was a haemophagocytic syndrome associated with immunodeficiency, given the presence of many large monocytes in the circulation, some with multiple cytoplasmic inclusions. The possibility of haemophagocytic lymphohistiocytosis was also considered. Finally, the possibility of ataxia telangiectasia was contemplated, being the prototype of an immunodeficiency disorder associated with non-Burkitt's acute lymphoblastic leukaemia. If this were the case, the positive serology could have been secondary to acquired autoimmunity or due to therapeutically administered intravenous immunoglobulin.

Further discussion and final diagnoses

Case 1

BP, on behalf of GL, reported that a red cell disorder had initially been suspected but further investigation showed the presence of a cryoglobulin; its effect on the FBC is shown in Table 1. An immunoglobulin M (IgM) kappa paraprotein was detected. A bone marrow film showed infiltration by lymphoplasmacytoid lymphocytes; many of these cells showed cytoplasmic vacuoles and some had very large cytoplasmic inclusions (Russell bodies) (Figure 7). A trephine biopsy confirmed the aspirate findings and, in addition, showed interstitial deposition of amyloid.

Table 1. Case 1, effects of storage of blood or plasma at room temperature or 37 °C

	Haemoglobin (g/dl)	Platelet count ($\times 10^{-9}/l$)
Fresh blood	10.8	162
Blood stored for 2 h at room temperature	11.0	1149
Blood stored for 2 h at 37 °C	10.8	161
Plasma stored for 2 h at room temperature	0.9	1414
Plasma stored for 2 h at 37 °C	0.3	481
Plasma stored for 2 h at room temperature then re-warmed to 37 °C	0.6	214

Final diagnosis. Lymphoplasmacytic lymphoma leading to cryoglobulinaemia and light-chain associated amyloidosis. Red cell changes and 'thrombocytosis' were artefactual.

Note. This condition is sometimes, perhaps erroneously, referred to as 'Waldenström's macroglobulinaemia' but in the condition that Waldenström actually described there was a very high concentration of an IgM paraprotein leading to hyperviscosity. Purists therefore prefer to confine the use of this term to patients with a lymphoplasmacytoid lymphoma with hyperviscosity attributable to a very high concentration of an IgM.

Case 2

F B-B stated that the first diagnosis was haemoglobin D-Punjab trait with iron deficiency, the second was

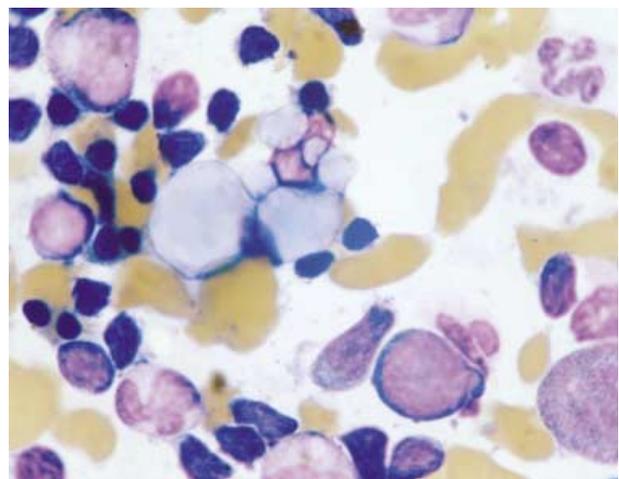


Figure 7. Bone marrow aspirate in case 1 showing Russell bodies in a myeloma cell.

hyposplenism [diagnosed on computer tomography (CT) scan and believed to be congenital] and the third was probable PRV with absent iron stores. Trisomies of chromosomes 8 and 9 were detected at this stage. Both of these trisomies are quite common in myeloproliferative disorders and their simultaneous occurrence is particularly frequent in PRV. The eosinophils did not show trisomy 8 and were therefore deemed to be nonclonal and secondary to coincidental asthma.

Final diagnoses. Asplenia (probably congenital); haemoglobin D-Punjab (D-Los Angeles) heterozygosity; polycythemia rubra vera; reactive eosinophilia (asthma).

Case 3

ML reported that the patient had gallstones. She had inherited a previously undescribed variant of dehydrated stomatocytosis, also known as hereditary xerocytosis. This is an autosomal dominant condition. Relevant investigations are shown in Table 2. Hereditary stomatocytosis was first described by Hardisty, Lock and Smith (1961). Subsequently, four related conditions were recognized, all characterized by abnormal cation flux: (i) overhydrated hereditary stomatocytosis; (ii) dehydrated hereditary stomatocytosis, (iii) cryohydrocytosis and (iv) familial pseudohyperkalaemia. Blood films in dehydrated hereditary stomatocytosis characteristically show target cells and small numbers of stomatocytes, echinocytes, irregularly contracted cells and cells with haemoglobin 'puddled' to one side of the cell. Despite the name, stomatocytes are often present only in fairly small numbers.

Final diagnosis. Dehydrated variant of hereditary stomatocytosis.

Case 4

TH presented the data on behalf of DO. They had also considered a morphological diagnosis of atypical CLL but the immunophenotype and karyotype were typical of

mantle cell lymphoma. The cells were monoclonal lambda-positive B cells expressing CD79a, CD79b and FMC7 (60%) but not CD23 (11%). They showed t(11;14)(q13;q32). There was subsequent disease evolution, with the large cells differing immunophenotypically from the small cells (Table 3 and Figure 8). The large and small cells showed the same rearrangement of the immunoglobulin VH gene but while the large cells had a TP53 (p53) mutation, the small cells expressed wild type TP53. At this stage, there was karyotypic evolution but interestingly the large cells were not tetraploid.

Final diagnosis. Mantle cell lymphoma with disease progression, associated with immunophenotypic and karyotypic evolution.

Case 5

GR showed evidence that this was indeed a reactive lymphocytosis, but with a clonal rearrangement of T-cell receptor beta genes detected both during and after the episode of lymphocytosis. The lymphocytosis resolved after 3 months and a definite aetiology had not been established. The titre of antibodies to adenovirus did not change and may not therefore have been relevant to the presenting illness. This case highlights the diagnostic problems that occur when molecular analysis shows evidence of a clonal lymphocyte population but the disease course does not suggest a neoplastic condition.

Table 3. Case 4, immunophenotype following disease evolution

Marker	Small cells	Large cells
CD19	+	+
CD20	+	+
CD5	+	+
CD10	-	+/-
CD25	+	-
CD38	-	+
HLA-DR	+	++

Table 2. Case 3, cation and ion flux studies

	Intracellular concentration (mmol/l cells)		K influx at 5 mM external K (mmol/l cells)		
	[Na]	[K]	NaK pump	NaK2Cl	Leak
Patient	11.69	3.7	7.68	0.73	0.431
Mother	7.9	109.7	1.77	0.16	0.134
Normal range	5-11	85-105	1-2	0-1	0.05-0.10

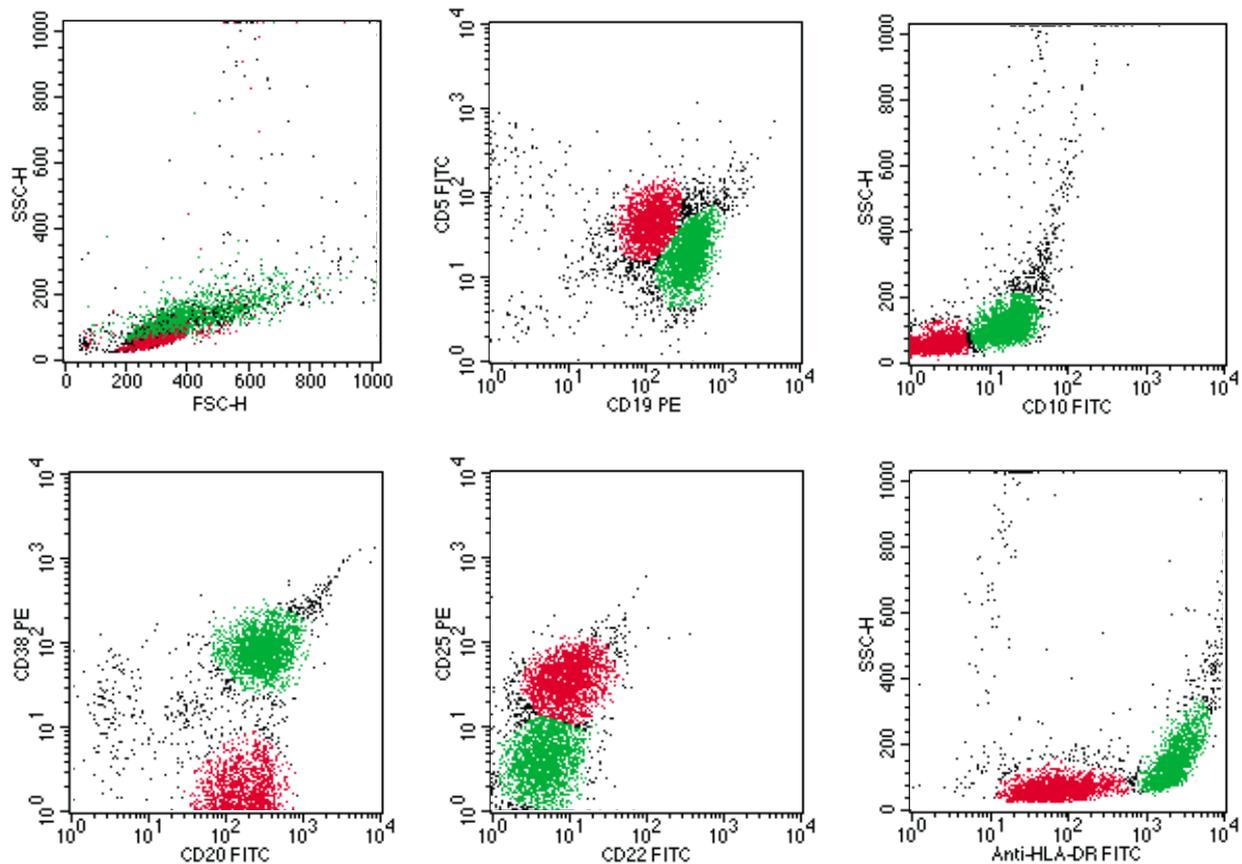


Figure 8. Flow cytometric immunophenotypic data, case 4.

Final diagnosis. Reactive T-cell lymphocytosis of unknown aetiology with molecular evidence of a clonal T-cell population.

Case 6

AT reported that the child was lacking signalling lymphocyte activation molecule (SLAM)-associated protein (SAP), although no mutation of the gene was demonstrated. This defect is usually associated with the X-linked lymphoproliferative disorder, previously known as Duncan's syndrome. This child is quite unusual for someone with deficiency of this protein as he had been exposed to the Epstein-Barr virus in the past, had had appropriate antibody responses and did not have detectable virus on PCR. In addition to autoimmune pancytopenia, other features of the disease were vasculitis and autoimmune hepatitis.

Final diagnosis. Autoimmune pancytopenia associated with lack of expression of SLAM-associated protein

(without the phenotype of X-linked lymphoproliferative disorder usually associated with deficiency of this protein).

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