

# Erythrocyte autoantibodies, autoimmune haemolysis, and myelodysplastic syndromes

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**SUMMARY** Immunohaematological investigations were carried out in 46 patients with erythrocyte autoantibodies associated with myelodysplastic syndromes. Eight patients had refractory anaemia, 17 refractory anaemia with ring sideroblasts, 11 refractory anaemia with excess of blasts, four chronic myelomonocytic leukaemia, five refractory anaemia with excess of blasts in transformation and one could not be classified. Standard agglutination direct antiglobulin tests showed that the red cells were most often coated with IgG and C3d, though increased amounts of IgM or IgA were also found in 15 of 35 cases (43%) when the more sensitive enzyme linked method was used. The IgG antibodies were predominantly of IgG1 subclass. Clinically important autoimmune haemolysis occurred in 15 patients, and was of "warm", "cold," and "mixed" types in seven, four, and four cases, respectively: it is important to recognise its presence in view of the good response to treatment. The increased incidence of erythrocyte autoantibodies in myelodysplastic syndromes is thought to be one manifestation of disturbed immune homeostasis.

The myelodysplastic syndromes are a group of acquired disorders which usually occur in middle or later life and result from clonal abnormalities of haemopoietic stem cells.<sup>1,2</sup> Attention has recently been drawn to the high incidence of erythrocyte autoantibodies in patients with myelodysplastic syndromes.<sup>3-5</sup> By providing a serological reference service for a population of 4.7 million,<sup>6</sup> the Trent Regional Blood Transfusion Centre is in a unique position to study a comparatively large number of such cases. We describe the immunohaematological findings in 46 patients with the association of myelodysplasia and red cell autoantibodies.

## Material and methods

From January 1 1983 to June 30 1988 blood samples from 1756 patients with suspected autoimmune haemolysis, or in whom red cell autoantibodies had been found, were referred to this centre for serological investigation. The records of the 46 patients with confirmed myelodysplastic syndrome were critically examined. Particular attention was paid to the type of myelodysplasia (FAB classification),<sup>1</sup> the immunohaematological profile, and whether there was any clinically important autoimmune haemolysis. The

latter was determined from the clinical and laboratory findings, including rate of transfusion, rate of fall in haemoglobin concentrations, reticulocyte count, blood and marrow film appearances, and measurements of red cell life span, serum haptoglobins, bilirubin concentration and lactic dehydrogenase activity.

The investigations carried out at this centre included direct antiglobulin tests (DAGT) using agglutination and enzyme linked methods, examination of red cell autoantibodies in serum and eluates, and detection of concomitant alloantibodies: full details have been described elsewhere.<sup>6-9</sup> The incidence of myelodysplastic syndromes in the Trent Region was obtained from the referring hospitals and from the Leukaemia Research Fund Centre for Clinical Epidemiology (University of Leeds).

## Results

Twenty of the patients were men and 26 women, ranging in age from 51 to 82 years and from 46 to 90 years, respectively; the median ages were 71 and 75 years.

Patient details are given in the table. Eight patients had refractory anaemia (RA), 17 refractory anaemia with ring sideroblasts (RARS), 11 refractory anaemia with excess of blasts (RAEB), four chronic myelomonocytic leukaemia (CMML), five refractory

Table Results of immunohaematological studies on patients with myelodysplastic syndromes

		Increased red cell bound proteins as shown by DAGT				
Case No	Age Sex	Agglutination immunoglobulins	DAGT complement	Enzyme linked immunoglobulins	Autoantibody type and specificity: immunoglobulins in eluate	Irregular alloantibodies
<b>Refractory anaemia:</b>						
1	54 M	Nil	C3d	NT	Cold 'not anti-Ii or Pr' active at 30°C + warm (wk)	None detected
2	64 M	Nil	C3d	IgG + IgM	Cold anti-I active at 30°C + warm (wk)	Anti-Jk <sup>a</sup>
3	69 F	IgG1	C3d	IgG, also occasionally IgA + IgM	Warm + cold anti-I active at 30°C: IgG1 + IgG3 + occasionally IgM	Anti-E Anti-c + E Anti-E + C <sup>w</sup> + Lu <sup>a</sup>
4	76 M	IgG	C3d	NT	Warm + cold (wk) active at 18°C	None detected
5	77 F	IgG	C3d	IgG	Warm: IgG1	None detected
6	79 F	Nil	Nil	Nil	Warm (wk) + cold (wk)	None detected
7	82 M	IgG + IgA	C3d	IgG + IgA + IgM	Warm + cold anti-I active at 18°C: IgG + IgA	None detected
8	87 F	IgG1	C3d	IgG	Warm	Anti-E + C <sup>w</sup> + Wr <sup>a</sup> + Bg <sup>a</sup> + Bg <sup>b</sup>
<b>Refractory anaemia with ring sideroblasts:</b>						
9	64 M	IgG	Nil	IgG	Warm: IgG	None detected
10	64 F	IgG	C3d	IgG	Warm + cold (wk) active at 18°C: IgG	Anti-E + C <sup>w</sup> + V <sup>w</sup>
11	69 F	IgG1 + IgM	C3d	IgG + IgM	Warm + cold anti-I active at 30°C: IgG + IgM	None detected
12	70 M	Nil	Nil	IgM	Cold, active at 18°C + warm (wk): IgM	None detected
13	70 F	IgG1	C3d	IgG	Warm + cold (wk) anti-I active at 18°C: IgG1	Anti-E + Wr <sup>a</sup> + Kp <sup>a</sup> + Mi <sup>a</sup> /V <sup>w</sup>
14	70 F	IgG	C3d	IgG + IgM	Warm (wk) + cold (wk), active at 18°C	Anti-E + K + Bg <sup>a</sup>
15	72 M	Nil	C3d	IgG	Cold anti-I active at 25°C + warm (wk)	None detected
16	72 F	IgG1	C3d	IgG	Warm + cold (wk) anti-I active at 18°C: IgG1	None detected
17	73 F	IgG1 + IgG2 + IgG3	C3d	IgG	Warm, some evidence of anti-e specificity: IgG1 + IgG2 + IgG3	None detected
18	73 F	IgG	C3d	NT	Cold anti-I active at 30°C + warm	Anti-K + Le <sup>a</sup>
19	75 M	IgG1	C3d	IgG	Warm: IgG	Anti-C <sup>w</sup>
20	76 M	Nil	Nil	IgG	NT	NT
21	78 F	IgG1	Nil	NT	Warm: IgG1	Anti-E + K
22	78 M	IgG	C3d	NT	Warm	Anti-K
23	78 F	IgG1	C3d	NT	Warm	Anti-E + Lu <sup>a</sup>
24	87 F	IgG1	Nil	NT	Warm	Anti-E
25	89 F	IgG	C3d	IgG + IgM	Cold 'not anti-Ii or Pr' active at 30°C + warm (wk)	None detected
<b>Refractory anaemia with excess of blasts:</b>						
26	51 M	IgG1	C3d	IgG	Warm + cold (wk) anti-I active at 18°C: IgG	Anti-K + Le <sup>a</sup>
27	59 M	IgG1	C3d	IgG + IgA + IgM	Warm + cold anti-Ii active at 30°C: IgG1 + IgG2	None detected
28	62 M	IgG	C3d	IgG	Nil: IgG1	None detected
29	64 F	IgG	C3d	NT	Warm (wk)	Anti-Bg <sup>a</sup>
30	68 F	IgG	C3d	IgG + IgM	Warm + cold anti-I active at 30°C: IgG1 + IgM	None detected
31	70 F	IgG	C3d	IgG + IgM	Cold anti-I active at 30°C + warm (wk): IgG1 + IgM	None detected
32	71 F	IgG	C3d	IgG + IgA + IgM	Warm: IgG	None detected
33	74 M	IgG	C3d	NT	Warm (wk)	Anti-K + Bg <sup>a</sup>
34	77 F	IgG + IgM	C3d	IgG + IgM	Warm + cold anti-i active at 18°C: IgG1 + IgM	Anti-K
35	81 F	IgG	C3d	NT	Warm (wk)	None detected
36	90 F	IgG1	C3d	IgG	Warm + cold (wk) active at 18°C: IgG1	Anti-E + Wr <sup>a</sup>
<b>Chronic myelomonocytic leukaemia:</b>						
37	69 M	IgG1	Nil	IgG	Warm (wk): IgG1	Anti-D + E + Wr <sup>a</sup>
38	74 M	IgG	C3d	NT	Warm (wk)	Anti-E
39	79 M	IgG	Nil	IgG	Warm: IgG1	Anti-C + D + E
40	84 F	IgG1	C3d	IgG + IgA	Warm, some evidence of anti-e specificity + cold (wk) anti-HI active at 18°C: IgG	None detected
<b>Refractory anaemia with excess of blasts in transformation:</b>						
41	46 F	IgG	C3d	IgG	Warm: IgG1	Anti-C + D + K
42	62 M	IgG	C3d	IgG + IgM	Warm + cold anti-I active at 18°C: IgG1	Anti-E
43	63 M	IgG1	C3d	IgG	Warm (wk) + cold anti-HI active at 18°C	None detected
44	73 M	IgG	C3d	IgG	Warm (wk)	None detected
45	77 F	IgG	C3d	IgG + IgM	Warm + cold anti-I active at 18°C	Anti-E + K + Le <sup>b</sup>
<b>Unclassified myelodysplasia:</b>						
46	79 F	IgG1	C3d	IgG + IgM	Warm + cold anti-I active at 30°C	Anti-E + A <sub>1</sub>

DAGT = direct antiglobulin test, NT = not tested, (wk) = antibody weakly reacting

anaemia with excess of blasts in transformation (RAEB-t) and one patient had myelodysplasia which could not be classified.

Clinically important autoimmune haemolysis occurred in 15 patients and was classified as "warm," "cold," and "mixed" types, depending on the reaction characteristics of the autoantibodies (table).<sup>6,10</sup> Three patients with "warm" autoimmune haemolysis had

cold agglutinins detectable at 18°C, and in three patients with the "cold" type, weak reacting warm autoantibodies were also found. Immune mediated haemolysis was possibly present in a further 12 subjects, but not in the remaining 19.

Almost all patients had been given multiple transfusions of blood. Only four had not been transfused and the transfusion history of one was unknown.

Fifteen of the women had been pregnant, while the obstetric history of the other 11 could not be obtained.

Polyclonal increases of serum gammaglobulins were seen in 21 (46%) patients and homogeneous bands were evident on electrophoresis in four (8.5%) cases.

Autoantibodies to leucocytes and platelets were found in nine patients out of the 11 tested, while alloantibodies were detected in 13 subjects but not in four.

The overall incidence of new cases of myelodysplastic syndromes was 2.4 per 100 000 of the population per year; it increased noticeably with age, the figure being 11.1 for those over 60 years.

### Discussion

Forty six patients with the recently recognised association of erythrocyte autoantibodies, autoimmune haemolysis, and myelodysplastic syndromes were seen over 66 months. This association was seen in about 7% of patients with primary myelodysplasia and accounted for 2.6% of cases with erythrocyte autoantibodies referred during this time. The age range, with 87% being over 60, was similar to that of other studies.<sup>2 11-15</sup>

Autoantibodies were found in all types of myelodysplasia (table). The classification was made at the time of referral and did not imply static biological entities as change to a more malignant form occurred in several patients. For this reason, no decision could be made regarding the tendency towards the formation of autoantibody in any particular type of myelodysplastic syndrome.

The agglutination DAGT showed that IgG and C3d most often coated the red cells, though in 15 of 35 patients (43%) increased amounts of IgM or IgA could also be shown using the more sensitive enzyme linked DAGT (table).<sup>8</sup> In 22 out of 25 cases IgG1 was the only subclass of IgG present (table). The proportion of cases with a positive DAGT varied in other studies of myelodysplastic syndromes, with figures of five out of 134,<sup>4</sup> eight of 98,<sup>3</sup> and eight of 37<sup>5</sup> being reported—a far higher incidence, as is 7% in our present study, than that for the general population of similar age.<sup>16</sup> Compared with our series, more cases had solely IgG or complement bound to their cells, but only agglutination DAGTs had been used.<sup>3-5</sup>

In the 15 patients with firm evidence of autoimmune haemolysis the diagnosis had usually been suspected on finding difficulty in compatibility testing, a positive DAGT, strongly reacting autoantibodies, increased transfusion requirements not related to disease progression, reticulocytosis and low haptoglobin concentrations. Similar presenting features were noted in other reports.<sup>4 17 18</sup> When haemolysis was severe, diagnosis was not usually difficult, but in some cases the

evidence was insufficient to determine whether clinically important immune mediated erythrocyte destruction was occurring. Measurement of red cell life span may be unhelpful because it is known to be shortened in most patients with myelodysplasia.<sup>11</sup> The finding of reticulocytosis can be of value, however, as reticulocyte counts are usually normal or reduced in myelodysplastic syndromes.<sup>2 12</sup> It is important to diagnose autoimmune haemolysis in such patients as there is a good response to treatment and consequent improvement in well-being; in some cases an empirical trial of suitable treatment, such as prednisolone, may be warranted.

The increased incidence of erythrocyte autoantibodies in myelodysplastic syndromes may be one manifestation of disturbed immune homeostasis. Others include polyclonal increases in gammaglobulins (seen in 46% of our patients) in about 32% of patients with myelodysplasia generally,<sup>3 13</sup> and in 66% of 35 cases of chronic myelomonocytic leukaemia<sup>19</sup>; monoclonal gammopathies were present in 8.5% of our cases and in 12% in other series.<sup>3 13</sup> A reduction in number and function of T cells (particularly T helper) has also been found, together with abnormalities of natural killer and B cells.<sup>2 15 20-22</sup> The immune dysfunction in myelodysplastic syndromes may be related to the large number of blood transfusions, to non-specific stimulation of B lymphocytes by substances released from mononuclear phagocytes, or to the clonal defect causing activation of previously silent cell lines.<sup>3-5</sup>

Red cell alloantibodies were found in 26 patients (57%); this was not surprising in view of the history of multiple transfusions in all but four, and pregnancies in at least 15 of the 26 women. Anti-E and anti-K occurred most frequently, either alone or in combination with other alloantibodies (table). A similar pattern has been reported in other studies of patients with erythrocyte autoantibodies, though concomitant alloantibodies were less common.<sup>9 23 24</sup> Most of the patients had been transfused elsewhere, and had our policy of giving Kell negative blood of matched Rh phenotype been followed,<sup>9</sup> alloimmunisation would have been less common.

Leucocyte and platelet antibody investigations were carried out in a few instances. The high incidence of both auto- and allo-antibodies was not unexpected in view of the probable disturbed immune homeostasis and the histories of pregnancies and multiple transfusions. These investigations were made either because of cytopenia or, more commonly, following a febrile transfusion reaction.

We thank Drs G Bardhan, M J Brown, R A Cartwright, E A French, M L Ghosh, R M Hutchinson, P A E Jones, E C M Logan, D C Mitchell, V Mitchell, D

R Prangnell, S Sobolewski, K R Speed, R M Stewart, P C Taylor, J G Tetley, D A Winfield, J K Wood and P J Wyld for providing clinical details and Mrs P Burke for secretarial assistance.

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