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Short Report

Comparative analysis of Shwachman-Diamond syndrome to other inherited bone marrow failure syndromes and genotype–phenotype correlation

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Our knowledge of the phenotypes of inherited bone marrow failure syndromes (IBMFSs) derives from case reports or case series in which only one IBMFS was studied. However, the substantial phenotypic overlap necessitates comparative analysis between the IBMFSs.
Shwachman–Diamond syndrome (SDS) is an IBMFS that the appreciation of what comprises its clinical phenotype is still evolving. In this analysis we used data on 125 patients from the Canadian Inherited Marrow Failure Study (CIMFS), which is a prospective multicenter population-based study. Thirty-four cases of SDS patients were analyzed and compared to other patients with the four most common IBMFSs on the CIMFS: Diamond Blackfan anemia, Fanconi anemia (FA), Kostmann/severe congenital neutropenia and dyskeratosis congenita (DC). The diagnosis of SDS, FA and DC was often delayed relative to symptoms onset; indicating a major need for improving tools to establish a rapid diagnosis.
We identified multiple phenotypic differences between SDS and other IBMFSs, including several novel differences. *SBDS* biallelic mutations were less frequent than in previous reports (81%). Importantly, compared to patients with biallelic mutations, patients with wild type *SBDS* had more severe hematological disease but milder pancreatic disease. In conclusion, comprehensive study of the IBMFSs can provide useful comparative data between the disorders. *SBDS*-negative SDS patients may have more severe hematological failure and milder pancreatic disease.

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Shwachman–Diamond syndrome (SDS) is an autosomal recessive multisystem disorder. Although the major components of the syndrome were described, the spectrum of the clinical phenotype is still unclear. Our understanding of the clinical features and genetic background derives from small case series or non-population-based studies. Based on these studies, the disorder is characterized by a variable phenotype which mainly includes abnormalities in the bone marrow, exocrine pancreas and bones (1–4), but may also involve other organs (1, 5–10). The hematological manifestations include cytopenia, myelodysplastic syndrome (MDS) and leukemia, particularly acute myeloid leukemia (AML) (1, 4, 9).

SDS is one of the common inherited bone marrow failure syndromes (IBMFSs) and the second most common cause of inherited pancreatic insufficiency (4, 5). Most patients with SDS are compound heterozygous for mutations in *SBDS* on 7q11.21 (11).

The major challenge in describing the spectrum of clinical manifestations of the IBMFSs is the substantial overlap between the various conditions. In the case of SDS, patients with marrow failure but without signs of malabsorption may not be diagnosed with SDS (12). On the other hand, patients with marrow failure and chronic diarrhea may not have SDS, but other conditions such as dyskeratosis congenita. In this study, we apply a novel methodology to study IBMFSs, in which we analyzed several IBMFSs from one comprehensive population-based registry to identify unique features of one IBMFS, SDS, and validate other features which were suggested in previous literature reviews or case series. The unbiased inclusion of patients also allowed us to study differences between SDS patients with and without mutations in the *SBDS* gene.

Methods

Registry

The Canadian Inherited Marrow Failure Study (CIMFS) is a multicenter study, which was approved by the Institutional Ethics Board of all the participating institutions. These centers care for >95% of the eligible pediatric IBMFS population in Canada. More than 90% of the patients in this study are from centers which enrolled >80% of the patients in their institutions. Patients who fulfill our diagnostic criteria for an IBMFS (12) are recruited.

Table 1. Criteria for diagnosis of Shwachman–Diamond syndrome

Fulfilling at least two of the following criteria
(1) At least two of the following:
  (a) Chronic cytopenia(s) detected on at least two occasions over at least 3 months
  (b) Reduced marrow progenitors (granulocyte–monocyte colony forming units, erythroid burst forming units and granulocyte, erythroid, monocyte, megakaryocyte-colony forming units)
  (c) Persistent elevation of hemoglobin F (on at least two occasions over at least 3 months apart)
  (d) Persistent red blood cell macrocytosis (on at least two occasions over at least 3 months apart) (not caused by hemolysis or a nutritional deficiency)[a]
(2) At least one of the following:
  (a) Evidence of pancreatic lipomatosis (for example, by ultrasound, computed tomography, magnetic resonance imaging or pathological examination of the pancreas e.g. by autopsy)
  (b) Reduced levels of at least two pancreatic enzymes adjusted to age (fecal elastase, serum trypsinogen, serum isoamylase, or duodenal enzymes following stimulation test)
(3) Positive genetic testing (*SBDS* and others once they become available)
(4) First degree-family member with Shwachman–Diamond syndrome

[a]Hemoglobinopathies with ineffective erythropoiesis and high hemoglobin F should be excluded by clinical or laboratory testing.
Supportive evidence of SDS include characteristic metaphyseal dysostosis.

The diagnosis of the specific IBMFS is determined according to the previously described diagnostic guidelines (13). The diagnostic criteria of SDS are provided in Table 1.

Patient information at study entry and at yearly follow-up was collected and included demographics, diagnosis, symptoms, family history, physical malformations, laboratory tests, genetic tests and imaging studies, treatment and outcomes.

Genetic testing was performed at the discretion of the treating physician. Genetic analysis of the *SBDS* gene was performed either in the clinical laboratory of the Hospital for Sick Children, Toronto or in a research laboratory (Y.D., Toronto). In each case, amplification primer sets flanking each of the five *SBDS* gene exons and sequencing primer sets for analysis of the polymerase chain reaction products were used.

Definitions

Abnormalities in peripheral blood cytopenias and mean corpuscular volume (MCV) were classified according to the published age-specific reference values (14). Severe aplastic anemia was defined when the bone marrow cellularity was <25% of the normal for age and at least two of the following: neutrophils $<0.5 \times 10^9$/l, platelets $<20 \times 10^9$/l, or absolute reticulocyte $\leq 40 \times 10^9$/l in anemic patients (15). As the percentage of cellularity could not be accurately quantified or was not available in all cases, we defined severe bone marrow failure as severe bilineage cytopenia that lasts longer than 3 months with hypocellular bone marrow.

MDS was defined based on the World Health Organization criteria of childhood MDS (16). The cytopathology of MDS was classified according to the Category–Cytology–Cytogenetics classification (17). AML was defined as documentation of ≥30% leukemic blasts in the bone marrow.

Data analysis

Student's *t*-test was used to determine statistical significance of differences in means. Mann–Whitney test was used to compare medians. Chi-squared test was used to determine associations between variables. Pearson correlation coefficient was used to calculate correlations between genotype and phenotype. p-Values of <0.05 were considered statistically significant. Survival was estimated by Kaplan–Meier analysis. Statistical analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago, IL).

Results

We analyzed data on 34 SDS patients registered on the CIMFS from July 2001 to December 2008 and compared it with the information obtained on patients with four other common IBMFSs on the registry: 34 FA patients, 8 DC, 39 DBA and 10 K/SCN. These IBMFSs constitute an important part of the differential diagnosis of SDS. Thirty-six IBMFS patients were previously published (12, 18).

Demographics

Ten of the tested patients were siblings. The ratio of males to females was 1 to 1.1, without statistical gender differences compared to other disorders (Table 2). The median age of SDS patients at presentation was 1.5 months with no statistical difference from the other four IBMFSs (Table 2). However, the median age at diagnosis of SDS was 17 months, which was significantly later than K/SCN and DBA (Table 2). The effect of having an older child on the time of diagnosis was difficult to assess in our series as in three of the

Table 2. Gender and ages of patients with Shwachman–Diamond syndrome compared to patients with other common groups of inherited bone marrow failure syndromes[a]

	SDS ($n = 34$)	FA ($n = 34$)	DC ($n = 8$)	DBA ($n = 39$)	K/SCN ($n = 10$)
Gender (%)					
Male	47	50	50	56	30
Female	53	50	50	44	70
		p = 1.00	p = 1.00	p = 0.486	p = 0.474
Median age at presentation in months (range)	$n = 25$ 1.5 (0–168)	$n = 22$ 0.25 (0–126) p = 0.86	$n = 7$ 6 (0–108) p = 0.789	$n = 30$ 1.1 (0–132) p = 0.307	$n = 8$ 0 (0–12) p = 0.204
Median age at diagnosis in months (range)	$n = 33$ 16.7 (0–188)	$n = 31$ 40.3 (0–146) p = 0.177	$n = 8$ 40.9 (8–201) p = 0.073	$n = 37$ 5.4 (0–184) p = 0.006	$n = 10$ 2.4 (0–13) p = 0.000
Median time of follow-up in months (range)	162 (24–528)	134 (0–318) p = 0.416	71 (0–35) p = 0.087	157 (0–496) p = 0.114	12.4 (0–27) p = 0.326

DBA, Diamond Blackfan anemia; DC, dyskeratosis congenita; FA, Fanconi anemia; K/SCN, Kostmann/severe congenital neutropenia; SDS, Shwachman–Diamond syndrome.

[a]$p < 0.05$ was considered statistically significant. Comparison of gender between SDS and the other IBMFSs was done by chi-squared test; comparison of age was done by Mann–Whitney test.

five families, either both siblings were diagnosed simultaneously or a second child was diagnosed after genetic screening. In the other two families, the older child was diagnosed earlier than the first child (17 *vs* 157 months and 2.5 *vs* 3.5 months, respectively).

Genetic analysis

Genetic analysis of the *SBDS* gene was carried out in 31 SDS cases. Compound heterozygosity for mutations in *SBDS* was found in 81% of patients tested (Figure 1). The most common alleles were 258+2T>C, followed by 183−184TA>CT and 183−184TA>CT+258+2T>C. Two novel mutations were identified: 388G>T and c.621+1G>A. The most common combinations of mutations were 183−184TA>CT/258+2T>C (12 patients) and 183−184TA>CT+258+2T>C/258+2T>C (6 patients).

In four cases, the diagnosis of SDS was established after genetic testing. Two of the four were diagnosed during family screening after identifying *SBDS* mutations in an index case.

Six of the 31 SDS patients had normal sequences of all five exons and flanking introns of the *SBDS* gene; two of them were identical twins. These patients met the diagnostic criteria for SDS (Table 8) but nonetheless had tests for several other disorders, which were negative (Table 9).

Hematological characteristics

Only four of the SDS patients (12%) presented with isolated mild neutropenia which was not seen in FA, DC and DBA. All other patients presented with types of cytopenia which were also seen in other syndromes. Twenty-four percent of the SDS patients exhibited bilineage cytopenia at diagnosis and 24% had trilineage cytopenia. At diagnosis, only 74% of SDS patients had neutropenia; without significant difference between SDS and FA or DC with regard to the frequency or severity of neutropenia (Table 3). However, neutropenia was less frequent and less severe in SDS compared to K/SCN.

At diagnosis, anemia was seen in 58% of the SDS patients. It was predominantly macrocytic and was accompanied by high hemoglobin F (HgF). Anemia was significantly less common in SDS than in FA or DBA, but without significant difference from K/SCN. However, the higher frequency of patients with high MCV and HgF in SDS and the normalization of hemoglobin after treatment of K/SCN patients with G-CSF suggest that the anemia in SDS is because of marrow failure, while in K/SCN it is not. It is noteworthy that both the mean MCV and the frequency of high MCV in SDS were lower than those in FA, DC and DBA. Thrombocytopenia in SDS patients was significantly less frequent compared to FA and DC.

The combination of isolated neutropenia and high MCV after the first year of life was seen in 20% of the SDS patients, but in none of the patients with FA, DC and DBA. The percentage was higher than K/SCN (9%), but without statistical differences. The combination of isolated neutropenia with high HgF after the first year of life was seen in 28% of the patients with

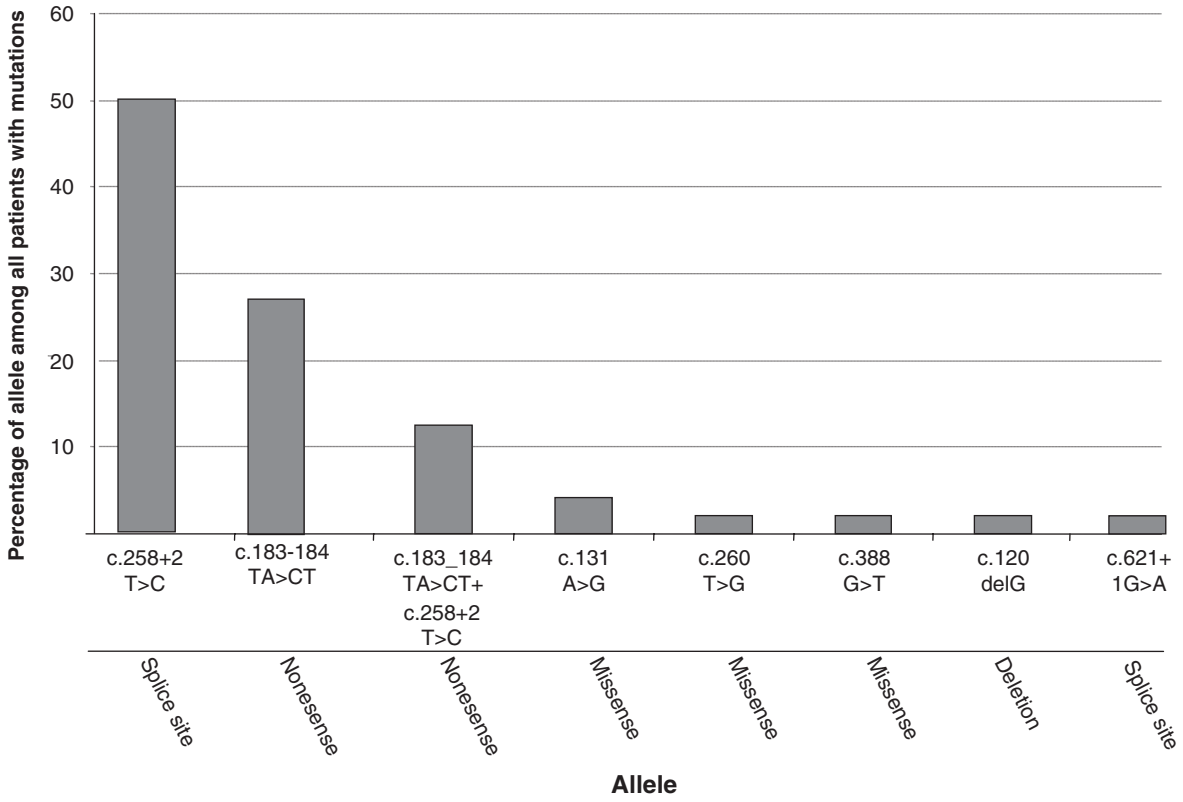

Fig. 1. The frequency of mutant *SBDS* alleles among patient with biallelic mutation.

SDS, but in none of the patients with the other conditions.

Initial bone marrow testing showed hypocellular specimens in 65% of the SDS cases (Table 4). At diagnosis, bone marrows from FA tended to be more severely affected, with significantly higher frequency of hypocellular specimens, reduced granulopoiesis, erythropoiesis and megakaryopoiesis. Bone marrow hypocellularity, decreased granulopoiesis and decreased megakaryopoiesis were more frequent in SDS compared to DBA. SDS patients had higher incidence of decreased bone marrow cellularity and megakaryocytes than K/SCN. Importantly, among the SDS patients whose bone marrow cell differential was available, none had maturation arrest at the promyelocyte–myelocyte stage, while all patients with K/SCN and available information had maturation arrest at this stage ($p < 0.0001$). DC bone marrows showed more similarities to SDS than the other conditions, and only megakaryopoeisis was less frequently decreased in SDS compared to DC.

At a median age of 26 months, 24% of SDS patients developed severe bone marrow failure (Table 5); this frequency was significantly lower in comparison to DC and FA, but significantly higher when compared to DBA.

The probability of developing clonal and malignant myeloid transformation to MDS and AML was statistically different among the IBMFSs. At a median age of 20 years, 18% of the SDS patients developed clonal and malignant myeloid transformation compared to 41%, 13%, 10% and 0% in FA, DC, K/SCN and DBA patients, respectively (Table 6; Figure 2).

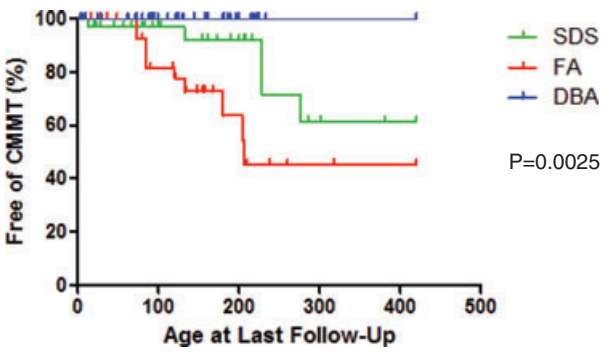

Fig. 2. Estimated risk of clonal marrow cytogenetic abnormalities, myelodysplastic syndrome and leukemia in patients with Shwachman–Diamond syndrome, Fanconi anemia, and Diamond Blackfan anemia in our study (CMMT, clonal and malignant myeloid transformation).

Table 3. Blood counts of Shwachman–Diamond syndrome patients compared to other inherited marrow failure syndromes at diagnosis[a]

	SDS ($n = 34$)	FA ($n = 34$)	DC ($n = 8$)	DBA ($n = 39$)	K/SCN ($n = 10$)
Neutropenia[b] (%)	74	63	62.5	31	100
Mild (%)	21	16	25	6	0
Moderate (%)	15	22	37.5	17	10
Severe (%)	38	25	37.5	8	90
		p = 0.527	p = 0.680	p = 0.001	p = 0.030
	($n = 34$)	($n = 32$)	($n = 8$)	($n = 36$)	($n = 10$)
Mean ANC $\times 10^9$/l (SD)	1.17 (1.03)	1.42 (1.22)	1.99 (2.62)	2.71 (2.20)	0.18 (0.22)
		p = 0.368	p = 0.158	p = 0.000	p = 0.005
Anemia[c] (%)	58	81	88	100	70
Mild-moderate (%)	46	31	75	32	70
Severe (%)	12	50	12.5	68	0
		p = 0.001	p = 0.175	p = 0.000	p = 0.356
	($n = 33$)	($n = 32$)	($n = 8$)	($n = 37$)	($n = 10$)
Mean hemoglobin (g/l) (SD)	108.8 (31.7)	85.0 (31.5)	92 (14.9)	58 (26.1)	110.8 (20.8)
		p = 0.004	p = 0.145	p = 0.000	p = 0.68
	($n = 33$)	($n = 32$)	($n = 8$)	($n = 37$)	($n = 10$)
Mean MCV (fl) (SD)	88.4 (8.3)	99.1 (7.6)	97.9 (13.8)	95.5 (6.2)	77.8 (9.1)
		p = 0.000	p = 0.015	p = 0.000	p = 0.002
	($n = 33$)	($n = 31$)	($n = 8$)	($n = 27$)	($n = 10$)
Increased MCV for age after the age of 1 year (%)	60	86	86	87	11
		p = 0.048	p = 0.363	p = 0.078	p = 0.020
	($n = 20$)	($n = 29$)	($n = 7$)	($n = 23$)	($n = 9$)
Mean HgF (SD)	7.95 (12.0)	13.6 (12.6)	11.6 (11.1)	9.3 (7.0)	1.23 (1.23)
		p = 0.113	p = 0.532	p = 0.755	p = 0.278
	($n = 28$)	($n = 22$)	($n = 5$)	($n = 9$)	($n = 4$)
Increased HgF for age after the age of 1 year (%)	72.2	100	100	100	0
		p = 0.042	p = 0.545	p = 0.136	p = 0.042
	($n = 18$)	($n = 21$)	($n = 5$)	($n = 9$)	($n = 3$)
Thrombocytopenia[d] (%)	38	75	100	11	0
Mild (%)	12	9	0	3	0
Moderate (%)	23.5	47	25	8	0
Severe (%)	3	19	75	0	0
		p = 0.009	p = 0.000	p = 0.063	p = 0.143
	($n = 34$)	($n = 32$)	($n = 8$)	($n = 36$)	($n = 10$)
Mean platelet count $\times 10^9$/l (SD)	213 (143)	122 (147)	18 (16)	395 (198)	479 (170)
		p = 0.012	p = 0.000	p = 0.000	p = 0.000
	($n = 34$)	($n = 32$)	($n = 8$)	($n = 36$)	($n = 10$)
Isolated neutropenia and high MCV after the age of 1 year (%)	20	0	0	0	11
		p = 0.021	p = 0.545	p = 0.025	p = 0.51
	($n = 20$)	($n = 30$)	($n = 7$)	($n = 28$)	($n = 9$)
Isolated neutropenia and high HgF after the age of 1 year (%)	28	0	0	0	0
		p = 0.015	p = 0.545	p = 0.136	p = 0.549
	($n = 18$)	($n = 21$)	($n = 5$)	($n = 9$)	($n = 3$)
Isolated anemia and high MCV after the age of 1 year (%)	5	13	0	50	0
		p = 0.626	p = 1.000	p = 0.001	p = 1.000
	($n = 20$)	($n = 30$)	($n = 7$)	($n = 28$)	($n = 10$)
Isolated anemia and high HgF after the age of 1 year (%)	6	19	0	100	0
		p = 0.349	p = 1.000	p = 0.000	p = 1.000
	($n = 18$)	($n = 21$)	($n = 5$)	($n = 9$)	($n = 3$)

ANC, absolute neutrophil count; DBA, Diamond Blackfan anemia; DC, Dyskeratosis congenita; FA, Fanconi anemia; HgF, haemoglobin F; K/SCN, Kostmann/severe congenital neutropenia; MCV, red blood cell mean corpuscular volume; NA, not available; SD, standard deviation; SDS, Shwachman–Diamond syndrome.

[a]p-Values were calculated by Student's *t*-test.

[b]Neutropenia was considered severe if absolute neutrophil count was $<0.5 \times 10^9$/l; moderate if >0.5 to <1.0 and mild if >1.0 to <1.5.

[c]Anemia was considered severe if hemoglobin was <70 g/l and mild-moderate if hemoglobin was >70 g/l but < lower limit of normal range for age and gender.

[d]Thrombocytopenia was considered severe if the platelet count was $<20 \times 10^9$/l; moderate if >20 to <100 and mild if >100 to <150.

Table 4. Results of bone marrow evaluation at the time of diagnosis[a]

	SDS	FA	DC	DBA	K/SCN
Bone marrow cellularity (%)	65	97	75	20	20
Decreased	23	3	25	49	30
Normal	8	0	0	20	20
Increased	4	0	0	11	20
Undetermined		p = 0.015	p = 0.88	p = 0.002	p = 0.048
	(*n* = 26)	(*n* = 30)	(*n* = 8)	(*n* = 35)	(*n* = 10)
Decreased granulopoiesis (%)	46	71	38	9	60
		p = 0.022	p = 0.898	p = 0.000	p = 0.271
	(*n* = 26)	(*n* = 27)	(*n* = 8)	(*n* = 35)	(*n* = 10)
Decreased erythropoiesis (%)	23	54	12.5	80	0
		p = 0.001	p = 0.976	p = 0.000	p = 0.232
	(*n* = 25)	(*n* = 28)	(*n* = 8)	(*n* = 36)	(*n* = 10)
Decreased megakaryopieisis (%)	35	86	88	0	0
		p = 0.000	p = 0.011	p = 0.000	p = 0.022
	(*n* = 25)	(*n* = 29)	(*n* = 8)	(*n* = 35)	(*n* = 10)

DBA, Diamond Blackfan anemia; DC, Dyskeratosis congenita; FA, Fanconi anemia; K/SCN, Kostmann/severe congenital neutropenia; SDS, Shwachman–Diamond syndrome.
[a]Comparison between SDS and the other IBMFSs was done by Chi-squared test.

Table 5. Disease progression and treatment of complications after study entry in patients with inherited bone marrow failure syndromes[a]

	SDS	FA	DC	DBA	K/SCN
Worsening cytopenia (%)	61	48	25	13	29
		p = 0.422	p = 0.114	p = 0.000	p = 0.207
	(*n* = 28)	(*n* = 27)	(*n* = 8)	(*n* = 31)	(*n* = 7)
Severe bone marrow failure (severe bilineage cytopenia with hypocellular bone marrow) (%)	15	56	88	0	0
		p = 0.000	p = 0.000	p = 0.015	p = 0.207
	(*n* = 34)	(*n* = 32)	(*n* = 8)	(*n* = 37)	(*n* = 10)
Median age of developing severe aplastic anemia (years) (range)	3.9	7.2	6.6	—	—
	(2–5)	(0.4–16)	(0.8–18)	—	—
		p = 0.182	p = 0.643	p = NA	p = NA
Treatment					
RBC transfusion (%)	28	67	88	95	0
		p = 0.002	p = 0.002	p = 0.000	p = 0.053
	(*n* = 32)	(*n* = 30)	(*n* = 8)	(*n* = 38)	(*n* = 10)
Platelet transfusion (%)	15	62	100	5	0
		p = 0.007	p = 0.000	p = 0.028	p = 0.255
	(*n* = 32)	(*n* = 30)	(*n* = 8)	(*n* = 38)	(*n* = 10)
G-CSF (%)	16	28	25	2.6	100
		p = 0.255	p = 0.533	p = 0.058	p = 0.000
	(*n* = 32)	(*n* = 29)	(*n* = 8)	(*n* = 39)	(*n* = 10)
HSCT (%)	9	55	63	5	0
		p = 0.000	p = 0.001	p = 0.533	p = 0.33
	(*n* = 34)	(*n* = 31)	(*n* = 8)	(*n* = 39)	(*n* = 10)
Crude mortality rate (%)	6	18	25	0	10
	7–43	7–18	2.5–11	—	7
		p = 0.132	p = 0.097	p = 0.125	p = 0.650

DBA, Diamond Blackfan anemia; DC, Dyskeratosis congenita; FA, Fanconi anemia; K/SCN, Kostmann/severe congenital neutropenia; SDS, Shwachman–Diamond syndrome.
[a]Comparison of medians between SDS and the other IBMFSs was done by Mann–Whitney test.

Non-hematological manifestations

Twenty-eight of the 33 SDS patients (85%) showed evidence of pancreatic dysfunction. Sixty-seven percent of these patients required treatment with pancreatic enzymes and ADEK vitamins. Various skeletal deformities were also seen (Table 7). One patient in this series developed insulin-dependent diabetes mellitus.

Table 6. Clonal marrow cytogenetic abnormalities/myelodysplastic syndrome

Disorder	Percentage of patients with clonal disease/MDS	Total number of patients with the disorder	p-Value[a]
SDS	18	34	
FA	38	34	0.059
DC	13	8	0.725
DBA	0	39	0.006
K/SCN	10	10	0.561

DBA, Diamond Blackfan anemia; DC, Dyskeratosis congenita; FA, Fanconi anemia; K/SCN, Kostmann/severe congenital neutropenia; SDS, Shwachman–Diamond syndrome.
[a]p-Value was calculated by Chi-Square test and represent comparison between SDS to other syndromes.

Table 7. Non-hematologic manifestations of 34 Shwachman–Diamond syndrome patients at diagnosis or at follow-up

Abnormality	Percentage of patients
Pancreatic dysfunction	85
Short stature	76
Skeletal abnormalities	73
Metaphyseal dysplasia	41
Osteoporosis/osteopenia	32
Short ribs	12
Scoliosis	9
Chest deformity	6
Eczema	63
Developmental delay	59
Oral	44
Carries	26
Hypoplastic teeth	9
Leukoplakia	3
Liver (hepatomegaly or elevated enzymes)	45
Abnormal gait	25
Lower back pain	15

Treatment

Thirty-five percent of the SDS patients needed treatment for cytopenia, compared to 85%, 100%, 100% and 95% of the patients with FA, DC, K/SCN and DBA, respectively (Table 5). The indications for treatment included anemia, neutropenia, thrombocytopenia and severe aplastic anemia, MDS and leukemia. Patients with SDS needed less blood products and HSCT than FA or DC patients. Although the degree of anemia at presentation was not different for SDS and K/SCN patients, SDS patients required more red blood cell transfusions. Patients with SDS were treated less frequently with G-CSF than patients with K/SCN.

Survival

Two patients with SDS (6%) died at the ages of 7 and 43 years (Figure 3). The former died of multi-organ failure 10 weeks after HSCT. The latter's death was secondary to a fungal infection, after receiving chemotherapy for AML and while awaiting HSCT.

Genotype–phenotype correlation for SDS patients

We first studied the differences between the SDS patients with (Group A) and without (Group B) mutations in the *SBDS* coding region or flanking intronic regions. Group B patients had significantly lower hemoglobin levels than Group A patients ($p = 0.005$), higher incidence of high HgF after the age of 1 year ($p = 0.02$) and higher mean HgF levels ($p = 0.01$). In contrast, severe pancreatic insufficiency requiring enzyme replacement was not seen in Group B compared to 75% of the patients in Group A ($p < 0.001$). At a median

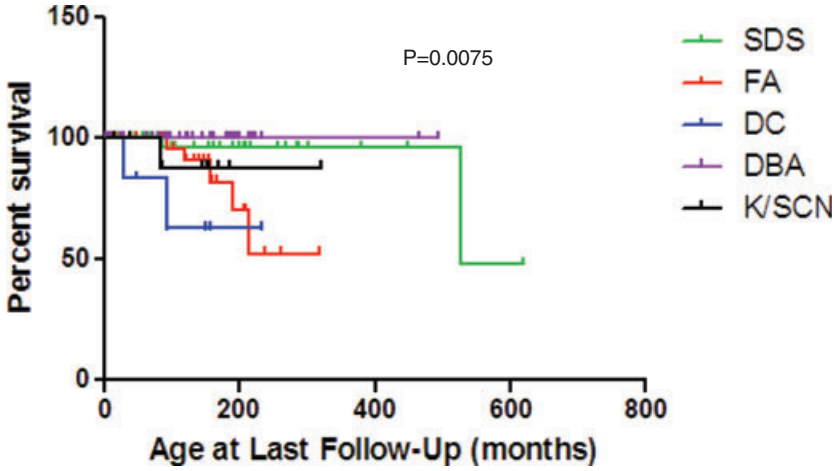

Fig. 3. Survival analysis of patients with Shwachman–Diamond syndrome, Fanconi anemia, dyskeratosis congenita, Diamond Blackfan anemia, Kostmann/severe congenital neutropenia.

Table 8. Clinical characteristics of the patients without mutations in the *SBDS* open reading frame

SBDS+/+ Patients	Chronic cytopenia	Reduced marrow progenitors	Elevated HbF	Elevated MCV	Short stature	Skeletal abnormalities characteristic of SDS	Pancreatic insufficiency
UPN 19	Trilineage cytopenia	Mild dyserythropoeisis, reduced megakaryocytes	Yes	Yes	Yes	Osteopenia	Pancreatic lipomatosis by ultrasound; abnormal fecal fat
UPN 20	Neutropenia and anemia	Mildly hypocellular marrow	Yes	Yes	Yes	Metaphyseal dysostosis	Pancreatic lipomatosis by ultrasound
UPN 24	Trilineage cytopenia	Hypoplastic marrow with dyserythropoeisis	Yes	Yes	Yes	Metaphyseal dysostosis, osteopenia	Pancreatic lipomatosis by ultrasound
UPN 176	Trilineage cytopenia	Moderately hypocellular with significantly decreased megakaryopoeisis and decreased erythropoeisis	Yes	Yes	Yes	Metaphyseal dysostosis	Pancreatic lipomatosis by ultrasound
UPN 204	Neutropenia	Hypocellular	Yes	No	Yes	Metaphyseal dysostosis	Pancreatic lipomatosis by ultrasound
UPN 212	Neutropenia and thrombocytopenia	Hypocellular with decreased megakaryocytes	Yes	Yes	Yes	Metaphyseal dysostosis	Pancreatic lipomatosis by ultrasound

Table 9. Negative testing of the patients without mutations in the *SBDS* open reading frame to rule out other IBMFSs

Genetic/screening tests for	UPN 19	UPN 20	UPN 24 (Twin of UPN 19)	UPN 176	UPN 204	UPN 212
Fanconi anemia	Chromosome fragility: spontaneous, MMC and DEB (peripheral blood)	Chromosome fragility spontaneous, MMC and DEB (peripheral blood)	Chromosome fragility: spontaneous, MMC and DEB (peripheral blood)	Chromosome fragility: spontaneous, MMC and DEB (peripheral blood)	Chromosome fragility: spontaneous, MMC and DEB (peripheral blood)	Chromosome fragility: spontaneous, MMC and DEB (peripheral blood)
Dyskeratosis congenita				DKC1 TERC		
Congenital amegakaryocytic thrombocytopenia				c-MPL		
Pearson syndrome	Mitochondrial deletion		Mitochondrial deletion		Mitochondrial deletion	Mitochondrial deletion
Cartilage-Hair Hypoplasia		RMRP		RMRP	RMRP	RMRP
Diamond Blackfan anemia	eADA RPS-19	eADA	eADA RPS-19	eADA RPS-19	eADA	eADA

follow-up of 3 years, the incidence of developing severe aplastic anemia was significantly higher in Group B (3 out of 6 *vs* 0 out of 25, $p < 0.0001$). In addition, the incidence of severe bone marrow failure was significantly higher in Group B (3 out of 6 *vs* 1 out of 25, $p = 0.002$). Five of the 6 patients in Group B needed treatment for their cytopenia compared to only 6 out of 25 Group A patients ($p = 0.005$). The differences in the incidences of developing clonal marrow cytogenetic abnormalities or MDS/AML were insignificant. The incidence of gait irregularities was significantly more prominent in Group B (6 out of 6 *vs* 2 out of 25, $p < 0.0001$).

We then analyzed the difference in phenotype between patients who have one truncation (e.g. c.183-184TA>CT) and one hypomorphic mutation (e.g. c.258+2T>C) versus patients who had two hypomorphic mutations. No differences were observed with regard to age of presentation, age of diagnosis, Hg, HgF, MCV, platelets, neutrophils, severe aplastic anemia and clonal marrow cytogenetic abnormalities.

Discussion

The diagnosis of IBMFS is often challenging. In the present study, the diagnosis of SDS, FA and DC was often delayed relative to symptom onset. These data show the need for raising the health care providers index of suspicion for an IBMFS and for improved clinical and laboratory tools to establish a rapid diagnosis of these IBMFS.

It is noteworthy that only 74% of the SDS patients had neutropenia at diagnosis; however, all the patients eventually manifested neutropenia which was either persistent or intermittent. Patients with IBMFSs can present with any severity and combination of cytopenias (4). This makes the diagnosis of a specific syndrome on the basis of hematological manifestations alone very difficult. Some important differences between SDS and other IBMFSs were noted in our study. First, the combinations of isolated neutropenia and high HgF or high MCV are more probably to be associated with SDS than with other IBMFSs. Second, in contrast to patients with K/SCN, patients with SDS are not probably to present with severe neutropenia. Third, compared to SDS, patients with DBA and FA are more probably to have severe anemia. Fourth, fewer patients with SDS have thrombocytopenia compared to those with DC and FA. Fifth, multilineage cytopenia does not differentiate SDS from DBA. These findings may help in early establishment of a correct clinical diagnosis and appropriately directed genetic testing.

SDS bone marrow morphology is not specific, but may help to differentiate from other IBMFS. The SDS bone marrow shows a higher degree of cellularity, granulopoiesis, erythropoiesis and megakaryopoiesis than FA. Although both SDS and DC are characterized by pancytopenia and have remarkably similar bone marrow morphology, patients with SDS typically present predominantly with neutropenia whereas patients with DC typically present predominantly with thrombocytopenia. None of the SDS patients had isolated erythroid hypoplasia, which can aid in the discrimination of DBA from SDS. Marrow testing can also help to differentiate between SDS and K/SCN as the latter is not probably to be characterized by reduced cellularity or megakaryopoiesis, but rather by maturation arrest at the promyelocyte–myelocyte stage. Our study validates results of previous series showing that the most common clonal marrow cytogenetic abnormalities in SDS patients are i(7q) and del (20q) (4).

Clinical and laboratory evidence of exocrine pancreatic insufficiency was reported in all SDS patients(1, 19). In contrast, our study showed that 15% of the patients had no evidence of pancreatic dysfunction, as concluded by their gastroenterologists. Furthermore, steatorrhea requiring treatment was reported only in 67% of the patients. This finding indicates that in a comprehensive registry in which all patients with IBMFSs are enrolled, cases with a partial phenotype can be identified.

We found major differences between patients with identifiable biallelic mutations in the *SBDS* open reading frame to those without. The patients without mutations had significantly more severe hematological disease and milder pancreatic disease. Recruitment of a larger population of patients is required to validate these results. It is noteworthy that our study did not include sequencing of non-coding regions, and these patients might constitute a group with unique alterations in *SBDS* rather than in novel other gene.

Most of the *SBDS* mutations in this series were reported as frequent in the literature (20) and only a few patients had rare mutations. 388G>T is a novel mutation, resulting in a missense change of valine to leucine at codon 130, nucleotide 388 in exon 3 of the *SBDS* gene. c.621+1G>A is also novel and alters the splice site.

This study has unique significance beyond the model of SDS. It provides an excellent example of how distinct features of a specific IBMFS can be better delineated using a comprehensive registry for all patients with IBMFSs and by comparing the features of the syndrome to other IBMFSs with overlapping phenotypes.

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Conflict of interest

None of the authors has conflict of interest.

References

1. Aggett PJ, Cavanagh NP, Matthew DJ, Pincott JR, Sutcliffe J, Harries JT. Shwachman's syndrome. A review of 21 cases. Arch Dis Child 1980: 55(5): 331–347.
2. Burke V, Colebatch JH, Anderson CM, Simons MJ. Association of pancreatic insufficiency and chronic neutropenia in childhood. Arch Dis Child 1967: 42: 147–157.
3. Pringle EM, Young WF, Haworth EM. Syndrome of pancreatic insufficiency, blood dyscrasia, and metaphyseal dysplasia. Proc R Soc Med 1968: 61: 776–777.
4. Dror Y. Shwachman-Diamond syndrome. Pediatr Blood Cancer 2005: 45(7): 892–901.

5. Hall GW, Dale P, Dodge JA. Shwachman-Diamond syndrome: UK perspective. Arch Dis Child 2006: 91(6): 521–524.
6. Mack DR, Forstner GG, Wilschanski M, Freedman MH, Durie PR. Shwachman syndrome: exocrine pancreatic dysfunction and variable phenotypic expression. Gastroenterology 1996: 111(6): 1593–1602.
7. Ginzberg H, Shin J, Ellis L et al. Shwachman syndrome: phenotypic manifestations of sibling sets and isolated cases in a large patient cohort are similar. J Pediatr 1999: 135(1): 81–88.
8. Dror Y, Ginzberg H, Dalal I et al. Immune function in patients with Shwachman-Diamond syndrome. Br J Haematol 2001: 114(3): 712–717.
9. Dror Y, Durie P, Ginzberg H et al. Clonal evolution in marrows of patients with Shwachman-Diamond syndrome: a prospective 5-year follow-up study. Exp Hematol 2002: 30(7): 659–669.
10. Dror Y, Durie P, Marcon P, Freedman MH. Duplication of distal thumb phalanx in Shwachman-Diamond syndrome. Am J Med Genet 1998: 78(1): 67–69.
11. Boocock GR, Morrison JA, Popovic M et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. Nat Genet 2003: 33(1): 97–101.
12. Teo JT, Klaassen R, Fernandez CV et al. Clinical and genetic analysis of unclassifiable inherited bone marrow failure syndromes. Pediatrics 2008: 122(1): 139–148.
13. Dror Y. Inherited bone marrow failure syndromes, 3rd edn. Oxford: Blackwell Publishing, 2006.
14. Dallman PR, Siimes MA. Percentile curves for hemoglobin and red cell volume in infancy and childhood. J Pediatr 1979: 94(1): 26–31.
15. Incidence of aplastic anemia: the relevance of diagnostic criteria. By the International Agranulocytosis and Aplastic Anemia Study. Blood 1987: 70(6): 1718–1721.
16. Hasle H, Niemeyer CM, Chessells JM, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia 2003: 17: 277–282.
17. Mandel K, Dror Y, Poon A, Freedman MH. A practical comprehensive classification for pediatric myelodysplastic syndromes: the CCC system. J Pediatr Hematol Oncol 2002: 24: 596–605.
18. Steele JM, Sung L, Klaassen R et al. Disease progression in recently diagnosed patients with inherited marrow failure syndromes: a Canadian Inherited Marrow Failure Registry (CIMFR) report. Pediatr Blood Cancer 2006: 47(7):918–925.
19. Cipolli M, D'Orazio C, Delmarco A, Marchesini C, Miano A, Mastella G. Shwachman's syndrome: pathomorphosis and long-term outcome. J Pediatr Gastroenterol Nutr 1999: 29(3): 265–272.
20. Majeed F, Jadko S, Freedman MH, Dror Y. Mutation analysis of SBDS in paediatric acute myeloblastic leukemia with and without chromosome 7 abnormalities. Pediatr Blood Cancer 2005: 45(7): 920–924.