Classification of and risk factors for hematologic complications in a French national cohort of 102 patients with Shwachman-Diamond syndrome

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ABSTRACT

Background
Patients with the Shwachman-Diamond syndrome often develop hematologic complications. No risk factors for these complications have so far been identified. The aim of this study was to classify the hematologic complications occurring in patients with Shwachman-Diamond syndrome and to investigate the risk factors for these complications.

Design and Methods
One hundred and two patients with Shwachman-Diamond syndrome, with a median follow-up of 11.6 years, were studied. Major hematologic complications were considered in the case of definitive severe cytopenia (i.e. anemia <7 g/dL or thrombocytopenia <20×10^9/L), classified as malignant (myelodysplasia/leukemia) according to the 2008 World Health Organization classification or as non-malignant.

Results
Severe cytopenia was observed in 21 patients and classified as malignant severe cytopenia (n=9), non-malignant severe cytopenia (n=9) and malignant severe cytopenia preceded by non-malignant severe cytopenia (n=3). The 20-year cumulative risk of severe cytopenia was 24.3% (95% confidence interval: 15.3%-38.5%). Young age at first symptoms (<3 months) and low hematologic parameters both at diagnosis of the disease and during the follow-up were associated with severe hematologic complications (P<0.001). Fifteen novel *SBDS* mutations were identified. Genotype analysis showed no discernible prognostic value.

Conclusions
Patients with Shwachman-Diamond syndrome with very early symptoms or cytopenia at diagnosis (even mild anemia or thrombocytopenia) should be considered at a high risk of severe hematologic complications, malignant or non-malignant. Transient severe cytopenia or an indolent cytogenetic clone had no deleterious value.

Key words: Shwachman-Diamond syndrome, genotype, aplastic anemia, secondary leukemia, cytopenia, myelodysplasia, monosomy 7.

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The online version of this article has a Supplementary Appendix.

Introduction

The Shwachman-Diamond syndrome (SDS) (OMIM 260400) is an autosomal recessive multisystem disorder characterized by exocrine pancreatic dysfunction and mild neutropenia and may be associated with metaphyseal dysostosis, mild intellectual retardation and various other organ dysfunctions.[1]

The *SBDS* gene, located on chromosome 7q11, is associated with the disease.[2] SBDS protein is an essential cofactor for elongation factor 1 (EFL1), and together they directly catalyze the release of eIF6 from nascent 60S subunits of the ribosome by a mechanism requiring both GTP binding and hydrolysis.[3] *SBDS* mutations arising from gene conversion are present in almost all patients with SDS, the compound heterozygous genotype p.[Lys62X]+[Cys84fs] being present in about 60% of patients.[2]

SDS is characterized by variable clinical phenotypes between and within families. Some patients with SBDS mutations may have normal blood cell counts, even though their siblings are severely neutropenic at a comparable age. Some patients have severe exocrine pancreatic deficiency, while in other cases this disorder is only diagnosed by routine screening. The severity and course of the disease also vary, with one third of patients developing major hematologic complications.[4] These hematologic complications are the main causes of early death and may necessitate hematopoietic stem cell transplantation.[5] No risk factors for these complications have been identified so far, although leukemia appears in the literature to be more frequent in males.[1,6,7] This prompted us to analyze a cohort of 102 genotyped patients with SDS belonging to 93 families, for whom there was exhaustive information on clinical features and hematologic and biological parameters which had been collected over a median follow-up of 11.6 years.

Design and Methods

Patients

Patients included in this study were all registered in the French severe chronic neutropenia registry. This registry was initially created in 1993; since then, enrollment has been prospective. Patients with all types of congenital neutropenia are included.[8] In 2008, the register was certified as a national registry by the health authorities, and completeness of cases was ascertained by controlling multiple sources. Thirty-five French pediatric hematology-oncology and gastroenterology units participate in the registry. Data monitoring, based on medical charts, is done by a clinical research associate who visits each center yearly. The patient must provide written informed consent to be included in the registry. Several reports of the register are available elsewhere.[4,5,9-11]

The common definition of SDS was used.[1] Briefly, SDS was diagnosed in patients with both neutropenia [with at least one complete blood count (CBC) showing an absolute neutrophil count below 1.5×10^9/L] and exocrine pancreatic deficiency. In addition to this phenotype, recessive mutations of the *SBDS* gene were considered as diagnostic criteria for SDS. Of the 111 patients included in the registry with a diagnosis of SDS, 105 were genotyped. No mutations were found in two patients and one patient was excluded because of lack of phenotypic information. Thus, this study involved 102 patients, all with proven *SBDS* mutations.

Clinical investigations

Demographic and auxological data, nutritional status, hematologic parameters, results of liver tests and immunological tests, and infectious status were recorded. Septicemia, cellulitis, bacterial or fungal pneumonia, osteitis, and liver abscess were considered as severe infections and were systemically recorded. Immunoglobulin levels were analyzed according to age.[12]

Developmental retardation was considered as severe if the patient was unable to attend a normal school, even late, and thus required special schooling. Prematurity was defined by a gestational age below 37 weeks, intrauterine growth retardation as a birth weight below three standard deviations (SD) for gestational age, and severe gastrointestinal complications as the need for nutritional support, either by the enteral route through a gastrostomy or by a parenteral route. Orthopedic complications were considered to have occurred when orthopedic surgery (i.e. for hip dysplasia or scoliosis) was required. Age at diagnosis was defined as the age at the first pathological manifestation leading to the diagnosis of SDS.

Definition of hematologic features and hematologic complications

The initial CBC values were the median values of the three first CBC collected in the life of the patient. Baseline CBC were considered if they were collected during routine consultations, with the exception of periods during granulocyte colony-stimulating factor (G-CSF) therapy and any hematologic complications defined below. Because neutropenia is part of the definition of the disease, hematologic complications take into account severe dysfunction in other hematopoietic lineages, i.e. anemia or thrombocytopenia. Severe cytopenia (SC) was considered in cases of profound anemia [hemoglobin (Hb) <7 g/dL)] or profound thrombocytopenia (platelet count $<20\times10^9$/L). The classification of SC was based on three criteria: bone marrow morphology and differential count, bone marrow cytogenetics and the duration of SC (less than or more than 3 months). Bone marrow smears were centrally reviewed (by OF) and classified according to the 2008 WHO classification, which is applicable to define acute leukemia and myelodysplastic syndrome (MDS).[13-15] Indeed, the complications were categorized as follows: (i) definitive malignant SC, i.e. myelodysplasia or acute leukemia according to the WHO 2008 classification; (ii) definitive non-malignant SC if the bone marrow smear did not show any malignant features according to the WHO 2008 classification and if the bone marrow cytogenetic analyses were normal; (iii) transient SC if the cytopenia lasted less than 3 months; and (iv) clonal bone marrow cytogenetics, when a bone marrow cytogenetic clone was detected on routine bone marrow examination and in the absence of SC. The onset of non-malignant SC and malignant SC was considered as two distinct events in the same patient if the two diagnoses were separated by at least 3 months. Myeloid blockage was defined according to a previous study.[11]

SBDS sequence analysis

The patients or their parents gave written informed consent to genetic testing. Genomic DNA was extracted from blood using standard procedures. The coding sequence and exon-intron boundaries of the *SBDS* gene were amplified by using the primers and polymerase chain reaction conditions described by Boocock *et al.*[2] The polymerase chain reaction products were sequenced in both directions with the ABI PRISM Big Dye Terminator v1.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems) on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Sequences were analyzed with Seqscape software v2.2 (Applied Biosystems). Mutations are numbered as recommended by the Human Genome Variation Society (*http://www.hgvs.org/*), using the reference sequence NM_016038.2.

Statistical methods

Stata* software version 10 was used for all statistical analyses. Lower and upper interquartile values (p25 and p75) and medians are used to describe the distribution of quantitative variables. Differences between groups of patients were analyzed with Fisher's exact test if the event was discrete and Wilcoxon's test for quantitative variables. Survival was compared between groups of subjects using the log-rank test, and a Cox model was used for multivariate analysis.[16] As we performed repeated tests, *P* values less than 0.001 were considered to indicate statistical significance, unless otherwise stated. For survival analyses the end-points were death and definitive SC (either malignant or non-malignant); the period taken into account was the interval from birth to the event or to the last examination when no event occurred. The Kaplan-Meier method was used to estimate survival rates. The cut-off date was 30 September, 2011.

Results

Demographic features and extra-hematopoietic characteristics

One hundred and two patients were studied. There was a slight male predominance (58 males; 57%). The median follow-up was 11.6 years (p25: 6.2 years - p75: 20 years), corresponding to 1446 person-years. The 102 patients belonged to 93 distinct families. There were eight multiplex families, with two cases in seven families and three cases in one family. The median age at diagnosis was 0.55 years (p25: 0.18 years - p75: 1.8 years). The median gestational age was 40 weeks (p25: 38 weeks - p75: 41 weeks). There were only 11 premature births (13%) before 37 weeks of gestation. The median birth weight was 2840 g (p25: 2440g - p75: 3190g) and 27 patients (26%) had intrauterine growth retardation ≥3 SD. Thirteen patients (16.7%) required nutritional assistance. Growth retardation (height ≥3 SD below the mean) was observed in 60 (61%). Severe bone complications leading to bone surgery were reported in only nine patients (9%). Severe mental retardation was present in 25 patients (34% of the 73 patients aged >7 years). Cardiac abnormalities were observed in 12 patients (12%) and consisted of heart malformations in six and cardiomyopathy in six. Labial cleft was observed in six patients. Finally, severe gastrointestinal symptoms requiring enteral or parenteral feeding or gastrostomy were noted in 19 patients (19%), but were always transient, and noted at a young age, at a median age of 0.6 years (p25: 0.2 years - p75: 0.9 years).

Immunohematologic features and infectious events

At diagnosis of SDS, the initial absolute neutrophil count (ANC) was 0.82×10^9/L (p25: 0.5×10^9/L - p75: 1.7×10^9/L). The median hemoglobin level was 11.4 g/dL (p25: 9.9 g/dL - p75: 12.2 g/dL) and the median platelet count was 217×10^9/L (p25: 142×10^9/L - p75: 319×10^9/L). At diagnosis, 38 patients had low blood cell count values with 23 patients having an ANC below 0.5×10^9/L, 11 having a platelet count below 100×10^9/L and 13 having a hemoglobin concentration below 9 g/dL.

During routine follow-up, a median of 17 baseline CBC values per patient were available (p25: 10 - p75: 28). In all cases with serial CBC, hematologic parameters fluctuated with time, without any detectable regular variation; *Online Supplementary Figure S1* shows the sequential variation of neutrophil counts in two patients followed over a 35-year period. The median ANC was 0.75×10^9/L (p25: 0.56×10^9/L - p75: 1.32×10^9/L). The median hemoglobin level was 11.7 g/dL (p25: 11.1 g/dL - p75: 12.4 g/dL) and the median platelet count was 185×10^9/L (p25: 145×10^9/L - p75: 238×10^9/L). During routine follow-up, 28 patients had low blood cell count values with 19 patients having a median ANC below 0.5×10^9/L, 11 having a platelet count below 100×10^9/L and 7 having chronic anemia (Hb below 9 g/dL). Bone marrow smears were available at baseline for 81 patients and granulopoietic blockage was observed in 20 patients (24.7%) while the others had differential counts within the normal range. Of note, routine bone marrow smears commonly showed granulopoietic abnormalities, with condensed chromatin and nuclear hyposegmentation (*Online Supplementary Figure S2*).

Among the 81 patients with assessable immunoglobulin levels, one patient had low values (between -2 SD and -3 SD for age), but did not require immunoglobulin infusions. In contrast, polyclonal hypergammaglobulinemia was found in 52 patients (65%). The median lymphocyte count was 3.3×10^9/L (p25: 2.5×10^9/L - p75: 4.5 $\times10^9$/L). Forty-three patients had at least one severe infection (42%). The median age at the first infection was 9.6 years (p25:2.4 years - p75: 16.9 years). The Kaplan-Meier plot (*data not shown*) showed that first severe infections were most frequent in childhood but continued to appear until the fourth decade. A total of 72 severe infections were recorded and consisted of pneumonia in 30 cases, cellulitis in 26 cases, sepsis with bacteremia in 19 cases (associated with other infections in 8 cases), osteoarthritis in 3 cases, meningitis in 1 case, and colitis in 1 case. Acute stomatitis occurred in 13 patients. No chronic periodontal disease was reported. Three cases of severe viral infections were reported: one of malignant varicella, one fatal case of measles, and one case of influenza with cardiomyopathy.

Hematologic complications

A total of 41 patients developed a hematologic complication, listed in detail in *Online Supplementary Table S1*. In 21 cases, definitive SC occurred and was classified as malignant cytopenia in nine cases, non-malignant in nine, while three cases first developed non-malignant SC and subsequently malignant SC, resulting in a total of 12 cases of malignant SC and 12 cases of non-malignant SC.

In the 12 patients with malignant SC according to the WHO 2008 classification, the cytological bone marrow features were acute myeloid leukemia with MDS-related changes in eight cases (FAB M2 in 2; FAB M4 in 2, FAB M6 in 3 and FAB M0 in one); refractory cytopenia with multilineage dysplasia in three cases, and MDS with refractory anemia with excess blasts (RAEB 1) in one case. The most frequent cytogenetic feature of patients with leukemia and MDS was monosomy 7, found in six of the 12 cases. Notably, the cytogenetic abnormality consisted of an isolated i(7)(q10) in two patients. In the 12 cases of non-malignant SC, two had presented with almost complete bone marrow aplasia, five with bone marrow hypoplasia involving all hematologic lineages, three with bone marrow hypoplasia and arrest of granulopoiesis, one with isolated maturation arrest and one with hypoplasia and mild dyserythropoiesis.

In three cases, non-malignant SC, managed by repeat transfusions and granulocyte colony-stimulating factor (G-CSF), was subsequently complicated by MDS. These three

patients, who had sequentially presented with non-malignant then malignant SC, were diagnosed at birth; the appearance of the bone marrow underwent dramatic changes, concomitant with the onset of a bone marrow cytogenetic clone.

Patients with malignant or non-malignant SC had different demographic patterns as malignant complications occurred at a median age of 11.1 years (p25: 4.1 years - p75: 24.6 years) while non-malignant SC occurred at a median age of 0.13 years (p25: 0.01 year - p 75: 3.3 years). Figure 1A depicts the risk of SC, showing that this risk is not constant throughout life; there is a high incidence below 1 year of age and then the risk decreases until the age of 15 years. It appears to be more constant below 15 years, but never achieves a plateau. The 20-year cumulative risk of SC was 24.3% (95% CI 15.3%-38.5%). The Kaplan-Meier plots of these two complications, shown in Figures 1B and 1C, highlight the different timings of onset: non-malignant SC occurred early in life and never after 15 years of age (Figure 1B), while malignant SC appeared throughout life, even after the age of 30 (Figure 1C). Overall, definitive SC was responsible for 15 of the 17 deaths observed in the cohort and the six survivors of SC all underwent hematopoietic stem cell transplantation (HSCT).

In contrast, the 21 patients who had transient SC or clonal bone marrow findings without SC had a good outcome. In 12 cases, the SC was transient, recovering in less than 3 months. Bone marrow smears and cytogenetic analyses were performed in six of these cases. In one case, morphological myelodysplasia was observed but without clonality, and the patient quickly recovered normal hemoglobin levels and platelet counts. Once hematologic recovery was achieved, the hematologic situation was stable, except in one patient who developed another new episode of definitive non-malignant SC 8 years later. Transient SC was suggestive of viral infection, but in all cases, common viral infections such as parvovirus, Epstein–Barr virus and cytomegalovirus were excluded. Such transient severe complications occurred at the median age of 0.83 years (p25: 0.28 years - p75: 3.4 years).

In nine patients without SC, the hematologic complications consisted of the presence of cytogenetic clones on routine bone marrow surveillance. Although bone marrow smears showed mild dysgranulopoietic features, they did not meet the criteria for MDS/acute leukemia. The cytogenetic clone consisted of i(7)(q10) in three patients and del(20q) in five patients. In two patients a more complex cytogenetic anomaly was observed with an indolent outcome. The cytological features observed in such cases were not different from those observed on routine bone marrow smears, involving condensed chromatin and nuclear hyposegmentation (*Online Supplementary Figure S2*), occasionally hypoplastic.

G-CSF was used to prevent infections in 17 patients (in addition to its use for SC or in the post-transplant period), at a mean dose of 4.8 μg/kg (0.5–10 μg/kg). In most cases, G-CSF was used "on demand", i.e. when an infection occurred. Only seven patients received long-term G-CSF therapy. HSCT was performed in 12 patients, for malignant (n=6) or non-malignant (n=6) SC, as reported previously.

Seventeen patients died (15%), at a median age of 6.5 years (minimum - maximum: 0.2 - 33.7 years). The survival plot showed higher mortality rates before 5 years of age and also in the third decade. The observed 20-year survival rate was 85% (95% CI: 76%-92%) (*Online Supplementary Figure S3*). The two deaths not related to SC were the consequence of measles with cardiomyopathy, and a traffic accident, potentially provoked by orthopedic difficulty at the age of 13 years.

SBDS *mutations and genotype-phenotype relationships*

The mutational spectrum in the 93 probands is reported in *Online Supplementary Tables S2A and S2B*. The p.Lys62X and p.Cys84fs mutations, resulting from conversion events between the *SBDS* gene and its highly homologous pseudogene, accounted for 86% (157/186 alleles) of the causal mutations. The genotypes p.[Lys62X]+[Cys84fs] and p.[Lys62X;Cys84fs]+[Cys84fs] were present in 61% and 6% of the patients, respectively.

The other molecular events were point mutations, consisting mainly of missense mutations (17/29), nonsense mutations (2/29), frameshift mutations (4/29), in-frame deletions (1/29) and splice defects (5/29). Two-thirds of rare events were novel and affected highly conserved residues (*Online Supplementary Table S2A*). The p.Cys84fs mutation was significantly more frequent than the p.Lys62X mutation in compound heterozygotes (27% and 5%, respectively). We compared the 67% of patients who had the two recurrent genotypes with patients who had other genotypes (33%). No significant difference was observed in the distribution of hematologic parameters, infectious events, death and non-hematologic features (*data not shown*). The rate of SC was not statistically different among patients with the frequent genotype p.[Lys62X]+[Cys84fs] than in patients with the other compound heterozygous genotypes (P=0.41). One important finding arguing against a close phenotype-genotype relationship in SDS is the lack of clinical concordance in the eight multiplex families (*Online Supplementary Table S3*), as five sibling pairs were concordant for severe cytopenia, while three were discordant.

Risk factors for leukemia and bone marrow failure

The following parameters were studied: gender, age at diagnosis, intrauterine growth retardation (≥3 SD), severe gastrointestinal complications, severe developmental retardation, initial and baseline blood parameters categorized as severe neutropenia (if absolute neutrophil count $<0.5\times10^9$/L), mild thrombocytopenia (if platelet count $<100\times10^9$/L), and mild anemia (if Hb < 9 g/dL), transient SC, cytogenetic clone, orthopedic complication, and prophylactic G-CSF use. The results of the univariate analyses are summarized in Table 1.

Of the 12 variables studied (Table 1), the analysis showed that three had deleterious prognostic value with a P value ≤0.001, namely age at diagnosis and hematologic parameters both at diagnosis and during routine follow-up. Severe gastrointestinal complications were associated with a high risk of SC but the P value was higher (P=0.016) than that for the other significant factors. Gender, intrauterine growth retardation, severe neurological development delay, transient SC, cytogenetic clone without SC, orthopedic complications, G-CSF prophylactic use and genotype did not have significant impact.

Finally, taking into consideration both the age and CBC at diagnosis, it was possible to distinguish three subgroups according to the risk of hematologic complications. The three groups are: (i) the low-risk group: patients ≥ 3

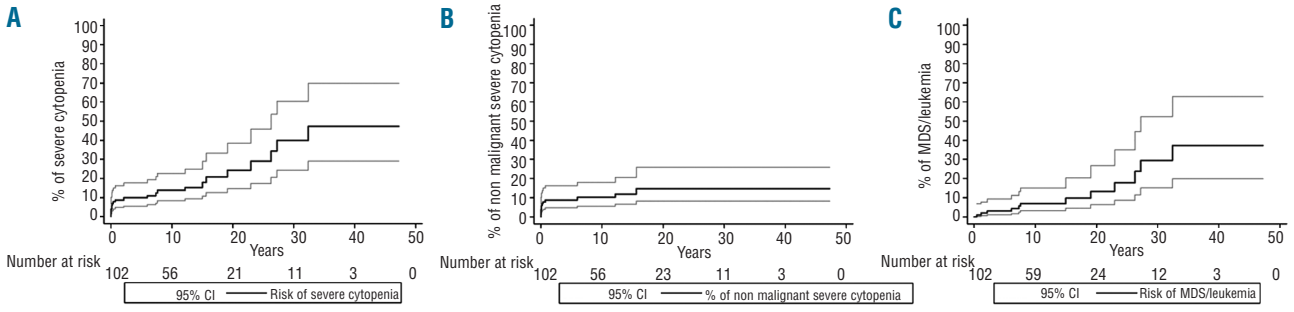

Figure 1. Kaplan-Meier plot showing the risk of severe cytopenia. The events analyzed are: (A) any severe cytopenia, (B) non-malignant severe cytopenia and (C) malignant severe cytopenia (MDS/ leukemia). The time is expressed in years since birth.

months old at diagnosis and no low value of the three hematologic parameters considered (ANC ≥ 0.5×10^9/L, Hb ≥9 g/dL and platelet count ≥100×10^9/L); (ii) the intermediate-risk group: patients <3 months old at diagnosis OR at least one low value on the initial CBC (ANC <0.5×10^9/L and/or Hb <9 g/dL and/or platelet count <100 ×10^9/L) and (iii) the high-risk group: patients <3 months old at diagnosis AND at least one low value on the initial CBC (ANC <0.5×10^9/L and/or Hb <9 g/dL and/or platelet count <100×10^9/L). The 10-year and 20-year risks of SC were 0%, 12.6%, 58.6% and 6.2%, 34.4% and 58.6%, respectively, in the three groups (P<0.0001, Figure 2). Lastly, the dynamics of severe hematologic complications were different in the three groups as the high-risk group was exposed in the first decade, the intermediate-risk group later, with SC presenting during the second decade, while the low-risk group developed SC in their third decade.

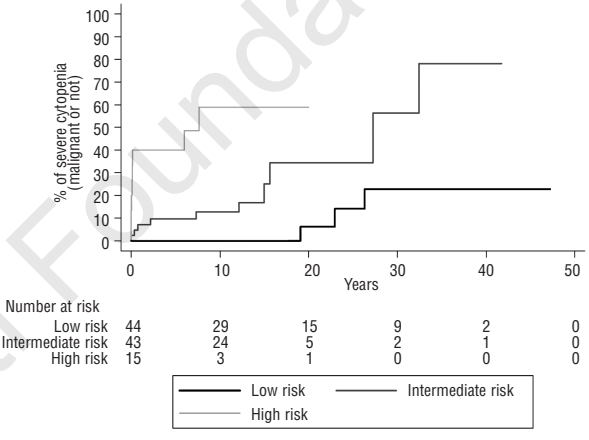

Figure 2. Kaplan-Meier plot showing the risk of severe cytopenia according to the severity score. The time is expressed in years since birth. The severity score is divided into three groups: high risk group if patients are diagnosed before the age of 3 months old AND if the first complete blood count exhibited either neutropenia <0.5×10^9/L or anemia <9 g/L or thrombocytopenia <100×10^9/L; intermediate risk group if patients are diagnosed before the age of 3 months old OR if the first complete blood count exhibited either a neutropenia <0.5 x10^9/L or anemia <9 g/L or thrombocytopenia <100×10^9/L; low risk if the patients are diagnosed after 3 months old and have no severe or mild hematologic abnormalities in their first blood count.

Discussion

We studied a large cohort of 102 patients with SDS through our national registry. The long-term follow-up period allowed us to observe hematologic complications and to identify risk factors for SC. In order to describe and classify the hematologic complications, for all patients we reviewed all hematologic parameters recorded longitudinally, the CBC, the bone marrow smears classified, after a central review, according to the more recent revision of the WHO classification of myelodysplasia[13,14] and the cytogenetic bone marrow findings. Our findings show that the major hematologic complications in SDS are definitive dysfunction of hematopoiesis, whether malignant or non-malignant, which is fatal in the absence of HSCT. Conversely, mild, non-fatal abnormalities are observed by the incidental detection of a cytogenetic clone on bone marrow and by a transient dysfunction of hematopoiesis. In our study, among the 21 patients who had definitive SC, only six patients survived, after undergoing HSCT. In contrast, among the 21 patients who had a mild hematologic complication, all survived, and only in one case did the patient present later with a severe hematologic complication (non-malignant SC – which was cured by HSCT). Our observations are concordant with those in case reports reviewed by Dror[1] and classified into four distinct groups: cytopenia, myelodysplasia, leukemia and cytogenetic abnormalities.

The situation described as cytopenia has also been called 'aplastic anemia' by several authors,[17-23] despite many cases not fulfilling the classical criteria of severe aplastic anemia.[24] In SDS, an additional complexity is related to cytogenetic abnormalities observed incidentally during routine bone marrow surveillance. Two recurrent cytogenetic anomalies have been observed, i(7)(q10) and del(20q), which can be indolent and sometimes even transient.[25-33] However, these same abnormalities can also announce or be associated with a frank malignant outcome,[34-36] as we observed in two cases in this survey. In addition to the patients with i(7)(q10) and del(20q), we also observed an indolent profile in two other patients, one with t(16;20) in addition to i(7)(q10) and one with a complex abnormality of chromosome 9 (*Online Supplementary Table S1*).

With regards to the difficulties in classifying the hematologic complications undoubtedly related to the underlying disease, our work shows that the WHO classification

of myelodysplasia can be applied to define the malignant complications of SDS[13-15] but needs to be completed by a category which can be simply defined as non-malignant SC, a category frequently named 'aplastic anemia'.[17-23] The terminology non-malignant SC was preferred to aplastic anemia, because routine bone marrow samples from SDS patients exhibited dysgranulopoietic features or abnormalities of granulopoietic maturation. As in Fanconi anemia, the dyserythropoiesis, hyposegmentation or condensed chromatin should not be considered as a sign of myelodysplasia,[37] unless it is observed in more than 50% of the neutrophils.

Indeed, the hematologic complications need to encompass mild abnormalities constituted by the incidental detection of cytogenetic clones on bone marrow and by transient dysfunction of hematopoiesis. In such cases, spontaneous recovery is a key feature and needs to be observed by month 3.

We then analyzed the risk factors that may influence the occurrence of severe hematologic complications, as they are the major causes of early death in patients with SDS. First, we analyzed the relationships between the genotype and the occurrence of hematologic complications. Until now, only four studies[38-41] had reported both information about *SBDS* genotypes and a survey of patients including cases with SC. These four studies comprised 47 patients, among whom nine cases of malignant SC and two cases of non-malignant SC were observed, showing no particular association between genotype and SC. In our large cohort of 102 genotyped patients, we excluded a correlation between genotype and the development of SC. This is not surprising since the two recurrent genotypes are observed in two-third of patients.

Secondly, we analyzed hematologic and clinical parameters. Our analysis showed that the prognostic factors for SC are significantly associated with age at first symptoms and with hematologic parameters, but did not correlate with gender or with other associated features characterizing the disease.

This led us to propose a classification of the severity of SDS based on the first blood counts (taking into account the first three CBC in order to exclude outlying values) and on the age of diagnosis (< or ≥3 months). Patients diagnosed as having SDS before the age of 3 months and with low values of hematologic parameters at diagnosis have a higher risk of major hematologic complications than patients diagnosed after the age of 3 months and without low values of CBC, or with only one of these features. Consequently, the occurrence of SC in the two groups was, respectively, 13% and 0% after 10 years and 59% and 33% after 20 years of evolution. These results need to be validated by other studies on different cohorts of patients but they offer a simple way to identify patients, at the time of their diagnosis, at high-risk of severe hematologic complications. Early identification of patients at risk of hematologic complications may be useful if considering the possibility of HSCT. To date HSCT is proposed only in cases of SC.[42] However, the results of transplants appear very variable, depending on the indication for the graft. Despite the small numbers of patients analyzed, there was a significant difference in survival between patients receiving HSCT for non-malignant SC and those undergoing the procedure for leukemic transformation. Taking into account 33 published original cases[5,18,22,23,25,43-55] of HSCT for SDS for which this information is mentioned, the survival after HSCT was significantly (P<0.001) different between the 22 patients with MDS/acute leukemia (17 deaths) and the 11 patients with

Table 1. Univariate analysis of prognosis. The end-point was severe cytopenia (malignant and non-malignant together) and the *P* value is for the log rank test.

Variables	N. at risk	Observed/ expected	Hazard ratio	*P* value
Gender				
Male	57	13/10.6	1.6	0.3
Female	45	8/10.4		
Age at diagnosis				
< 3 months	35	12/4.3	7.3	0.0001
3 months – 3 years	49	6/11.5	0.99	
3 years	18	3/5.2	1	
Genotype				
[Cys84fs]+[Lys62X]	62	13/14.4	0.75	0.41
Others	38	8/6.5		
CBC at diagnosis: ANC < 0.5x10^9/L				
Yes	23	8/4	2.54	0.02
No	79	13/19		
CBC at diagnosis: platelets < 100x10^9/L				
Yes	11	7/1.9	5.9	<0.0001
No	91	13/18		
CBC at diagnosis: hemoglobin < 9 g/dL				
Yes	13	4/2.1	2.2	0.15
No	89	17/19		
CBC at diagnosis – at least one low value				
Yes	38	14/6.6	4.4	0.0005
No	64	7/14.3		
Routine CBC: ANC < 0.5x10^9/L				
Yes	19	9/2.6	5.5	<0.0001
No	83	12/18.4		
Routine CBC platelets < 100x10^9/L				
Yes	11	9/.2	7.3	<0.0001
No	88	11/17.9		
Routine CBC hemoglobin <9 g/dL				
Yes	3	2/0.16	17	<0.0001
No	90	143/14.73		
Routine CBC – at least one low chronic value				
Yes	25	14/3.65	10.2	<0.0001
No	77	7/17.35		
Transient severe cytopenia				
Yes	12	1/2.5	0.35	0.29
No	90	20/18.4		
Cytogenetic clone without severe cytopenia				
Yes	9	0/2	*	0.19
No	93	21/19		
G-CSF^s – prophylactic – at baseline				
No	85	2/3.51	0.52	0.37
Yes	17	19/17.5		
Gastrostomy or parenteral nutrition				
Yes	19	6/2.5	3.25	0.013
No	83	15/18.5		
Severity score:				
Low risk	44	3/11.3	1	
Intermediate risk	43	10/7.8	5.9	< 0.0001
High risk	15	8/1.65	27.5	

*CBC: complete blood count; ANC: absolute neutrophil count; G-CSF: granulocyte-colony-stimulating factor; Severity score: high risk if age at diagnosis < 0.25 years and if at diagnosis CBC with a low value /intermediate if age at diagnosis < 0.25 years or if at diagnosis CBC with a low value / low risk if diagnostic age ≥ 0.25 years and no low value in the CBC at diagnosis.. *Intrauterine growth retardation (≥3 SD), severe developmental retardation, orthopedic complications were assessed and did not provide any prognostic information*

non-malignant severe cytopenia (1 death). Several factors may explain the poor results of HSCT for myelodysplasia/acute leukemia in SDS, with age appearing to be an important factor, as malignant SC occurred in older patients, while several associated morbidities progress with age in this setting, including the nutritional consequences of exocrine pancreatic insufficiency. In order to improve the results of HSCT, it has been proposed that pre-emptive transplantation be performed early in life[56] so as to prevent hematologic complications, with limited toxicity. Our study is the first to identify prognostic factors for severe hematologic complications in SDS. If our findings are confirmed, it would then be possible to consider a program of pre-emptive HSCT for SDS patients at high-risk of severe cytopenia, early in life.

In conclusion, based on a central review of all available material that can be collected in SDS patients, our study defines the hematologic complications observed in a population-based survey of SDS patients. This study showed that the risk of severe and potentially fatal complications in SDS is extremely high, being up to 25% by the age of 20 years, whereas, in contrast to the situation in patients with Fanconi anemia, no other malignancies are observed. The hematologic complications were correlated with the blood counts at diagnosis and with the age of onset of first symptoms, offering a simple method for classifying patients with SDS according to their risk of hematologic complications. A potential use of this risk stratification could be pre-emptive HSCT for the patients at high risk of hematologic complications.

Authorship and Disclosures

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