Basophils in acute myeloid leukaemia

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SUMMARY Thirty four out of 750 patients entered into the Medical Research Council's (MRC) 9th Acute Myeloid Leukaemia Trial had more than 1% basophils (range 1-27%) often with bizarre granulation and primitive forms, a rare finding in this disease. Both normal and abnormal karyotypes were present including abnormalities of 6p, 12p, and the Philadelphia chromosome. Basophilia was found in both "monolineage" and "multilineage" leukaemias and the commonest French-American-British (FAB) classification group was M2, followed by M4. Basophilia did not seem to be associated with a worse prognosis, although cases with abnormalities of 6p died of disease that was resistant to first line conventional chemotherapy.

Basophils are rare in normal bone marrow, comprising less than 0.2% of all nucleated cells.¹ Their role in immediate hypersensitivity reactions is well known but they are not common in other diseases. Increased numbers may be found in myeloproliferative disorders, in particular, chronic myeloid leukaemia (CML), where an increase in numbers may herald the onset of blast transformation.² In 1984 Pearson drew attention to the presence of increased numbers of marrow basophils (> 1%) in association with a t(6;9) (p23;q34) translocation in acute myeloid leukaemia (AML)³ and in 1985 Daniel *et al* reported evidence of blast cell maturation towards basophils in cases of AML with a deletion of the short arm of chromosome 12.⁴ Basophils have also been noted in Philadelphia positive "de novo" AML.⁵ In 750 cases entered into the MRC's 9th AML trial only 34 cases showed 1% or more basophils of any stage of maturity in the bone marrow.

Material and methods

Patients were entered into the trial from several participating centres in the United Kingdom and Ireland, at the discretion of the local physician or haematologist.

Patients were randomly allocated to 1 + 5 or 3 + 10 DAT as induction chemotherapy. DAT chemotherapy consisted of daunorubicin 50 mg/m² by intravenous bolus on day 1 or days 1, 3, and 5; cytosine arabinoside 100 mg/m² by intravenous bolus 12 hourly for five or 10 days; and 6-thioguanine 100 mg/m² orally 12 hourly for five or 10 days. Patients entering remission were given 2 + 7 DAT then randomised to either MAZE (m-amsa 100 mg/m² intravenously, 5-azacytidine 100 mg/m², and etoposide 100 mg/m²

intravenously, each daily for five days), or COAP (cyclophosphamide 600 mg/m² intravenously, vincristine 1.5 mg/m² (maximum 2 mg) intravenously, cytosine arabinoside 100 mg/m² intravenously for five days and prednisolone 60 mg/m² orally for five days). After completing consolidation, patients were randomised for the last time to stop treatment, or continue with eight further monthly courses of cytosine and 6-thioguanine followed by four courses of COAP.

Bone marrow smears were routinely stained with Romanowsky, Sudan black B, periodic acid Schiff reagent (PAS), dual esterase and iron according to standard cytological methods.⁶ If sufficient slides were available smears were also stained with toluidine blue.⁷ Differential counts of 500 cells were performed on Romanowsky stained preparations and further 500 cell counts on toluidine blue stained smears if available.

Cytogenetic studies were performed in Cambridge or at the referring centre using standard culture methods and G-banding. Two patients with Philadelphia chromosomes presented as "de novo" AML but were withdrawn from the trial when the karyotype became known.

Results

Thirty four out of 750 patients entered into the trial showed more than 1% basophils in bone marrow smears and 20 patients (59%) achieved remission (64% for all patients (750) in the same trial). Median survival was about 282 days and median duration of remission has not been reached but is in the region of 18-24 months. Ten patients are still alive at the time of writing. Four patients (case 4, 29, 31, 34) died in remission during consolidation or maintenance

Table Karyotype and morphological details of patients with basophilia

Case No	Sex/ Age	White cell count	FAB	Cell lines affected	Percentage blasts	Percentage basophils	Morphology of basophils	Percentage of bone marrow cells toluidine positive	Karyotype	Re-mission	Survival (days)
1	M 33	8.2	NC	?G,Mega,B	60	27	P,G	30	46XYt(3;6)(q21;p21)	RD	71
2	M 23	17.7	2	G,Eo,B	39	26	P,G,D	ND	46XYt(9;22)(q34;q11)	RD	175
3	F 61	1	1	G,B	51	23	D,P	ND	46X+8,del121(q22)	ID	45
4	M 67	28.5	2	G,E,Mega,Eo,B	67	19	N,P,D	16	46XY	Y	42 (ICH in CR)
5	M 57	1.6	NC	G,E,Mega,B	49	14	G,P	29	42XY,-5,-16,-17,-18,-20+mar	Y	240+
6	M 52	6.1	2	G,Eo,B	70	14	P,N	13	46XY/47XY+8	Y	717
7	M 70	8.0	1	G,E,B	71	9	G,P	11	46XY,del17(?q24)	RD	36
8	M 53	3.8	4	M,G,Eo,B	33	8	G,P	ND	46XY/45X,-Y	Y	267+
9	M 18	34.1	2	G,B	71	7	N,P	ND	47XY+15,t(6;9)(p23;q24)	RD	48
10	M 42	4.1	2	M,G,B	59	6	G,P	8	Failed	Y	71+
11	M 51	1.3	2	G,Eo,B	67	6	P,G	7	Failed	ID	13
12	F 36	4.9	2	G,B	31	6	P,N	2	47XX+8	Y	150+
13	M 71	8.0	4	M,G,B	71	5	P,G	ND	Failed	Y	793
14	M 64	75.3	4	M,G,B	62	5	N,D	4	Failed	RD	177
15	M 64	27.7	NC	?M/G,B	88	5	P,G	ND	Failed	ID	18
16	M 66	3.3	2	G,B	54	5	N,P,G,D	5	45XY,-7,del(12)(p11;p13)	ID	33
17	F 66	1.1	2	G,E,B	60	4	N,P	7		Y	282
18	M 43	4.8	2	M,G,B	77	4	P,G	ND		Y	759+ (in 2nd CR)
19	F 67	9.0	NC	G,E,Mega,Eo,B	43	4	D,G,P	ND		Y	182+
20	M 43	7	7	Mega,B	61	3	N	ND	46XYt(9;22)(q34;q11)	RD(PR)	751
21	M 53	2.5	2	G,E,Mega,Eo,B	58	3	P,G	6		Y	408+
22	M 72	5.5	2	G,Eo,B	40	3	N,D	ND	46XY(12p-)	ID	20
23	M 72	40.8	NC	M,G,Mega,Eo,B21		3	P,G	ND	46XYiso dic(17)(p11-2)/47XYdic(17)+13	RD	141
24	M 43	8.0	4	M,G,Eo,B	68	3	N,D,P	3		RD	53
25	M 60	9.2	1	G,E,Mega,B	94	3	P,G,N	ND	46XY	Y	150+
26	F 53	10.0	4	M,G,Mega,B	82	3	G,P	ND	46XX	Y	334+
27	F 65	25.3	1	G,Mega,B	73	2	N,G,P,D	ND	45XX,4q-,5q-,t(6;12)(q13;p11),-7,11q+,-17,+E,21q+/?22q+	RD	64
28	M 31	148	2	G,B	52	2	G,N	ND		Y	1011+
29	F 57	24.6	4	M,G,E,B	42	2	G,N,P	5	47XX+21	Y	125 (in CR)
30	M 40	49.8	4	M,G,Eo,B	43	2	P,G	3		Y	560
31	F 56	65.7	4	M,G,E,Mega,B	69	1	N,D	2	46XX	Y	371 (in CR)
32	F 72	38.0	4	M,G,B	88	1	N	ND		Y	452
33	M 41	19.0	1	G,B	78	1	G,P	5	45XY,6q-,-16,-17+t(1;17)(q25;q11),21q+	Y	311
34	F 32	4.6	NC	B	99	0	P	30	Failed	Y	180 (in CR)

Cell lines: M = monocytic; G = granulocytic; E = erythroid; Mega = megakaryocytic; Eo = eosinophil; B = basophil; NC = not classifiable. Key: Morphology of basophils (listed in descending order of prevalence). N = normal; D = degranulated; G = heavily granulated; P = primitive; RD = resistant disease; ID = induction death.

therapy. Basic data including some morphological details and karyotypes are summarised in the table.

CLINICAL DETAILS

Twenty four men and 10 women are included in the data. Median age was 55 years (range 18–72 years) for all patients, 61 years for non-remitters and 56 years for remitters. Splenomegaly was seen in only five patients (cases 1, 2, 20, 28, 32). Case 32 had meningeal leukaemia at presentation and case 30 relapsed with central nervous system disease. Four patients were classifiable as “secondary” leukaemias. Case 14 had had pelvic radiotherapy for bladder carcinoma two years previously, case 10 had had a myeloproliferative disorder for nine months treated with hydroxyurea, case 27 had had carcinoma of the ovary treated with thiotepa and methotrexate three years previously and

case 32 had polycythaemia rubra vera treated with ³²P, busulphan, and chlorambucil over a period of 15 years. Case 12 presented with “appendicitis” and was found to have leukaemic infiltration of the bowel and case 2 had an intracerebral granulocytic sarcoma, which on examination of the biopsy specimen was Philadelphia positive. The data on cases 2 and 20 who were both Philadelphia positive is included in this report as they both presented as de novo AML, although we recognise that this may be the blast crisis of CML.

Five patients had skin diseases present at diagnosis which may or may not be associated with basophilia. These included case 13—dermatitis herpetiformis treated with dapson; case 14—psoriasis; case 24—lichen planus; case 25—erythema nodosum and patient 28—eczema (and asthma) since childhood.

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Case 11 had been treated for tuberculosis 10 months previously. None of the patients had urticarial or hyperpigmented rashes.

Median haemoglobin concentration was 7.8 g/dl (range 3.8–14.5 d/l), median white cell count was $8.6 \times 10^9/l$ (range 1.1 – $148 \times 10^9/l$), and median platelet counts were $49 \times 10^9/l$ (range 11 – $255 \times 10^9/l$). Distribution of haemoglobin, white cells, and platelets of cases with basophilia was similar to that of the de novo cases (750) in the trial. Only seven cases had basophils in the peripheral blood; cases 16, 17, 21, and 29 had 1% basophils, case 8, 3%, case 4, 11% and case 2, 32%.

MORPHOLOGY

All cases showed varying numbers of morphologically abnormal basophils (table and figures). Abnormal basophils could be divided into three groups: (i) degranulated forms (12 cases)—most severe in case 3; (ii) heavily granulated with large abnormal granules (23 cases)—most severe in cases 1, 2, 5 and 26; and (iii) primitive basophil precursors (27 cases)—most severe in cases 1, 2, 5 and 11. Cases 4, 6, 9, 14 and 20 showed clinically important numbers of “normal” basophils. Toluidine blue staining generally confirmed the numbers of basophils seen in Romanowsky stained preparations and permitted better assessment of gran-

All slides stained with Romanowsky stain unless otherwise stated.

1 Case 9 [t(6;9)]: blasts mainly agranular, but showing chunky basophil granules in blast (centre right), mature normal basophil (centre lower left), and probable basophil myelocyte (centre upper left).

2 Case 1 [t(3;6)]: agranular blast and primitive basophils with large abnormal granules. The centre basophil also has some residual basophilic cytoplasm visible.

3 Case 5: blast cells showing typical chunky basophil granules often localised.

4 Case 24 (46XY): agranular blast cells with large partially degranulated basophil.

5 Case 16 (12p-): Sudan black stain showing metachromatic pink staining of a few basophil granules.

6 Case 10: from left to right—mature granulated (but heavily stained) basophil, basophil precursor with extensive small basophil granules, agranular blast, eosinophil promyelocyte and monocytoid cell (bottom).

7 Case 16 (12p-): Sudan black stain showing mainly negative basophil with a faint scattering of metachromatic granules, an eosinophil metamyelocyte, and two neutrophil metamyelocytes.

8 Case 10: Toluidine blue stained smear showing sharp contrast between positive basophils (with normal granulation in this case) and negative blast cells.

9 Case 9 [t(6;9)]: PAS stained smear with negative blast cells but typical mature basophil with heavily positive coarse granules.

10 Case 4 (46XY): agranular blast cells and possible eosinophil-basophil (top left).

- 11 Case 24 (46XY): agranular blast cell, two relatively normal basophils, and an unidentifiable cell.
- 12 Case 34: PAS stained smear showing coarse PAS positive granules compatible with, but not diagnostic of, a pure basophil leukaemia.
- 13 Case 34: blast cell with a few coarse localised granules.
- 14 Case 10: possible hybrid eosinophil-basophil.
- 15 Case 16 (12p-): Toluidine blue stained smear showing heavily granulated basophil with large abnormal granules.
- 16 Case 22 (12p-): degranulated basophil.
- 17 Case 16 (12p-): atypical basophil but with relatively normal granulation.
- 18 Case 16 (12p-): primitive basophil with typical chunky or angular granules.
- 19 Case 7 (del 17): mature basophil with broad lobulated nucleus and large discrete basophil granules.
- 20 Case 15: polyploid mature basophil with abnormal granulation.
- 21 Case 34: Toluidine blue stained smear showing metachromatic nature of granules in blast cells.

ule size. A Romanowsky stained smear from case 34 showed a cluster of chunky basophilic granules in about 20% of blasts; the granules stained weakly with toluidine blue, metachromatically with Sudan black, and positively with PAS. This case was felt to be a pure basophil leukaemia of primitive origin (figs 12, 13, 21).

Neutrophil granulocytic involvement was seen in all but three cases. Case 34 is described above, case 15 had an undifferentiated leukaemia with blast cells that were negative for Sudan black, PAS, and dual esterase but had 5% basophils in the marrow, and case 1 had blast cells which were negative for Sudan black, PAS, and dual esterase but 5% were positive for α -naphthyl acetate esterase. Auer rods occurred in 13 cases.

Eleven patients had probable eosinophil disease with increased numbers of precursors and mature forms containing large round mixed eosinophilic and basophilic granules as in the *inv* 16 abnormality. Case 30 had severe eosinophilia (39%). In 14 cases cells were seen with both eosinophil or basophil granules in the same cell, possibly representing combined eosinophil-basophils (fig 14). Here the basophil granules were different from the basophil granules seen in the eosinophils in *inv* 16, in that they were not so round and darker in colour.

Ten patients had probable erythroid disease, nine of whom (cases 4, 5, 7, 17, 19, 21, 25, 29, 31) had some PAS positive erythroblasts. Increased numbers of abnormal megakaryocytes (micromegakaryocytes, megakaryoblasts, multiple separate nuclei) were seen in 10 patients.

The two cases (cases 1 and 9) with abnormalities of 6p23-p21 and the three cases with abnormalities of 12p (cases 16, 22 and 27) had similar blasts with round nuclei, fine chromatin, and agranular basophilic cytoplasm. A few blasts in each case showed a cluster of chunky basophilic granules. None of these cases showed Auer rods either with Romanowsky or Sudan black stains. Blast cells from case 1 (t(3;6)) were Sudan black negative, but cases 9, 16, 22 and 27 showed 27%, 43%, 40% and 18% with localised sudanophilia, respectively. Cases 1, 9, and 27 were negative with dual esterase stains but cases 16 and 22 showed 20% and 40% chloroacetate esterase positively. Basophils stained variably with Sudan black including metachromatically but were all strongly PAS positive with a typical coarse granular block pattern. Erythroid precursors were rare in all cases but cases 16 and 22 also showed 6% eosinophils. Cases 1 and 27 showed increased numbers of megakaryoblasts and micromegakaryocytes.

Discussion

Increased numbers of basophils (with normal morphological detail) have been associated with a t(6;9)

translocation in AML³ but have not been seen in all cases with t(6;9).⁸⁻¹⁰ Initially it was thought that basophilia may have been due to the involvement of 9q34 and the *abl* oncogene as basophilia is common in CML. Two patients in this series (cases 1 and 9), however, had karyotypic abnormalities of 6p23/6p21, suggesting that the 6p breakpoint may be more strongly associated with basophilia than 9q34 in AML. Case 1 of this series has been reported in detail elsewhere.¹¹

Fleishman initially drew attention to the presence of a common breakpoint at 6p23 in myeloid leukaemias in a series of five cases which included two with t(6;9) and a case of myelomonocytic leukaemia with t(X;6)(p11;p23).¹² No comment on the presence or absence of basophils is made in this paper, although the two cases with t(6;9) were later included in the series of nine cases by Pearson associating t(6;9) with basophilia.³ Pearson also gave the karyotypes of five other patients with increased marrow basophils (range 1.75-6.0%). They were as follows: case 83, 46XY, -2, -5, -16, +3 mar; case 39 46XY/45XY -7; Patient 166 46XY; case 170 45XY-7, del(12)(p11p13)/44XY -7, -13, del(12); and case 168 46Xdel(Y)(q12), inv16(p13q22). There seem to be some chromosomal abnormalities in common between these cases and our series and the deletion of 12p has already been associated with basophilia.⁴

Rothenberg reported two cases of AML developing from Philadelphia negative myeloproliferative disorders with prominent basophilia.¹³ One of these cases had a normal karyotype and the other trisomy 8. It is very unlikely, despite three patients in our series also having trisomy 8, that this abnormality is associated with basophilia as trisomy 8 is the commonest chromosomal abnormality in haematological malignancies whereas basophilia is relatively rare.

A case of megakaryoblastic leukaemia in a child with Down's syndrome—48XY+11+21,t(1;15) (breakpoints not specified)—has been described,¹⁴ which on culture differentiated into basophils. The diagnosis of megakaryoblastic leukaemia was based on the distribution of platelet peroxidase (PPO) in the perinuclear space and endoplasmic reticulum on ultrastructural studies, the blasts being undifferentiated and cytochemically negative on light microscopic examination. A similar pattern of PPO with basophil/mast cell granules in the same cell was found in a few cases of blast cell crisis of CML in a series of 41 patients studied by Parkin *et al.*¹⁵ Some cases of lymphoid blast crisis (CALLA positive) had basophil/mast cell granules ultrastructurally and some "lymphoid" blasts contained large azurophilic granules which also stained with toluidine blue and PAS.¹⁵ In another study basophil/mast cell granules were seen ultrastructurally in five cases of ALL with a t(4;11)

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abnormality.¹⁶ This suggests that primitive basophil leukaemias may well be underdiagnosed.

Wick also commented on the fact that basophil differentiation may be missed and patients erroneously classified as having ALL.¹⁷ Wick described four cases, similar to case 34 in this series, which had a small percentage of blasts with azurophilic granules but the blasts were negative for MPO, Sudan black, PAS, esterase and positive for toluidine blue and acid phosphatase. Three of the four cases had a normal karyotype. Recognition of basophil differentiation by metachromatic staining with toluidine blue is important so that the correct chemotherapy can be given.

Bone marrow basophilia was found in six out of 375 cases reviewed by May and Waddell,¹⁸ three of whom had non-malignant conditions and had 1.5%, 2%, and 9% marrow basophils. The authors speculated that bone marrow basophilia in dysplasia and leukaemia may reflect a local response of normal marrow lymphocytes to long term exposure to abnormal secretory or cell surface antigens of the leukaemic cell population, leading to increased T cell derived factors. The *in vitro* differentiation of basophils from marrow precursors has been shown to be enhanced by the presence of T cell derived factors.¹⁹ One factor, termed basophil-like cell promoting activity,²⁰ stimulates long term growth and also induces differentiation of cells which morphologically and functionally resemble basophils. A second factor, which in contrast to the previous factor stimulates murine IL-3 dependent cells,²⁰ induces the growth of human meta-chromatically staining cells of an immature morphology with a certain resemblance to mast cells. Basophil-like cell promoting activity does not share biochemical similarities with other well defined human growth factors, while the IL-3 activity has strong resemblance to pluripotential haemopoietic CSF (m-CSF/IL3).²⁰

None of the 34 in this series had increased marrow lymphocytes suggestive of a role for T cell driven proliferation of basophils, but immunophenotyping was not performed. Neither was there a notable increase in clinically important infections at diagnosis which might stimulate the immune system. In case 28 the basophilia may have been related to his concomitant asthma and eczema.

The basophilia in some of these patients, particularly those with a relatively low percentage, may be a result of stimulation by non-lineage specific growth factors or gene products. Multi-CSF or IL3 has been located to 5q23-q31,²¹ which does not seem to have a specific role in this series. Those cases with the most noticeably increased numbers of basophils may be stimulated by more specific growth factors. Serum factors have been shown to have no effect on basophilopoiesis in the culture of marrow from patients

with CML, however,²² and it was suggested that increased basophil production was the result of a primary change in the transformed cell.

At present the chromosomal location of any basophil growth factor is not known, but the cytogenetic data suggest that the short arms of chromosomes 6 and 12 may be candidate regions. Genes already associated with 6p21 include tumour necrosis factors α and β ,²³ which help mediate the acute phase response and may also be cytotoxic to certain tumour cells, and the genes for the major histocompatibility complex (MHC); but their relevance to basophilia is not obvious. An oncogene *c-kirsten ras 2* has been assigned²⁴ to 12p11-1-p12-1 and may be relevant to the pathogenesis.

Recently the MIC Cooperative Group²⁵ proposed a complex classification of AML incorporating morphology, immunophenotype, and cytogenics. Specific subgroups designated M2 baso/12p- and M1 t(9;22) have been proposed. The Philadelphia positive cases in our series did not fit this nomenclature, being M7 and M2, respectively, although the cases with a deletion of 12p(p11;p13) were classifiable as M2. Four other cases entered into the AML trial had abnormalities of 12p but showed no proliferation or abnormalities of basophils. The brief karyotypes of the patients were as follows: man aged 33, 46XY/45XY, dic(12;21)(p11-2;p13); man aged 71, t(12;17)(p12,q11) + complex; woman aged 64, t(?;12)(?p?) + complex; and man aged 64 t(3;12)(p21;p12?p13) + complex. The last case had pronounced eosinophilia (52%) and is similar to other patients with eosinophilia and abnormalities of 12p13 described by Reese *et al.*²⁶ Abnormalities of 12p12 have also been found in 23 cases of ALL,²⁷ although many of them were CALLA positive, ultrastructural studies looking for basophil granules were not done, and it is not possible to say whether any of these cases were basophil leukaemias that were misdiagnosed, a possibility commented on by Wick.¹⁷ In the original series by Daniel⁴ only five of 16 patients with abnormalities of 12p had basophil disorders, four of whom had deletions and one a translocation at 12p13. Although specific deletions of No 12 at p11p13 may be associated with basophilia, breakpoints of 12p13 (or p11/12) do not seem to be associated with basophilia.

Basophilis do not seem to be associated with a worse remission rate but those cases with chromosome abnormalities of 6p and 12p do seem to fare badly. The two cases with abnormalities of 6p had disease that was resistant to conventional first line treatment. Cases of AML with abnormalities of 6p^{3 8 10 12 28-33} have done poorly with only eight out of 22 achieving remission, which was shortlived. The longest survivor relapsed after allogeneic bone marrow transplantation and died 18 months after diagnosis. At the moment

there are two few documented cases with deletions of 12p to assess the prognosis.

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