



## Cytogenetic characterization of acute myeloid leukemia in Shwachman's syndrome. A case report

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### ABSTRACT

We report on a case of acute myeloid leukemia in a 17-year old boy affected by Shwachman Diamond syndrome (SDS). Conventional cytogenetics at diagnosis revealed an abnormal clone with complex karyotypic changes including typical myeloid aberrations, such as monosomy 5, tetrasomy of chromosome 8, trisomy 9, and deletion of the short arm of chromosome 12. The boy was treated with conventional chemotherapy and reached complete remission of leukemia, confirmed by cytogenetics and fluorescence *in situ* hybridization. Nevertheless he failed to regenerate normal marrow cellularity and blood cell count. Cytogenetic information on hematologic malignancies in SDS patients are discussed.

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Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, somatic abnormalities (particularly metaphyseal dysostosis), and bone marrow dysfunction. It was first described in 1964 by Shwachman *et al.*<sup>1</sup> and is now recognized as the second most frequent cause of pancreatic insufficiency in children. Hematologic abnormalities consist of constant or intermittent neutropenia, associated in 50% of cases with megaloblastic anemia and in 70% of cases with thrombocytopenia. Furthermore, raised values of fetal hemoglobin (HbF) and impaired chemotaxis have been described.<sup>2</sup> The bone marrow can be normo- or hypo-cellular. Sometimes the syndrome is characterized by remarkable vacuolization of erythroid and myeloid precursors, hemosiderosis, and ringed sideroblasts.<sup>3</sup> SDS can therefore be considered as a constitutional marrow failure syndrome. Patients have an increased frequency of myelodysplasia and leukemic transforma-

tion. A recent analysis of 21 patients with SDS showed that the incidence of myelodysplasia and transformation to acute myeloid leukemia is 33% and 24%, respectively.<sup>4</sup> A few cases of acute lymphoblastic leukemia have also been described<sup>5-7</sup> as well as one case of a juvenile form of chronic myeloid leukemia.<sup>8</sup> When leukemia develops, due to the preceding bone marrow failure, serious complications may be induced by chemotherapy and, because of the underlying organ dysfunctions, these patients should be managed with extreme caution when treated with allogeneic bone marrow transplantation.<sup>9-13</sup> We describe here the cytogenetic characterization and clinical course of an acute myeloid leukemia occurring in a young patient affected by Shwachman's syndrome.

### Case report

The patient was investigated for failure to thrive in early childhood. Diagnosis of SDS was made at the age of thirteen months and he was treated with pancreatic supplements. Recurrent infections were not noted from the past history. In 1993, when he was 14 years old, a mild pancytopenia was evidenced: WBC  $3.9 \times 10^9/L$ , PMN  $1.2 \times 10^9/L$ , Hb 118 g/L, Plts  $127 \times 10^9/L$ . Neutrophil chemotaxis was within the normal range, HbF was just above normal range (2.8% of total hemoglobin). At that time radiography showed growth retardation, metaphyseal chondroplasia, and short stature. In June 1997, when he was 17 years old, he was diagnosed as having an acute myeloid leukemia, M2 according to the FAB classification. Blood cell count was as follows: Hb 8.4 g/dL, WBC  $1.4 \times 10^9/L$ , with 8% myeloid blasts, Plts  $9 \times 10^9/L$ . Marrow aspirate showed high cellularity with massive infiltration by blast cells with a high nucleus/cytoplasmic ratio and a basophilic hypergranulated cytoplasm, an irregularly shaped nucleus with homogeneous chromatin, and rare nucleoli. Marrow blast cells were Sudan black and naphthol ASD acetate esterase positive. The blast population displayed the following immunophenotype:

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CD13 85%, CD33 94%, CD34 14%, CD11b 81%, CD14 61%, CD2 24%, CD3 19%, CD20 neg, CD22 neg, CD7 50%. A bone marrow sample was processed for cytogenetic analysis after 24h cultures. Chromosomes were G banded using Wright's stain. A complex karyotype was found in 12 out of 15 metaphases: 49-52, XY, +X, -5, +8, +8, +9, del(12p), -13, +mar1-4 (cp12) (Figure 1). The constitutional karyotype on PHA-stimulated peripheral blood cultures was 46,XY. Treatment was started according to the ongoing protocol for acute myeloid leukemia: LANL 93, GIMEMA random 2 (mitoxanthrone + aracytin + etoposide). The period of aplasia was characterized by numerous complications, including persistent fever despite empiric antibacterial and antifungal therapy, respiratory insufficiency, impaired kidney function, hematemesis, and melena. Marrow recovery was extremely slow. The patient was considered in remission 51 days after the beginning of chemotherapy, in the presence of peripheral cytopenia and hypocellular bone marrow without evidence of blast cells. Conventional cytogenetics at that time showed a normal karyotype and fluorescence *in situ* hybridization (FISH) with a biotin-labeled centromeric alpha-satellite probe for chromosome 8 (D8Z1, Oncor, Gaithersburg, MD, USA) only showed disomic nuclei. On day +66 the boy was admitted to undergo a course of chemotherapy with aracytin and idarubicin. Two months later

he was still pancytopenic and in a poor general condition while the bone marrow remained morphologically and histologically free of leukemia. He refused further consolidation chemotherapy. In March 1998 he developed a progressive anemia which required red cell transfusions. In September 1998 severe thrombocytopenia ( $<7 \times 10^9/L$ ) was also noticed. A marrow aspirate in December 1998 showed absence of blasts and only disomic nuclei with a centromeric FISH probe for chromosome 8. At the last follow-up, in September 1999, the patient was in a good clinical condition. His blood cell count was: WBC  $3.5 \times 10^9/L$  (neutrophils = 57%), Hb 101 g/L, Plts  $35 \times 10^9/L$ .

### Discussion

The primary defect responsible for SDS is unknown. A spectrum of clinical abnormalities has been described, including metaphyseal dysostosis, epiphyseal dysplasia, immune dysfunction, liver disease, growth failure, renal tubular defects, insulin-dependent diabetes mellitus, and psychomotor retardation. Bone marrow dysfunction and pancreatic insufficiency, however, are constant. Speculations about an *in utero* insult during development of both bone marrow and pancreas at around the 5th month of pregnancy have been put forward.<sup>14</sup> Similarly to other congenital bone marrow disorders, such as Fanconi's anemia, SDS is an interesting

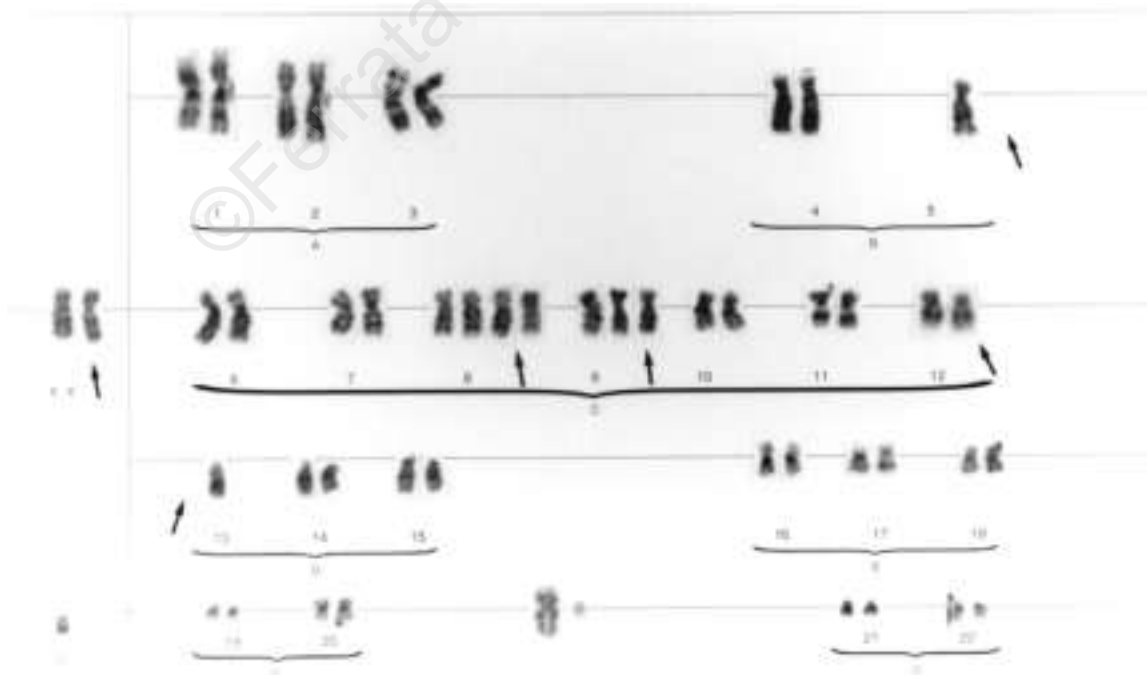


Figure 1. G-banded bone marrow karyotype at time of diagnosis of acute myeloid leukemia.

Table 1. Cytogenetic abnormalities reported in patients with Shwachman-Diamond syndrome and hematologic malignancies.

Patient n.	Hematologic Diagnosis	Age*/sex (years)	Preceding MDS	Karyotype	Treatment/response	Survival > 1 year	Ref.
1	AML-M6	42/M	+	46,XY,der(20)(q11)	n.r./-	n.r.	4
2	AML-M5	5/M	+	47,XY,der(7)t(4;7)(q31,q11),+21	Allo-BMT/+	+	4
3	MDS	4/M		46,XY,i(7q)	n.r.	n.r.	4
4	MDS	11/F		46,XX,der(7)(q11.2q32)(not clonal)	Allo-BMT/+	n.r.	4
5	AML-M5	11/M	+	47,XY,+1, del(9)(q22)	n.r./-	n.r.	4
6	AML-M2	8/M	+	46,XY,add(11p),-15,-22,+mar1,+mar2	n.r./-	n.r.	4
7	LAL-L1	1.9/M	-	53,XY,+G,+G	Vcr+Pdn+L-Asp/+	+	6
8	AML	9/M	+	45,XY,-7,der(18)	Adria+Ara-C/-	-	6
9	AML-M4	24/F	+	46,XX,inv(9)(?)	Allo-BMT/+	-	9
10	MDS	8/M		45,XY,-7	Allo-BMT/n.e	-	10
11	MDS	9/M		46,XY,i(7q)	allo-BMT/n.e	-	10
12	AML-M6	43/M	+	46,XY,del(20q)/46,XY,-2,-4,del(5q),del(7q),+mar+ring	Dauno+Ara-C+6-TG		
	-	13					
13	HYPOPLASIA	6.5/M	-	46,XY,i(7)(q10)	No	n.r.	17
14	MDS	5/M		46,XY,i(7q),del(20)(q11)	No	n.r.	17
15	MDS	7/M		46,XY,i(7)(q10)	No	+	18
16	MDS	7/M		46,XY,del(7)(q22-34)	n.r.	n.r.	19
17	MDS	1.4/F		45,XX,t(6;13)(q21;q31),-7	n.r.	n.r.	19
18	AML-M4	38/M	+	45-50,XY,-18,t(21;?) (q22;?) ,dic(22;?) (p11?)	Allo-BMT/+	n.r.	22
19	AML-M2	17/M	-	49-52,XY,+X,-5,+8,+8+9,del(12p),-13,+markers	Mitox+Ara-C+VP-16/+	+	Present case

AML=acute myeloid leukemia; ALL=acute lymphoblastic leukemia; MDS=myelodysplastic syndrome; BMT= bone marrow transplantation; n.r.=not reported; n.e.=not evaluable. \*Age at time of diagnosis of AL or MDS with karyotypic results.

*in vivo* model of leukemic transformation. The molecular pathogenesis of bone marrow dysfunction and leukemia evolution is unknown. One case of an eighteen-month old girl with a *de novo* constitutional t(6;12)(q16.2;q21.2) was described, suggesting that either the 6q16.2 or 12q21.2 region may contain critical genes for SDS.<sup>15</sup> More recently, however, 6q and 12q were not involved in the Shwachman-Diamond syndrome by linkage.<sup>16</sup> Droor *et al.*<sup>17</sup> showed that bone marrow failure in MDS is due to both a hematopoietic stem cell and a stromal defect.

Different FAB subtypes of acute myeloid leukemia (M2;M4;M5;M6) have been described, often following a period of myelodysplasia.<sup>4,9,10,13</sup> A review of the literature revealed 18 cases of SDS patients with an abnormal karyotype at the time of evolution (Table 1). The most striking finding is a male predominance (16 out of 18 cases) which should be further investigated in prospective multicenter studies in order to avoid bias related to publications. A consistent proportion of patients (12 out of 18) showed a chromosome 7 abnormality, such as an i(7q) (5 out of 18), a der(7), -7, t(4;7)(q21;q11), del(7q) either isolated or associated with other changes.<sup>4,6,12,13,18-20</sup> Indeed, Droor *et al.*<sup>18</sup> hypothesized a critical role for chromosome 7 abnormalities in the multistep pathogenesis of the malignant transformation.

In our patient diagnosis of acute leukemia was made when he was 17 years old, a preceding MDS was not documented, and chromosomes 7 were normal. Karyotyping of bone marrow

blasts did, however, show complex changes typically found in MDS/AML, such as monosomy 5, tetrasomy 8, trisomy 9, and del(12p). Monosomy 5 and deletion of 12p, which are frequently found in secondary AML/MDS, could have originated from specific toxic insults to the bone marrow stem cell. Tetrasomy of chromosome 8 has never been observed in SDS. It is also a rare event in adult AML in which it is likely preceded by a trisomy through a mechanism of double non-dysjunction.<sup>21,22</sup> Since trisomy 8 in bone marrow cells of MDS and AML may originate from a constitutional mosaicism, we performed cytogenetics on PHA-stimulated blood and FISH with a centromeric probe for chromosome 8. The constitutional karyotype was normal and no masked constitutional trisomy 8 emerged from interphase nuclei, suggesting that the numerical change of chromosome 8 in this case of SDS was an acquired event during leukemic evolution.

By analogy with other cases of acute leukemia in patients with SDS, the clinical course of the disease in our patient was characterized by multiple complications after chemotherapy, and by a delayed hematologic reconstitution.<sup>4,9-13</sup> Indeed blood cell count and marrow cellularity have never recovered to normal levels and for a long period he remained dependent on red blood cell and platelet transfusions. Experiences with allogeneic bone marrow transplantation are extremely limited. At the time of writing only 10 patients with SDS who received a marrow

allograft have been reported.<sup>4,9-13,23,24</sup> Severe to fatal complications were encountered in six patients, one of whom died on day 23 post-transplant because of cardiac toxicity.<sup>11</sup> Although the correct clinical approach for leukemic SDS patients is still to be determined, our case shows that a stable remission may be obtained by chemotherapy, even in the presence of an adverse complex bone marrow karyotype.

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