CYTOGENETIC ABNORMALITIES IN PATIENTS WITH SEVERE APLASTIC ANEMIA

Natalia Mikhailova, Mario Sessarego, Giuseppina Fugazza, Attilio Caimo, Sandor De Filippi, Maria Teresa van Lint, Stefania Bregante, Antonella Valeriani, Nicola Mordini, Teresa Lamparelli, Francesca Gualandi, Domenico Occhini, Andrea Bacigalupo

Divisione Ematologia II and Servizio Citogenetica, Ospedale San Martino; Dipartimento Medicina Interna (DIMI) Università, Genoa, Italy

ABSTRACT

Background. Cytogenetic abnormalities have been described in a few patients with otherwise typical severe aplastic anemia (SAA), and the possible clonal nature of this disease is a controversial issue.

Materials and Methods. Sixty-nine patients with acquired severe aplastic anemia underwent cytogenetic examination on bone marrow cells at the time of diagnosis (n=34) and/or at least twice after immunosuppressive therapy (IS) (n=35).

Results. We identified 2 major groups. Group A: 51 patients (74%) were normal and remained normal. Group B: 18 patients (26%) had at least one abnormal cytogenetic analysis. This second group could be further subdivided as follows: (B1) chromosomal abnormalities not present at first examination and acquired in the course of the disease (n=7); (B2) clonal cytogenetic abnormalities present at first examination and persisting (n=3); (B3) reversible cytogenetic abnormalities (n=8). The most frequent abnormality was trisomy 8 (n=8) followed by monosomy 7 (n=2); 82% of patients are alive in group A and 61% in group B. Three patients developed acute leukemia, all from group B. This represents 4% of all patients or 17% of those with at least one abnormal cytogenetic test.

Conclusions. Thus the majority of SAA patients have normal karyotypes in marrow cells at presentation and at follow-up. Patients with abnormal karyotypes exist and can be further subdivided into those with reversible and those with persistent abnormalities. The latter are at risk of developing myelodysplasia or acute leukemia.

Significant progress has been made over the past decade in the understanding of severe aplastic anemia (SAA): quantitative stem cell defects,[1] defective microenvironment,[2,3] abnormal cellular interactions[4] and immune suppression of bone marrow function[5,6] have all been described and postulated as being relevant to the pathogenesis of marrow aplasia.[7]

The key question is the following: is the defect *intrinsic* or *extrinsic* to the stem cell. Marsh and coworkers[8] showed that purified CD34+ cells from SAA patients were unable to sustain long-term *in vitro* hematopoiesis, even when grown over normal stromal cells, and even after correction for their numerical deficiency. At the same time van Kamp and coworkers[9] reported a high rate of SAA females with clonal hematopoiesis. Shortly thereafter a study by the EBMT appeared on the high risk of developing hematologic malignancies in SAA patients treated with anti-lymphocyte globulin (ALG) and/or cyclosporin (CyA).[10] Finally, the Ulm group highlighted the

high percentage of patients with defective expression of GPI-linked proteins,[11] confirming the possible association between SAA and the well-known clonal disorder paroxysmal nocturnal hemoglobinuria (PNH). Taken together, these observations suggest that aplastic anemia is a preleukemic disorder with clonal hematopoiesis and defective stem cells. However, this intepretation may be restrictive because clonal does not necessarily mean leukemic or malignant; clonality may be the result of extrinsic selection rather than genetic mutation.[12]

One way of looking at this problem is to assess whether clonality is present at diagnosis of the disease, or whether a clone emerges after IS therapy during follow-up. The use of X-chromosome inactivation patterns to identify clonal hematopoiesis has been a matter of debate,[13] and a very recent contribution suggests that most SAA patients have polyclonal hematopoiesis.[14] Cytogenetic analysis is another method of marking clonal hematologic disease, and cytogenetic abnormalities have been described in a few patients with otherwise typical AA.[15]

We now report an analysis of cytogenetic data from 69 patients with SAA at diagnosis and during or after the first course of immunosuppressive therapy.

Patients and Methods

Patients

Sixty-nine patients with SAA underwent cytogenetic examination between 1987 and 1995. The median age was 30 (range 7-70); 38 were males and 31 females.

The first cytogenetic examination was performed at diagnosis prior to the first IS treatment in 34 patients, while 35 patients were analyzed for the first time during or after IS therapy, with a median follow-up time of 3 years (range= 0.5 to 11) after diagnosis. Diagnosis of SAA and response to treatment were established according to published criteria.[16] AA was considered severe if it presented a hypocellular bone marrow and cytopenia with at least two of the following criteria: a granulocyte count below 0.5×10^9/L, a platelet count below 20×10^9/L and/or a reticulocyte count below 2×10^9/L. A marrow biopsy was carried out in all patients at diagnosis.

Treatment

All patients were given immunosuppression (IS): 40 were treated with antilymphocyte globulin (ALG) with or without 6-methylprednisolone (6MP) and with or without androgens, as previously described;[17] 8 received only 6MP, and 21 patients were given ALG + CyA + corticosteroids + growth factors, as described.[18,19]

Cytogenetics

Cytogenetic examinations were performed using unstimulated 24-48-hour bone marrow cultures. The most commonly used procedures were Q-banding and R-banding; 15 or more metaphases were analyzed at each examination. A cytogenetic clone was thought to exist when two or more cells had the same structural chromosome change or extra chromosome. At least three cells with the same missing chromosome were considered to constitute a clone.[20]

Results

Sixty-nine patients with SAA underwent cytogenetic examination at the time of diagnosis (n=34) and/or at least twice after IS therapy (n=35). Two major groups were identified regarding the patterns of chromosome changes (Table 1).

Group A: normal-normal pattern. Fifty-one out of 69 patients (74%) always presented normal cytogenetics (Table 1). Twenty-four of them were investigated for cytogenetic abnormalities

Table 1. Patterns of cytogenetic changes.

Group	Cytogenetics	No. of pts.	(%)	Alive (%)
A	normal - normal	51	(74)	41 (80)
B1	normal - abnormal	7	(10)	4 (57)
B2	persistent cytogenetic abnormalities	3	(4)	0
B3	reversible cytogenetic abnormalities	8	(12)	7 (87)

at presentation of SAA and twenty-seven after IS therapy was completed; 41 of these 51 patients (80%) are alive. Five patients failed to achieve remission and are alive but transfusion dependent. Ten patients died: nine with complications due to cytopenia and 1 of an HIV infection acquired from her partner.

Group B included 18 patients (26%) with at least one abnormal chromosome analysis and was further subdivided into:

Group B1: normal - abnormal pattern. 7/69 patients (10%) were normal at the first cytogenetic examination but developed an abnormal karyotype during the course of the disease that persisted during follow-up (Table 1). Four patients showed trisomy 8, one monosomy 7, one i (7q) monosomy 19, and one trisomy 14 (Table 2). Three patients achieved transfusion independence and survive with a cytogenetic abnormality but no clinical or hematological features of MDS. Four patients were resistant to IS therapy. One of them (trisomy 8) is alive with supportive care. Another (monosomy 7) died of

SAA complications, and two developed acute leukemia [i (7q), monosomy 19 and trisomy 8].

Group B2: persistent cytogenetic abnormalities. Three out of 69 patients (4%) were abnormal at the first examination and remained abnormal. One of them was examined at the time of diagnosis and two after IS therapy. The chromosome changes were the following: trisomy X, trisomy 8, monosomy 7. There were no responders in this group. Two patients (trisomy X, trisomy 8) died with cytopenia early in the course of the disease. MDS transformed into acute leukemia in one patient (monosomy 7).

Group B3: reversible cytogenetic abnormalities. Eight out of 69 patients (11%) showed transient cytogenetic abnormalities. Three of them (37%) with cytogenetic abnormalities at first examination improved hematologically and cytogenetically after IS therapy and have not been transfused for more than 3 years. In two patients the abnormal clone disappeared within the first year of IS therapy and in one patient, 2 years after IS. Peripheral blood counts and bone marrow mor-

Table 2. Characteristics of cytogenetic abnormalities in 18 patients with at least one abnormal karyotype.

Group	Pts	time of first cytogen. test	first cytogenetics	last cytogenetics	chromosome abnormality	time from Dx (years)	number of abn cytogen.tests	max% abn. metaphases	out-come	cause death	days FU from Tx
B1	1	at FU	N	ABN	trisomy 8	4	7	100	a		3541
B1	2	at FU	N	ABN	monosomy 7	7	5	70	d	AA	2656
B1	3	at FU	N	ABN	trisomy 8	5	6	50	a		3335
B1	4	Dx	N	ABN	trisomy 14	1	1	15	a		2272
B1	5	Dx	N	ABN	trisomy 8	< 1	3	40	d	AL	335
B1	6	Dx	N	ABN	trisomy 8	1	1	80	a		432
B1	7	Dx	N	ABN	i (7q), monosomy 19	1	1	100	d	AL	242
B2	8	at FU	ABN	ABN	trisomy 8	3	8	100	d	AA	2898
B2	9	at FU	ABN	ABN	monosomy 7	5	11	100	d	AL	2488
B2	10	Dx	ABN	ABN	trisomy X	0	2	20	d	AA	1
B3	11	at FU	N	N	monosomy 16	4	2	15	d	AA	2794
B3	12	at FU	N	N	monosomy 12	9	1	5	a		3997
B3	13	at FU	ABN	N	monosomy 21	5	1	15	a		4315
B3	14	Dx	ABN	N	trisomy 8	0	1	10	a		21
B3	15	Dx	ABN	N	del 5q	0	4	60	a		1274
B3	16	Dx	N	N	trisomy 8	2	1	15	a		1056
B3	17	Dx	N	N	trisomy 8	< 1	2	20	a		1378
B3	18	Dx	N	N	monosomy 3	2	1	15	a		2366

Abbreviations: pts = patients; FU = follow-up after IS therapy; Dx = at diagnosis; Tx = treatment; N = normal; ABN = abnormal; a = alive; d = dead; time from Dx = interval between diagnosis and first cytogenetic abnormality; AL = acute leukemia. ; AA = aplastic anemia.

phology confirmed complete hematologic reconstitution. Five out of the 8 patients (63%) were normal at first chromosome examination: four at presentation of SAA, one after IS therapy. In two patients a cytogenetic abnormality (monosomy 16, trisomy 8) was detected twice during the course of IS; in three others (monosomy 12, trisomy 8, monosomy 3) it was found only once, after IS was completed. Overall, 15% of metaphases carried a chromosome abnormality. Subsequent examinations showed a normal karyotype. Seven of the 8 patients (87%) responded to IS therapy and are alive 21-3997 days from the initiation of IS therapy to the last cytogenetic examination in PR/CR. One patient (monosomy 16) was resistant and died of SAA complications.

Discussion

Cytogenetic studies on marrow samples are not commonly performed in patients with marrow aplasia, mainly because of the difficulties in obtaining sufficient cells for analysis, especially at the onset of the disease. There are thus few publications on cytogenetic abnormalities in acquired AA. In one large series chromosomal abnormalities were found in 4% of 183 cases.[15]

We have shown in this study that cytogenetic patterns can vary greatly over time in patients with acquired SAA treated with immunosuppressive therapy. We could identify two major groups: one with normal karyotypes at presentation and during the course of the disease, and a second presenting clonal chromosomal abnormalities in at least one cytogenetic analysis at some time point before and/or after immunosuppressive therapy.

The majority of patients, 74%, fell into the first group; the median follow-up is over 5 years and most of them have been studied at least once a year. There were no cases of myelodysplasia or leukemia in this group. This would indicate that a large proportion of patients have normal karyotypes both at presentation and during follow-up, and do not show the emergence of abnormal clones. This is in keeping with the recent finding of polyclonal hematopoiesis in the majority of females with X-linked polymorphism studied,[14] and with the hypothesis that a defect extrinsic to the stem cell is involved in the pathogenesis of the disease.[12]

The second group of patients (26%) showed clonal chromosomal abnormalities in at least one cytogenetic analysis, either at diagnosis of the disease or during follow-up. The most common cytogenetic abnormality was trisomy 8, which was seen in eight patients: 1 at diagnosis, 7 during follow-up, with the percentage of metaphases ranging from 10% to 100%. Two patients were young (7 and 23 years old), and six were elderly (50 years old or over). The two young patients died, one of leukemia and the other of cytopenia, while the six elderly patients survive 184 to 2265 days from detection of the first abnormal karyotype. It should be noted that 3/6 improved their peripheral blood counts, in particular the platelet count, at the time when trisomy 8 was detected. After the first detection there was a trend toward an increase of +8 metaphases with time. One patients showed 10% in 1990 and 100% +8 metaphases in 1994. Five out these 6 patients are transfusion independent, suggesting that hematopoiesis compatible with life can be carried on for a considerable amount of time by stem cells with the +8 marker. One unexpected finding was the disappearance of the clonal abnormality in some patients. One patient presented with del 5q (60% of metaphases) in September 1991 after achieving a partial remission with cyclosporin A; the percentage of del 5q metaphases was 40% in February 1992, 0% in July 1993, 100% in January 1995 and 0% in May 1995. Although these results may be the expression of sampling error, they suggest two considerations: the trend is not always from 0% to 100%, and there may be times in which stem cells with a normal karyotype predominate. This in turn points to the coexistence of normal and abnormal stem cells, in keeping with data recently reported for patients with myelodysplasia.[21] It also suggests that our capability of detecting abnormal clones depends on the number of marrow cells with a normal karyotype: the greater this number, the less our chance of identifying clonal abnormalities. If one can expand normal cells by means of immunosuppressive therapy, then the abnormal

clone may be reduced or even disappear.

Finally, one should also consider the hypothesis that in some patients an abnormal clone may exist or emerge and take over normal hematopoiesis, eventually terminating in acute leukemia. The actuarial risk of developing a malignant hematologic disease after IS therapy is high.[22,23] We observed 10 cases (group B1 and B2) with stable chromosome abnormalities and an extremely low rate of hematologic recovery; only 2 patients (both with trisomy 8) became transfusion independent. Acute leukemia following MDS occurred in three of these patients (37%). The persistent chromosomal abnormality and the high incidence of clonal malignant diseases is in keeping with intrinsic stem cell damage in this group of patients.

In conclusion, our results suggest that the majority of SAA patients have normal karyotypes in marrow cells at presentation and at follow-up. Patients with abnormal karyotypes exist and can be further subdivided into those with reversible and those with persistent abnormalities. The latter, rather than the former, are at risk of developing myelodysplasia or acute leukemia, and this might be relevant to the use of myeloid growth factors such as G-CSF.[24]

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