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Early Release Paper

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

Jean Donadieu,<sup>1</sup> Odile Fenneteau,<sup>2</sup> Blandine Beaupain,<sup>1</sup> Sandrine Beauvils,<sup>3</sup> Florence Bellanger,<sup>3</sup> Nizar Mahlaoui<sup>4</sup>, Anne Lambilliotte<sup>5</sup>, Nathalie Aladjidi<sup>6</sup>, Yves Bertrand<sup>7</sup>, Valérie Mialou<sup>7</sup>, Christine Perot<sup>8</sup>, Gérard Michel<sup>9</sup>, Fanny Fouyssac<sup>10</sup>, Catherine Paillard<sup>11</sup>, Virginie Gandemer<sup>12</sup>, Patrick Boutard<sup>13</sup>, Jacques Schmitz<sup>14</sup>, Alain Morali<sup>15</sup>, Thierry Leblanc<sup>16</sup>, Christine Bellanné-Chantelot,<sup>3</sup> and the associated investigators of the French Severe Chronic Neutropenia Registry\*

<sup>1</sup>AP-HP Registre Français des Neutropénies Congénitales, Hôpital Trousseau, Service d'Héματο-oncologie Pédiatrique, APHP, Paris, France; <sup>2</sup>AP-HP, Hôpital R. Debré Laboratoire d'Hématologie, Paris, France; <sup>3</sup>AP-HP, Hôpital Pitié-Salpêtrière, Département de Génétique, Université Pierre et Marie Curie, Paris, France; <sup>4</sup>AP-HP, Hôpital Necker Enfants Malades, Unité d'Immuno-hématologie Pédiatrique, Paris, France; <sup>5</sup>CHU Lille, Hôpital Jeanne de Flandre, Héματο-oncologie Pédiatrique, Lille, France; <sup>6</sup>CHU Bordeaux, Service d'Héματο-oncologie Pédiatrique, Bordeaux, France; <sup>7</sup>CHU Lyon, Institut d'Hématologie et Oncologie Pédiatrique, Service de pédiatrie, Lyon, France; <sup>8</sup>AP-HP, Hôpital Saint-Antoine, Laboratoire de cytogénétique, Paris, France; <sup>9</sup>CHU Marseille, Hopital La Timone, Service d'Héματο-oncologie Pédiatrique APHM, Marseille, France; <sup>10</sup>CHU Nancy, Hôpital de Brabois, Héματο-oncologie Pédiatrique Nancy, France; <sup>11</sup>CHU Clermont Ferrand, Service d'Héματο-oncologie Pédiatrique, Clermont Ferrand, France; <sup>12</sup>CHU Rennes, Hôpital Sud Service d'Héματο-oncologie Pédiatrique, France; <sup>13</sup>CHU Caen, Service d'Héματο-oncologie Pédiatrique, Caen, France; <sup>14</sup>AP-HP, Hôpital Necker Enfant Malades, Service de Gastro-entérologie Pédiatrique, Paris, France; <sup>15</sup>CHU Nancy, Hôpital de Brabois, Gastro-entérologie Pédiatrique Nancy, France, and <sup>16</sup>AP-HP Hôpital R Debré, Service d'hématologie pédiatrique, Paris, France

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

## **Abstract**

**Background.** To study the hematological complications of patients with the Shwachman-Diamond syndrome and their risk factors.

**Design and Methods.** 102 patients, with a median follow-up of 11.6 years, were studied. Major hematological complications were considered in the case of definitive severe cytopenia (i.e. anemia < 7g/dL or thrombocytopenia < 20 G/L), classified as malignant (myelodysplasia/leukemia) according to the 2008 World Health Organization classification or as non-malignant.

**Results.** Severe cytopenia were observed in 21 patients and classified as malignant severe cytopenia (n=9), non-malignant severe cytopenia (n=9) and in 3 cases malignant severe cytopenia was preceded by non-malignant severe cytopenia. 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 hematological parameters both at diagnosis of the disease and during the follow-up were associated with severe hematological complications ( $P<0.001$ ). Fifteen novel *SBDS* mutations were identified. Genotype analysis showed no discernable 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 hematological complications, malignant or non-malignant. Transient severe cytopenia or indolent cytogenetic clone had no deleterious value.

## Introduction

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

The *SBDS* gene, located on chromosome 7q11, is associated with the disease.<sup>2</sup> *SBDS* protein is an essential cofactor for elongation factor 1 (EFL1), and together they directly catalyze eIF6 release from nascent 60S subunits of the ribosome by a mechanism requiring both GTP binding and hydrolysis.<sup>3</sup> *SBDS* mutations arising from gene conversion are present in almost all patients, the compound heterozygous genotype p.[Lys62X]+[Cys84fs] being present in about 60% of patients.<sup>2</sup> SDS is characterized by variable clinical phenotypes between and within families. 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, one third of patients developing major hematological complications.<sup>4</sup> The latter are the main causes of early death and warrant a hematopoietic stem cell transplantation.<sup>5</sup> No risk factors for these complications have been identified so far, although leukemia appears in the literature to be more frequent in males.<sup>1;6;7</sup> This prompted us to analyze a cohort of 102 genotyped patients with SDS belonging to 93 families, for which exhaustive clinical features, hematological and biological parameters have 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. The registry was initially created in 1993; since then, enrollment has been prospective. All types of congenital neutropenia were included<sup>8</sup>. Since 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 gastro enterology units participated to this register. Data monitoring, based on medical charts, was done by a clinical research associate who visited each centre yearly. The

patient must provide written informed consent to be included in the registry. Several reports of the register are available elsewhere<sup>4,5,9-11</sup>

The common definition of SDS was used.<sup>1</sup> Briefly, SDS was diagnosed in patients with both neutropenia (with at least one complete blood count showing an absolute neutrophil count below 1.5 G/L) and exocrine pancreatic deficiency. In addition to this phenotype, recessive mutations of the *SBDS* gene were considered as diagnostic criteria of SDS. Of the 111 patients included in the registry with SDS diagnosis, 105 patients 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 investigation*

Demographic, auxologic, nutritional status, hematological parameters, liver tests, 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.<sup>12</sup>

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 3 standard deviations (SD) for gestational age, and severe gastrointestinal complications as the need for nutritional support, either by enteral route with gastrostomy or by 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 by the age at the first pathological manifestation leading to the diagnosis of SDS.

### *Definition of hematological features and hematological complications*

Initial complete blood count (CBC) value was the median value of the three first CBC collected in the life of the patients. Baseline CBC were considered if they were collected during routine consultations, with the exception of periods with granulocyte colony-stimulating factor (G-CSF) therapy and any hematological complications defined below. Because neutropenia is part of the definition of the disease, hematological complications take into account a severe dysfunction in other hematopoietic lineages, i.e. anemia or thrombocytopenia. Severe cytopenia (SC) was considered in cases of profound anemia (hemoglobin [Hb] < 7g/dL) or profound thrombocytopenia (platelets < 20 G/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 or more than 3 months). Bone marrow smears were centrally reviewed (OF) and classified according to the 2008 WHO classification, which is applicable to define acute leukemia and myelodysplastic syndrome (MDS).<sup>13-15</sup> Indeed, the complications were categorized as follows: a) malignant definitive SC, i.e. myelodysplasia or acute leukemia according to the WHO 2008 classification; b) non-malignant definitive 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; c) transient SC if the latter lasted less than 3 months; d) clonal bone marrow cytogenetics, when a bone marrow cytogenetic clone was detected on routine bone marrow examination and in the absence of SC. Onset of non-malignant SC and malignant SC were considered as two distinct events in the same patient, if the two diagnoses were separated by at least three months. Myeloid blockage was defined according to previous study<sup>11</sup>.

### *SBDS sequence analysis*

The patients or their parents gave their written informed consent for genetic testing. Genomic DNA was extracted from blood with standard procedures. The coding sequence and exon-intron boundaries of the *SBDS* gene were amplified by using the primers and polymerase chain reaction (PCR) conditions described by Boocock *et al.*<sup>2</sup> PCR 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 the 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<sup>®</sup> software version 10 was used for all statistical analyses. Lower and upper interquartile, and median values depict the distribution of quantitative variables. Differences between groups of patients were analyzed using 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 Cox model was used for multivariate analysis.<sup>16</sup> As we performed repeated tests, *P* values <0.001 were considered to indicate statistical significance, unless otherwise stated. For survival analyses the endpoints were death, definitive SC (either malignant or non-malignant) and 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. 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. Median birth weight was 2840g (p25: 2440g - p75: 3190g) and 27 patients (26%) had intrauterine growth retardation  $\geq 3$  SD. Thirteen patients (16.7%) required nutrition assistance. Growth retardation (height  $\geq 3$  SD) 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). Heart 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 was observed in 19 patients (19%), but were always transient, and observed in young age, at a median age of 0.6 years (p25: 0.2 years p75 0.9 years).

#### *Immuno-hematological features and infectious events*

At diagnosis of SDS, the initial absolute neutrophil count (ANC) was 0.82 G/L (p25: 0.5 - p75: 1.7 G/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 G/L (p25 142 - p75 319 G/L). At diagnosis, 38 patients had low blood cell count values with 23 patients with ANC below 0.5 G/L, 11 with a platelet count below 100 G/L and 13 with hemoglobin 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, hematological parameters fluctuated with time, without any detectable regular variation; *online supplementary figure 1* shows the sequential variation of neutrophil count in two patients followed over a 35-year period. The median ANC was 0.75 G/L (p25: 0.56 G/L - p75: 1.32 G/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 G/L (p25: 145 G/L - p75 238 G/L). During routine follow-up, 28 patients had low blood cell count values with 19 patients with median ANC below 0.5 G/L, 11 with a platelet count below 100 G/L and 7 with chronic anemia (hemoglobin 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 presented granulopoietic abnormalities, with condensed chromatin and nuclear hyposegmentation (*online supplementary figure 2*). Among the 81 patients with assessable immunoglobulin levels, one patient had low values (between -2SD and -3SD 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 G/L (p25: 2.5 G/L - p75: 4.5 G/L).

Forty-three patients presented at least one episode of severe infection (42%). 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 varicellae, one lethal case of measles, and one case of influenza with cardiomyopathy.

### *Hematological complications*

A total of 41 patients presented a hematological complication, listed in detail in table 1. In 21 cases, definitive SC occurred and was classified as malignant cytopenia in nine cases, non-malignant in nine, while three cases presented first a non-malignant SC and subsequently a 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 cytologic bone marrow features were acute myeloid leukemia (AML) 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, MDS with refractory anemia with excess blasts (RAEB 1) in one case. The most frequent cytogenetic feature of patients with leukemia and MDS was the monosomy 7, found in six of the twelve 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 hematological 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 G-CSF, was subsequently complicated by MDS. The three patients, who had sequentially presented with non-malignant then malignant SC, were newborns at diagnosis; the bone marrow aspect exhibited dramatic changes, concomitant with the onset of a bone marrow cytogenetic clone.

Malignant and non-malignant SC had a different demographic pattern as malignant complications occurred at a median age of 11.1 years (p25: 4.1 years - p75: 24.6 years) while the non-malignant SC occurred at median ages of 0.13 years (p25: 0.01 year - p 75: 3.3 years). Figure 1a depicts the occurrence of the risk of SC and shows that the risk is not constant throughout life; there is a high incidence below 1 year of age and then the risk decreases with age until 15 years. It appears to be more

constant below 15 years, but never achieves a plateau. Twenty-year cumulative risk of SC was 24.3% (95% CI 15.3%-38.5%). The Kaplan-Meier plot of these two complications included in figures 1b and 1c accentuates the different timing 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 6 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 three months. Bone marrow smears and cytogenetic analyses were performed in six such cases. In one case, morphological myelodysplasia was observed but without clonality, and the patient shortly recovered normal hemoglobin and platelet counts. However, once hematological recovery was achieved, the hematological situation was stable, except in one patient who presented with another new episode of definitive non-malignant SC eight years later. Transient SC was suggestive of viral infection, but in all cases, common viral infections such as parvovirus, Epstein-Barr virus (EBV), and cytomegalovirus (CMV) were excluded. Such transient severe complications occurred at the median ages of 0.83 years (p25: 0.28 years - p75: 3.4 years).

In nine patients without SC, the hematological complications consisted of the presence of cytogenetic clones on routine bone marrow surveillance. Although bone marrow smears showed mild dysgranulopoietic features, they did not exhibit MDS/acute leukemia criteria. The cytogenetic clone consisted of *i*(7)(q10) in 3 patients and *del*(20 q) in 5 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 to those observed on routine bone marrow smears, involving condensed chromatin and nuclear hyposegmentation (supplementary figure 2), occasionally hypoplastic.

G-CSF was used to prevent infections in 17 patients (in addition to its use for SC or in a 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.<sup>5</sup>

Seventeen deaths were recorded (15%), at a median age of 6.5 years (min-max: 0.2 - 33.7 years). The survival plot showed higher mortality rates before five years of age and also in the third decade and the

20-years survival rate observed was 85% (95% CI: 76%- 92%) (Supplementary figure 3). The two deaths, not related to a SC, were the consequence a measles infection with a myocardiopathy, and a traffic injury, potentially provoked by orthopedic difficulty at age of thirteen.

#### *SBDS mutations and genotype-phenotype relationships*

The mutational spectrum in the 93 probands is reported in tables 2A and 2B. The p.Lys62X and p.Cys84fs mutations, resulting from conversion events between the *SBDS* gene and its highly homologous pseudogene, represented 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 (table 2A). 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 hematological parameters, infectious events, death and non-hematological 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 1*), as five sib 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 ( $\leq 3$ SDS), severe gastrointestinal complications, severe developmental retardation, initial and baseline blood parameters categorized as severe neutropenia (if absolute neutrophil count  $< 0.5$  G/L), mild thrombocytopenia (if platelet count  $< 100$  G/L), and mild anemia (if Hb  $< 9$  g/dL), transient SC, cytogenetic clone, orthopedic complication, prophylactic G-CSF use. Univariate analysis is summarized in table 3.

Among 12 variables studied (table 3), the analysis showed that three of them had deleterious prognostic value with a  $P$  value  $\leq 0.001$ , namely the age at diagnosis and the hematological parameters both at diagnosis and during routine follow-up. Severe gastrointestinal complications are associated with a high risk of SC but the  $P$  value is higher than for other significant factors ( $P=0.016$ ). 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 complete blood count at diagnosis, it was possible to distinguish three subgroups according to the risk of hematological complications. The three groups are the (i) the low-risk group: patients with age of diagnosis  $\geq 3$  months and no low value on the three parameters (ANC  $\geq 0.5$  G/L and/or Hb  $\geq 9$  g/dL and/or platelets  $\geq 100$  G/L); (ii) the intermediate-risk group: patients with age of diagnosis  $< 3$  months or at least one low value on the initial CBC (ANC  $< 0.5$  G/L and/or Hb  $< 9$  g/dL and/or platelets  $< 100$  G/L) and (iii) the high-risk group (patients with age of diagnosis  $< 3$  months and at least one low value on the initial CBC (ANC  $< 0.5$  G/L and/or Hb  $< 9$  g/dL and/or platelets  $< 100$  G/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 three groups also had a different dynamic of severe hematological complications 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 presented SC in their third decade.

## 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 the hematological complications and to identify risk factors for SC.

To describe and to classify the hematological complications, we reviewed for all patients all the hematological parameters obtained longitudinally, the complete blood counts, the bone marrow smears classified, after a central review, according to the more recent revision of the WHO classification of myelodysplasia<sup>13;14</sup> and the cytogenetic bone marrow findings. Our findings show that the major hematological complications in SDS are a definitive dysfunction of hematopoiesis, either malignant or non-malignant, which is lethal in the absence of HSCT. Conversely, mild abnormalities, not lethal, are observed by the incidental detection of cytogenetic clone on bone marrow and by a transient dysfunction of hematopoiesis. In our study, among the 21 patients who experienced a definitive SC, only six patients survived, after receiving a HSCT. In contrast, among the 21 patients who presented a mild hematological complication, all survived, and only in one case, the patient had presented later with a severe hematological complication (a non malignant SC – which was cured by a HSCT). Our observations are concordant with case reports reviewed by Dror<sup>1</sup> and classified into 4 distinct groups: cytopenia, myelodysplasia, leukemia and cytogenetic abnormalities. The situation described as cytopenia was also named by several authors as ‘aplastic anemia’,<sup>17-23</sup> despite many cases not fulfilling the classical criteria of severe aplastic anemia.<sup>24</sup> In SDS, an additional complexity is related to cytogenetic abnormalities observed incidentally during routine bone marrow surveillance. Two recurrent cytogenetic anomalies are observed, i(7)(q10) and del(20q), which can be indolent and eventually transient.<sup>25-33</sup> But the same abnormalities can also announce or be associated with a frank malignant outcome,<sup>34-36</sup> as we observed in two cases in this survey. In addition to i(7)(q10) and del(20q), we observed a same indolent profile in two patients, one with t(16;20) in addition to i(7)(q10) and one with a complex abnormality of the chromosome 9 (table 1).

With regards to the difficulties in classifying the hematological 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<sup>13-15</sup> but needs to be completed by a category which can be simply defined as non-malignant SC, a category frequently named ‘aplastic anemia’.<sup>17-23</sup> The terminology

non-malignant SC was preferred to aplastic anemia, because routine bone marrow of SDS patients exhibited dysgranulopoietic features or abnormalities of granulopoietic maturation. As in Fanconi anemia, the dyserythropoiesis hyposegmentation or condensed chromatin have not be considered as a sign of myelodysplasia,<sup>37</sup> except if it was observed in more than 50% of the neutrophils.

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

We then analysed the risk factors that may influence the occurrence of severe hematological complications, as they constitute so far the major causes of early death in patients with SDS. First, we analysed the relationships between the genotype and the occurrence of hematological complications. Until now, only four studies<sup>38-41</sup> had both reported information about *SBDS* genotypes and a survey of patients including cases with SC. These four studies represent a total of 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 this large cohort of 102 genotyped patients, we excluded a correlation between the genotype and the development of SC. This is not surprising since the two recurrent genotypes are observed in two-third of patients.

Secondly, we analysed hematological and clinical parameters. Our analysis showed that the prognostic factors of SC are significantly associated with the age at first symptoms and with hematological 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 3 CBC in order to exclude outlier values) and on the age of diagnosis (< or ≥ 3 months). Patients diagnosed as SDS before the age of three months and with low hematological parameters at diagnosis have a higher risk of major hematological complications than patients diagnosed after three months and without low values of CBC, or with only one of these features. Consequently, the occurrence of SC was respectively in the two groups 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 it offers a simple way to identify patients at high-risk of severe hematological complications, at the time of their diagnosis.

Early identification of patients at risk for hematologic complications may be useful if any consider the possibility offered by the hematopoietic stem cell transplantation (HSCT). To date HSCT is proposed only in case of severe cytopenia<sup>42</sup>. But the results of transplants appear highly contrasted, according to the indication of the graft. Despite the small number, 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<sup>5;18;22;23;25;43-55</sup> of HSCT for SDS for which this information is mentioned, the survival after HSCT is significantly ( $p < 0.001$ ) different among the 22 patients with MDS / acute leukemia (17 deaths) vs. the 11 patients with non malignant severe cytopenia (1 death). Several factors may explain the poor results of HSCT for myelodysplasia/acute leukemia in SDS and age appeared 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 the HSCT, pre-emptive transplantation has been proposed early in life<sup>56</sup> in order to prevent hematological complications, with a limited toxicity. Our study is the first to identify prognostic factors of severe hematological complications in SDS. If these results are confirmed, it would be possible then to consider a program of pre-emptive HSCT for SDS patients at high-risk of severe cytopenia, early in life.

In conclusion, our study defines in a population-based survey, the hematological complications observed in SDS, based on the central review of all available materials which can be collected in SDS patients. This study showed that the risk of severe and potential lethal complications in SDS is extremely high, up to 25% by age of 20 years, while no other malignancy is observed, in contrast to Fanconi anemia. The hematological complications were correlated with the hematological blood counts collected at diagnosis and with the age of first symptoms, offering a simple tool to classify patients with SDS with regards to the risk of hematological complications. A potential use could be a pre-emptive hematopoietic stem cell transplantation program for the patients at high risk of hematological complications.



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### **Authorship and Disclosures**

The original design of the study was drawn up by J Donadieu and C Bellanné Chantelot. Odile Fenneteau reviewed the bone marrow smears of patients with severe hematological complications. The French SCN register is coordinated by J Donadieu and the data management is realized by B Beaupain. Genetics analysis was performed by C Bellanné-Chantelot, S. Beaufils and F Bellanger in France. All the authors contributed to the writing and revision of the manuscript.

*\*Associated investigators of the French Severe Chronic Neutropenia Registry in alphabetic order.*

Treating physicians: Dalila Adjaoud<sup>17</sup>, Sophie Ansoborlo<sup>18</sup>, Brigitte Bader-Meunier<sup>4</sup>, Vincent Barlogis<sup>9</sup>, Françoise Bellmann<sup>1</sup>, Philippe Bensaïd<sup>19</sup>, Claire Berger<sup>20</sup>, Christian Berthou<sup>21</sup>, Stéphane Blanche<sup>4</sup>, Monique Blayo<sup>22</sup>, Pascale Blouin<sup>23</sup>, Pierre Bordigoni<sup>10</sup>, Joel Cardona<sup>24</sup>, Jean Laurent Casanova<sup>4</sup>, Jean-Pierre Cezard<sup>25</sup>, Jean Pierre Chouraqui, Philippe Colombat<sup>23</sup>, Alain Dabadie<sup>26</sup>, Marianne Debré<sup>4</sup>, Anne Deville<sup>27</sup>, Viviane Dubruille<sup>28</sup>, Frank DuGravier<sup>29</sup>, Zairi El Mostefa<sup>30</sup>, Jean-Pierre Fermant<sup>31</sup>, Claire Fieschi<sup>31</sup>, Alain Fischer<sup>4</sup>, Claire Galambrun<sup>9</sup>, Claire Gay<sup>20</sup>, Carine Garcette<sup>32</sup>, Jean-Louis Ginies<sup>33</sup>, Muriel Girard<sup>14</sup>, Frédéric Gottrand<sup>34</sup>, Oliver Goulet<sup>14</sup>, François Labarthe<sup>35</sup>, Guy Leverger<sup>1</sup>, Martine LeMerrer<sup>36</sup>, Olivier Hermine<sup>37</sup>, Florence Lacaille<sup>14</sup>, Alain Lachaux<sup>38</sup>, Armelle Lamour<sup>39</sup>, Jeanne Languépin<sup>40</sup>, Mustapha Layadi<sup>41</sup>, Odile Lejars<sup>23</sup>, Edouard Legall<sup>12</sup>, Philippe Lemoine<sup>42</sup>, Anne Lienhardt Roussie<sup>40</sup>, Aude Marie-Cardine<sup>43</sup>, Evelyne Marinier<sup>25</sup>, Chantal Maurage<sup>35</sup>, Françoise Mazingue<sup>5</sup>, Françoise Mechinaud<sup>44</sup>, Etienne Merlin<sup>11</sup>, Marguerite Michau<sup>6</sup>, Fabrice Monpoux<sup>27</sup>, Martine Meyer<sup>11</sup>, Brigitte Nelken<sup>5</sup>, Marie Ouache<sup>16</sup>, Isabelle Pellier<sup>33</sup>, Perle Pages<sup>1</sup>, Odile Peter<sup>45</sup>, Christophe Piguet<sup>40</sup>, Maryline Poiree<sup>27</sup>, Yves Perel<sup>6</sup>, Corinne Pondarré<sup>7</sup>, Xavier Rialland<sup>33</sup>, Bertrand Roquelaure<sup>46</sup>, Jacques Sarles<sup>46</sup>, Gérard Socié<sup>47</sup>, Felipe Suarez<sup>37</sup>, Jean Louis Stephan<sup>20</sup>, Caroline Thomas<sup>44</sup>, Patrick Tounian<sup>32</sup>, Valérie Triolo<sup>27</sup>, Olivier Tournilhac<sup>48</sup>, Sébastien Trouillier<sup>48</sup>, Stéphane Van der Becken<sup>49</sup>, Francis Witz<sup>50</sup>, AND hematologists: Chantal Brouze<sup>51</sup>, Marie Paule Callat<sup>52</sup>, Xavier Charmes<sup>53</sup>, Marion Eveillard<sup>54</sup>, Chantal Fossat<sup>55</sup>, Richard Garand<sup>54</sup>, Franck Geneviève<sup>56</sup>, Sandrine Girard<sup>57</sup>, Hélène Lapillonne<sup>58</sup>, Véronique Latger-Cannard<sup>59</sup>, Béatrice Ly-Sunnaram<sup>60</sup>, Maria-Elena Noguera<sup>61</sup>, Gabrielle Mareynat<sup>62</sup>, Marie-Pierre Pagès<sup>57</sup>, André Petit<sup>63</sup>, Franck Trimoreau<sup>64</sup>.

17 CHU Grenoble, Département de Pédiatrie, Grenoble France

18 CH Saintes, Service de Pédiatrie, Saintes, France

19 CH Argenteuil, Service de Pédiatrie, Argenteuil, France

20 CHU Saint Etienne, Service de Pédiatrie, Génétique et Hémato Oncologie, Saint Etienne, France

21 CHU Brest, Service d'Hématologie, Brest, France

- 22 CH Quimper, Service de Pédiatrie, Quimper, France
- 23 CHU Tours, Hemato Oncologie Pédiatrique, Tours, France
- 24 CHU Poitiers La miletrie, Service de Pédiatrie, Poitiers, France
- 25 AP-HP Hôpital R Debré, Service de Gastro Enterologie pédiatrique, Paris, France
- 26 CHU Rennes, Gastro Entérologie Pédiatrique, Rennes, France
- 27 CHU Nice, Département de Pédiatrie, Nice, France
- 28 CHU Nantes, Hématologie, Nantes, France
- 29 Cabinet de Pédiatrie, Pessac, France
- 30 CH Hyeres, Service de Pédiatrie, France
- 31 APHP, Hôpital Saint Louis, Département d'Immuno-Hematologie, Paris, France
- 32 AP-HP, Hôpital Trousseau, Service de gastro entérologie Pédiatrie, Paris, France
- 33 CHU Angers, Département de Pédiatrie, Angers, France
- 34 CHU Lille, Hôpital Jeanne de Flandre, Service de gastro entérologie Pédiatrique, Lille, France
- 35 CHU Tours, Hôpital Clocheville, Service de pédiatrie – unité de gastro entérologie – Tours, France
- 36 AP-HP, Hôpital Necker, Centre de référence des maladies osseuses, Paris, France
- 37 AP-HP, Hôpital Necker, Service d'hématologie, Paris, France
- 38 CHU Lyon, Hôpital Mère et Enfants, Gastro entérologie Pédiatrique, , Lyon, France
- 39 CH Morlaix, Service de Rhumatologie, Morlaix, France
- 40 CHU, Département de Pédiatrie, Limoges, France
- 41 CH, Service de Pédiatrie, Guéret, France
- 42 CHU, Département de Pédiatrie, Brest, France
- 43 CHU, Hôpital Charles Nicolle, Rouen, France
- 44 CHU Nantes, Service d'Hémato Oncologie Pédiatrique, Hotel Dieu, Nantes, France
- 45 CH Mulhouse, Service de Pédiatrie, Mulhouse, France
- 46 CHU Marseille, Hôpital La Timone, Service de gastro enterologie Pédiatrique, Marseille, France
- 47 AP-HP, Hôpital Saint Louis, Service de transplantation médullaire, Paris, France
- 48 CHU de Clermont Ferrand, Hotel Dieu, Hématologie Medecine Interne, Clermont Ferrand, France
- 49 CH de la Réunion, Hôpital sud, Département de pédiatrie, La réunion, France

50 CHU de Nancy, Hématologie, Nancy, France

51 AP-HP, Hôpital Necker, Laboratoire d'hématologie, Paris, France

52 CHU de Rouen, Laboratoire d'Hématologie, Rouen, France

53 CH Aurillac, Laboratoire de Biologie, Aurillac, France

54 CHU Nantes, Laboratoire d'hématologie, Nantes, France

55 CHU Marseille, Hôpital La Timone, Laboratoire d'hématologie Marseille, France

56 CHU Angers, Laboratoire d'Hématologie, Angers, France

57 CHU Lyon, Institut d'Hemato Oncologie Pédiatrique, Laboratoire d'hématologie et cytogénétique, Lyon, France

58 AP-HP, Hôpital Trousseau, Laboratoire d'Hématologie, Paris, France

59 CHU Nancy, Laboratoire d'Hématologie, Nancy, France

60 CHU Rennes, Laboratoire d'hématologie, Rennes, France

61 AP-HP, Hôpital Saint Louis, Laboratoire d'hématologie, Paris, France

62 CHU Clermont Ferrand, Laboratoire d'hématologie, Clermont Ferrand France

63 CHU Tours, Laboratoire d'Hématologie, Tours, France

64CHU Limoges, Laboratoire d'Hématologie, Limoges, France

## References

1. Dror Y. Shwachman-Diamond syndrome. *Pediatr.Blood Cancer* 2005;45(7):892-901.
2. Boocock GR, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. *Nat.Genet.* 2003;33(1):97-101.
3. Finch AJ, Hilcenko C, Basse N, Drynan LF, Goyenechea B, Menne TF, et al. Uncoupling of GTP hydrolysis from eIF6 release on the ribosome causes Shwachman-Diamond syndrome. *Genes Dev.* 2011;25(9):917-29.
4. Donadieu J, Leblanc T, Bader-Meunier B., Barkaoui M, Fenneteau O, Bertrand Y, et al. Analysis of risk factors for myelodysplasias, leukemias and death from infection among patients with congenital neutropenia. Experience of the French Severe Chronic Neutropenia Study Group. *Haematologica* 2005;90(1):45-53.
5. Donadieu J, Michel G, Merlin E, Bordigoni P, Monteux B, Beaupain B, et al. Hematopoietic stem cell transplantation for Shwachman-Diamond syndrome: experience of the French neutropenia registry. *Bone Marrow Transplant.* 2005;36(9):787-92.
6. Mack DR. Shwachman-Diamond syndrome. *J.Pediatr.* 2002;141(2):164-5.
7. Smith OP, Hann IM, Chessells JM, Reeves BR, Milla P. Haematological abnormalities in Shwachman-Diamond syndrome. *Br.J.Haematol.* 1996;94(2):279-4.
8. Donadieu J, Fenneteau O, Beaupain B, Mahlaoui N, Bellanne CC. Congenital neutropenia: diagnosis, molecular bases and patient management. *Orphanet.J Rare.Dis.* 2011;6(1):26.
9. Anon. The French national registry of primary immunodeficiency diseases. *Clin.Immunol.* 2010;135(2):264-2.

10. Beaupain B, Leblanc T, Reman O, Hermine O, Vannier JP, Suarez F, et al. Is pegfilgrastim safe and effective in congenital neutropenia? An analysis of the French Severe Chronic Neutropenia registry. *Pediatr.Blood Cancer* 2009;53(6):1068-73.
11. Bellanne-Chantelot C, Clauin S, Leblanc T, Cassinat B, Rodrigues-Lima F, Beaufile S, et al. Mutations in the ELA2 gene correlate with more severe expression of neutropenia: a study of 81 patients from the French Neutropenia Register. *Blood* 2004;103(11):4119-25.
12. Plebani A, Ugazio AG, Avanzini MA, Massimi P, Zonta L, Monafò V, et al. Serum IgG subclass concentrations in healthy subjects at different age: age normal percentile charts. *Eur.J.Pediatr.* 1989;149(3):164-7.
13. Arber DA, Brunning RD, Orazi A et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. In: Swerdlow SHCEHNLJESPSASHTJVJW, ed.: WHO; 2008:124-6.
14. Germing U, Aul C, Niemeyer CM, Haas R, Bennett JM. Epidemiology, classification and prognosis of adults and children with myelodysplastic syndromes. *Ann.Hematol.* 2008;87(9):691-9.
15. Niemeyer CM, Baumann I. Myelodysplastic syndrome in children and adolescents. *Semin.Hematol.* 2008;45(1):60-70.
16. Collet D. Modelling survival data in medical research. London: Chapman and Hall; 1994.
17. Black LV, Soltau T, Kelly DR, Berkow RL. Shwachman-Diamond syndrome presenting in a premature infant as pancytopenia. *Pediatr.Blood Cancer* 2008;51(1):123-4.
18. Fleitz J, Rumelhart S, Goldman F, Ambruso D, Sokol RJ, Pacini D, et al. Successful allogeneic hematopoietic stem cell transplantation (HSCT) for Shwachman-Diamond syndrome. *Bone Marrow Transplant.* 2002;29(1):75-9.
19. Kuijpers TW, Nannenbergh E, Alders M, Bredius R, Hennekam RC. Congenital aplastic anemia caused by mutations in the SBDS gene: a rare presentation of Shwachman-Diamond syndrome. *Pediatrics* 2004;114(3):387-91.

20. Vibhakar R, Radhi M, Rumelhart S, Tatman D, Goldman F. Successful unrelated umbilical cord blood transplantation in children with Shwachman-Diamond syndrome. *Bone Marrow Transplant.* 2005;36(10):855-61.
21. Woods WG, Krivit W, Lubin BH, Ramsay NK. Aplastic anemia associated with the Shwachman syndrome. In vivo and in vitro observations. *Am.J.Pediatr.Hematol.Oncol* 1981;3(4):347-51.
22. Barrios NJ, Kirkpatrick DV. Successful cyclosporin A treatment of aplastic anaemia in Shwachman-Diamond syndrome. *Br.J.Haematol.* 1990;74(4):540-1.
23. Tsai PH, Sahdev I, Herry A, Lipton JM. Fatal cyclophosphamide-induced congestive heart failure in a 10-year-old boy with Shwachman-Diamond syndrome and severe bone marrow failure treated with allogeneic bone marrow transplantation. *Am.J.Pediatr.Hematol.Oncol.* 1990;12(4):472-6.
24. Camitta BM. Criteria for severe aplastic anaemia. *Lancet* 1988;1(8580):303-4.
25. Cunningham J, Sales M, Pearce A, Howard J, Stallings R, Telford N, et al. Does isochromosome 7q mandate bone marrow transplant in children with Shwachman-Diamond syndrome? *Br.J.Haematol.* 2002;119(4):1062-9.
26. Dror Y, Durie P, Ginzberg H, Herman R, Banerjee A, Champagne M, et al. Clonal evolution in marrows of patients with Shwachman-Diamond syndrome: a prospective 5-year follow-up study. *Exp.Hematol.* 2002;30(7):659-9.
27. Maserati E, Minelli A, Pressato B, Valli R, Crescenzi B, Stefanelli M, et al. Shwachman syndrome as mutator phenotype responsible for myeloid dysplasia/neoplasia through karyotype instability and chromosomes 7 and 20 anomalies. *Genes Chromosomes.Cancer* 2006;45(4):375-82.
28. Minelli A, Maserati E, Nicolis E, Zecca M, Sainati L, Longoni D, et al. The isochromosome i(7)(q10) carrying c.258+2t>c mutation of the SBDS gene does not promote development of myeloid malignancies in patients with Shwachman syndrome. *Leukemia* 2009;23(4):708-11.

29. Porta G, Mattarucchi E, Maserati E, Pressato B, Valli R, Morerio C, et al. Monitoring the isochromosome i(7)(q10) in the bone marrow of patients with Shwachman syndrome by real-time quantitative PCR. *J Pediatr.Hematol.Oncol* 2007;29(3):163-5.
30. Pressato B, Marletta C, Montalbano G, Valli R, Maserati E. Improving the definition of the structure of the isochromosome i(7)(q10) in Shwachman-Diamond Syndrome. *Br.J Haematol.* 2010;150(5):632-3.
31. Smith A, Shaw PJ, Webster B, Lammi A, Gaskin K, Diaz S, et al. Intermittent 20q- and consistent i(7q) in a patient with Shwachman-Diamond syndrome. *Pediatr.Hematol.Oncol.* 2002;19(7):525-8.
32. Sokolic RA, Ferguson W, Mark HF. Discordant detection of monosomy 7 by GTG-banding and FISH in a patient with Shwachman-Diamond syndrome without evidence of myelodysplastic syndrome or acute myelogenous leukemia. *Cancer Genet.Cytogenet.* 1999;115(2):106-13.
33. Mellink CH, Alders M, van der LH, Hennekam RH, Kuijpers TW. SBDS mutations and isochromosome 7q in a patient with Shwachman-Diamond syndrome: no predisposition to malignant transformation? *Cancer Genet.Cytogenet.* 2004;154(2):144-9.
34. Dror Y, Squire J, Durie P, Freedman MH. Malignant myeloid transformation with isochromosome 7q in Shwachman-Diamond syndrome. *Leukemia* 1998;12(10):1591-5.
35. Maserati E, Minelli A, Olivieri C, Bonvini L, Marchi A, Bozzola M, et al. Isochromosome (7)(q10) in Shwachman syndrome without MDS/AML and role of chromosome 7 anomalies in myeloproliferative disorders. *Cancer Genet.Cytogenet.* 2000;121(2):167-71.
36. Raj AB, Bertolone SJ, Barch MJ, Hersh JH. Chromosome 20q deletion and progression to monosomy 7 in a patient with Shwachman-Diamond syndrome without MDS/AML. *J Pediatr.Hematol.Oncol* 2003;25(6):508-9.



37. Cioc AM, Wagner JE, MacMillan ML, DeFor T, Hirsch B. Diagnosis of myelodysplastic syndrome among a cohort of 119 patients with fanconi anemia: morphologic and cytogenetic characteristics. *Am.J Clin.Pathol* 2010;133(1):92-100.
38. Kawakami T, Mitsui T, Kanai M, Shirahata E, Sendo D, Kanno M, et al. Genetic analysis of Shwachman-Diamond syndrome: phenotypic heterogeneity in patients carrying identical SBDS mutations. *Tohoku J.Exp.Med.* 2005;206(3):253-9.
39. Makitie O, Ellis L, Durie PR, Morrison JA, Sochett EB, Rommens JM, et al. Skeletal phenotype in patients with Shwachman-Diamond syndrome and mutations in SBDS. *Clin.Genet.* 2004;65(2):101-12.
40. Taneichi H, Kanegane H, Futatani T, Otsubo K, Nomura K, Sato Y, et al. Clinical and genetic analyses of presumed Shwachman-Diamond syndrome in Japan. *Int.J.Hematol.* 2006;84(1):60-2.
41. Maserati E, Pressato B, Valli R, Minelli A, Sainati L, Patitucci F, et al. The route to development of myelodysplastic syndrome/acute myeloid leukaemia in Shwachman-Diamond syndrome: the role of ageing, karyotype instability, and acquired chromosome anomalies. *Br.J Haematol.* 2009;145(2):190-7.
42. Dror Y, Donadieu J, Kogmeier J, Dodge J, Toiviainen-Salo S, Makitie O, et al. Draft consensus guidelines for diagnosis and treatment of Shwachman-Diamond syndrome. *Ann.N.Y.Acad.Sci.* 2011;1242(1):40-55.
43. Arseniev L, Diedrich H, Link H. Allogeneic bone marrow transplantation in a patient with Shwachman-Diamond syndrome. *Ann.Hematol.* 1996;72(2):83-4.
44. Bunin N, Leahey A, Dunn S. Related donor liver transplant for veno-occlusive disease following T-depleted unrelated donor bone marrow transplantation. *Transplantation* 1996;61(4):664-6.

45. Cesaro S, Guariso G, Calore E, Gazzola MV, Destro R, Varotto S, et al. Successful unrelated bone marrow transplantation for Shwachman-Diamond syndrome. *Bone Marrow Transplant.* 2001;27(1):97-9.
46. Davies SM, Wagner JE, DeFor T, Blazar BR, Katsanis E, Kersey JH, et al. Unrelated donor bone marrow transplantation for children and adolescents with aplastic anaemia or myelodysplasia. *Br.J.Haematol.* 1997;96(4):749-6.
47. Dokal I, Rule S, Chen F, Potter M, Goldman J. Adult onset of acute myeloid leukaemia (M6) in patients with Shwachman-Diamond syndrome. *Br.J.Haematol.* 1997;99(1):171-3.
48. Faber J, Lauener R, Wick F, Betts D, Filgueira L, Seger RA, et al. Shwachman-Diamond syndrome: early bone marrow transplantation in a high risk patient and new clues to pathogenesis. *Eur.J Pediatr.* 1999;158(12):995-1000.
49. Hsu JW, Vogelsang G, Jones RJ, Brodsky RA. Bone marrow transplantation in Shwachman-Diamond syndrome. *Bone Marrow Transplant.* 2002;30(4):255-8.
50. Mitsui T, Kawakami T, Sendo D, Katsuura M, Shimizu Y, Hayasaka K. Successful unrelated donor bone marrow transplantation for Shwachman-Diamond syndrome with leukemia. *Int.J.Hematol.* 2004;79(2):189-2.
51. Okcu F, Roberts WM, Chan KW. Bone marrow transplantation in Shwachman-Diamond syndrome: report of two cases and review of the literature. *Bone Marrow Transplant.* 1998;21(8):849-51.
52. Park SY, Chae MB, Kwack YG, Lee MH, Kim I, Kim YS, et al. Allogeneic bone marrow transplantation in Shwachman-Diamond syndrome with malignant myeloid transformation. A case report. *Korean J.Intern.Med.* 2002;17(3):204-6.
53. Ritchie DS, Angus PW, Bhathal PS, Grigg AP. Liver failure complicating non-alcoholic steatohepatitis following allogeneic bone marrow transplantation for Shwachman-Diamond syndrome. *Bone Marrow Transplant.* 2002;29(11):931-3.

54. Seymour JF, Escudier SM. Acute leukemia complicating bone marrow hypoplasia in an adult with Shwachman's syndrome. *Leuk.Lymphoma* 1993;12(1-2):131-5.
55. Smith OP, Chan MY, Evans J, Veys P. Shwachman-Diamond syndrome and matched unrelated donor BMT. *Bone Marrow Transplant.* 1995;16(5):717-8.
56. Bhatla D, Davies SM, Shenoy S, Harris RE, Crockett M, Shoultz L, et al. Reduced-intensity conditioning is effective and safe for transplantation of patients with Shwachman-Diamond syndrome. *Bone Marrow Transplant.* 2008;42(3):159-65.

## Legends

**Table 1.** Detailed description of the patients with hematological complications, sorted by type of complications: Severe cytopenia malignant, Severe cytopenia non malignant, Cytogenetic clone without severe cytopenia, transient severe cytopenia.

**Table 2.** A: Details of allelic changes, including the nucleotide sequence changes and deduced consequences at the protein level. The results are given for the 102 patients belonging to 93 families. Each allele was counted only once per multiplex family.

B: Distribution of the genotypes among the 93 affected families, classified by the consequences of allelic changes.

**Table 3.** Univariate prognosis analysis. The end point was severe cytopenia (malignant and non-malignant together) and the p value is the p value of the log rank test.

Table 1.

	UPN	SBDS Genotype	Age*	Bone marrow cytology Review	Bone marrow Karyotype	Follow-up after the event (y)	Outcome (alive/HSCT/ cause of death)
Malignant SC	5617	p.[Lys62X]+[Cys84fs]	2.5	AML with MDS related changes FAB type AML2	Monosomy 7	0.3	Death / no HSCT
	5057	p.[Lys62X]+[Cys84fs]	26.3	AML with MDS related changes FAB type AML2	43,XY,-5,-7,-13,-16,-18,-20,+3mar[5] / 51,XY,+1,-5,-7,+10,+10+21,+3mar[2] / 46,XY,del(20)(q11q13)[2]	0.5	Death / AML / no HSCT
	5081	p.[Lys62X]+[Cys84fs]	27.2	AML with MDS related changes FAB type AML4	45,XX,add(1)(p11),-7,add(14)(q32),add(21)(q22)[20]	0.58	Death / HSCT / viral infection after HSCT
	5073	p.[Lys62X]+[Cys84fs]	32.4	AML with MDS related changes FAB type AML6	41-46,XY,-5,-7,-15,-16,-19,+der(3)(?) ,+der(6)(?) ,del(13)(q13q33),der(20)(?) ,+r(?) ,+mar1 ,+mar2 [cp31]	1.29	Death / AML/ no HSCT
	5253	p.[Cys84fs]+c.[290-1G>A]	7.3	AML with MDS related changes FAB type AML6	45,XY,del(5)(q ?q?),add(9)(q ?),+11,add(17)(p ?),-20,+22	0.42	Death / HSCT / viral infections after HSCT
	5038	p.[Lys62X]+[?]	19.1	AML with MDS related changes FAB type AML6	46,XY[1]/45,XY,del(5)(q15q33),-7,+?mar[1]/44,XY,der(3)t(3;6)(?;?) ,del(5)(q?q?),-6,-7[19]	1.9	Death/ HSCT / Death related to MDS relapse
	5023	p.[Lys62X]+[Cys84fs]	22.9	AML with MDS related changes FAB type AML0	46,XY,add(15)(p?) / 44,idem,-20,-21,-22,+mar[20]/46,XY[3]	1.41	Death / no HSCT
	5117	p.[Lys62X]+[Cys84fs]	7.6	Refractory cytopenia with multilineage dysplasia	46,XY,del(1)(p36),t(3;21)(q26;q11),del(5)(q21q24),del(7)(q21q35),+del(8)(p21),-18[15]/46,XY[10]	1.36	Death / HSCT / Death related to MDS relapse
	5082	p.[Cys84fs]+[Gln94_Val95del]	15.9	Refractory cytopenia with multilineage dysplasia	isochromosome 7q	12.8	Alive / HSCT
Non-malignant followed by malignant SC	5737	p.[Cys84fs]+c.[129-71_140del83]	0.1	Hypoplastic	no abnormality	1.5	Death // no HSCT – supportive care
			1.1	MDS (AREB 1)	Monosomy 7	0.43	Death after HSCT / toxicities of HSCT Cond. regimen
	5208	p.[Lys62X]+[Cys84fs]	0.1	Hypoplastic granulopoietic maturation arrest	no abnormality	1.01	Death / secondary MDS 0.5 year after initial event
			0.4	Refractory cytopenia with multilineage dysplasia	46,XY,i(7)(q10)[10]/46,XY[10]	0.57	Death from MDS / no HSCT
	5437	p.[Cys84fs]+[Arg169Leu]	0.2	Hypoplastic granulopoietic maturation arrest	no abnormality	6.30	Death / no HSCT / secondary AML 6 y after initial event
			6	AML with MDS related changes FAB type AML4	46,XY,add(7)(q31)[15]/46,XY[1].ish der(7)t(1;7)(q32;q31)(WCP7+,WCP1+)	0.5	Death from AML /no HSCT
Non-malignant definitive SC	5263	p.[Lys62X]+[Cys84fs]	0.05	Aplastic	no abnormality	10.4	Alive after HSCT
	5855	p.[Lys62X]+[Cys84fs]	0.1	Aplastic	no abnormality	0.21	Death / supportive care respiratory distress
	5170	p.[Lys62X]+[Cys84fs]	0.4	Hypoplastic	no abnormality	10.3	Alive after HSCT
	5512	p.[Lys62X]+[Cys84fs]	0.2	Hypoplastic	no abnormality	8.3	Alive after HSCT
	5128	p.[Cys84fs]+[Cys119Arg]	12.2	Hypoplastic	no abnormality	22.1	Alive after HSCT
	5184	p.[Lys62X]+[?]	5.9	Hypoplastic with mild dyserythropoiesis features	no abnormality	17.1	Alive after HSCT
	5589	p.[Cys84fs]+[Glu99fs]	0.1	Hypoplastic with dysgranulopoietic features	no abnormality	1.9	Death / no HSCT
	5770	p.[Cys84fs]+[exon 2 deletion]	0.73	Normally rich bone marrow no blast Granulopoietic Maturation arrest	no abnormality	0.37	Death / no HSCT
Transient then definitive SC	5098	p.[Cys84fs]+c.[624+1G>C]	8	No BM smear	Not done	17.2	Definitive not clonal SC 7/9 years after
			15.6	Hypoplastic	no abnormality	9.67	Alive after HSCT
Transient SC	5154	p.[Lys62X]+[Cys84fs]	1.6	No BM smear	Not done	13.8	Stable situation
	5139	p.[Lys62X]+[Cys84fs]	0.4	Hypoplastic	no abnormality	16.4	Stable situation
	5171	p.[Lys62X]+[Cys84fs]	6.5	No BM smear	Not done	8.3	Stable situation
	5207	p.[Lys62X]+[Cys84fs]	0.28	Hypoplastic	no abnormality	19.3	Stable situation
	5504	p.[Lys62X]+[Lys148Thr]	0.9	Dysmyelopoietic features hemophagocytosis	no abnormality	8.4	Stable situation
	5777	p.[Cys84fs]+[Pro6Leu]	3.4	No BM smear after CBC normalisation hypoplastic with dysgranulopoietic features + neutrophil phagocytosis	Not done	4.3	Stable situation

	5180	p.[Lys62X]+[Cys84fs]	0.2	Erythroblastopenia	No abnormally	20	Stable situation
	5254	p.[Cys84fs]+[Cys119Tyr]	0.4	No BM smear	Not done	49	Stable situation
	5620	p.[Lys62X]+[Cys84fs]	0.13	No BM smear	Not done	6.31	Stable situation
	7011	p.[Lys62X]+[Cys84fs]	0.1	Maturation arrest	No abnormally	0.3	Stable situation
	5764	p.[Lys62X]+[Cys84fs]	0.75	Maturation arrest	no abnormality	3.25	Stable situation
Bone marrow cytogenetic clone without SC	5074	p.[Lys62X]+[Cys84fs]	26.5	Rich mild dysgranulopoiesis and dysmegacaryopoietic	46,XX,del(20)(q11q13)[10]/46,XX[20]	10.7	Stable situation
	5321	p.[Lys62X]+[Cys84fs]	5.6	Rich mild dysgranulopoiesis with bilobated nuclei / segmentation defect	46,XY,del(20)(q1?1)[6]/46,XY[28]	3.7	Stable situation
	5519	p.[C84fs]+[Arg218Gln]	6.5	Poor Mild dysgranulopoiesis with bilobated nuclei / segmentation defect	46,XY,t(16;20)(q24;q11.2)[2]/46,XY,i(7q) [1]/46,XY[17]	7.4	Stable situation
	5571	p.[C84fs]+[Arg218X]	13.6	Poor Mild dysgranulopoiesis with bilobated nuclei / segmentation defect	46,XY,del(20)(q?)[7]/46,XY[23]	5.3	Stable situation
	5670	p.[C84fs]+[Phe57Leu]	3.7	Rich Mild dysgranulopoiesis with bilobated nuclei / segmentation defect	isochromosome 7q	1.1	Stable situation
	5715	p.[C84fs]+[Gln103fs]	2.8	Rich Mild dysgranulopoiesis with bilobated nuclei / segmentation defect	46,XY,del(20)(q1?2)[16]/46,XY[4]	2.14	Stable situation
	5579	p.[C84fs]+[exon 2 deletion]	7.56	Rich Mild dysgranulopoiesis with bilobated nuclei / segmentation defect	46,XX,?dup(9)(p13)[?6]/46,XX[24] 44,X,-X,add(9)(p22),-13,-14,-14,+2mar[2]/46,XX[18]	4.8	Stable situation
	6251	p.[Cys84fs]+[Cys19Thr]	22.5	Rich Mild dysgranulopoiesis with bilobated nuclei / segmentation defect	46,XX,i(7)(q10)[12]/46,XX[8]	0.5	Stable situation
	5451	p.[Lys62X]+[Cys84fs]	5.5	Poor Mild dysgranulopoiesis with bilobated nuclei / segmentation defect	46,XX,del(20)(q12)[4]/46,XX[29]	6	Stable situation

Abbreviation: HSCT: Hematopoietic Stem Cell transplantation; AML: Acute Myeloid Leukemia; MDS: Myelodysplasia; FAB: French American British classification of leukaemia; RAEB:

Refractory anemia with excess blasts; BM, bone marrow; \* age at eve

**Table 2A.**

Location	Nucleotide sequence change	Protein effect	Type of mutation	Number of alleles	Percentage	References
Exon 1	c.13delA	p.Thr5fs	Frameshift mutation	1	< 0.01	This report
Exon 1	c.17C>T	p.Pro6Leu	Missense mutation	1	< 0.01	This report
Exon 1	c.56G>A	p.Arg19Gln	Missense mutation	1	< 0.01	48
Exon 1	c.95 A>G	p.Tyr32Cys	Missense mutation	1	< 0.01	49
Intron 1	c.129-2A>G	p.?	Splice defect	1	< 0.01	This report
Intron 1	c.129-1G>A	p.?	Splice defect	1	< 0.01	This report
Exon 2	c.129-71_140del83	p.?	Splice defect	1	< 0.01	This report
Exon 2	c.164C>A	p.Ser55X	Nonsense mutation	1	< 0.01	This report
Exon 2	c.171T>A	p.Phe57Leu	Missense mutation	1	< 0.01	This report
Exon 2	c.183_184delinsCT	p.Lys62X	Nonsense mutation	58	0.31	2
Exon 2	c.[183_184delinsCT; 258+2T>C]	p.[Lys62X;Cys84fs]	Nonsense mutation	6	0.03	2
Exon 2	c.129-?_258+?	p.?	Exon 2 deletion	1	< 0.01	This report
Intron 2	c.258+1G>A	p.Cys84fs	Splice defect	1	< 0.01	2
Intron 2	c.258+2T>C	p.Cys84fs	Splice defect	90	0.49	2
Exon 3	c.279_284del	p.Gln94_Val95del	In-frame deletion	1	< 0.01	48
Exon 3	c.297_300delAAGA	p.Glu99fs	Frameshift mutation	2	0.01	50
Exon 3	c.307_308delCA	p.Gln103fs	Missense mutation	1	< 0.01	49
Exon 3	c.355T>C	p.Cys119Arg	Missense mutation	1	< 0.01	This report
Exon 3	c.356G>A	p.Cys119Tyr	Missense mutation	3	0.02	This report
Exon 3	c.385A>G	p.Thr129Ala	Missense mutation	1	< 0.01	This report
Exon 3	c.443A>C	p.Lys148Thr	Missense mutation	1	< 0.01	48
Exon 3	c.453A>C	p.Lys151Asn	Missense mutation	1	< 0.01	This report
Exon 3	c.461C>T	p.Ala154Val	Missense mutation	2	0.01	This report
Intron 3	c.460-1G>A	p.?	Splice defect	1	< 0.01	48
Exon 4	c.506G>T	p.Arg169Leu	Missense mutation	2	0.01	This report
Intron 4	c.624+1G>C	p.?	Splice defect	1	< 0.01	48
Exon 5	c.652C>T	p.Arg218X	Nonsense mutation	1	< 0.01	51
Exon 5	c.653G>A	p.Arg218Gln	Missense mutation	1	< 0.01	This report
			Undetermined mutation*	2	0.01	
				186		

\*In two patients with a documented *SBDS* phenotype, only one heterozygous *SBDS* mutation was found.

**Table 2B.**

Genotype	Occurrence	Percentage
p.[Cys84fs]+[Lys62X]	56	62
p.[Cys84fs]+[Lys62X;Cys84fs]	6	6.5
p.[Cys84fs]+[Cys84fs]	1	1
p.[Cys84fs]+[nonsense, frameshift mutation]	8	9
p.[Cys84fs]+[splice defect]	5	5
p.[Cys84fs]+[missense mutation]	12	13
p.[Cys84fs]+[in-frame deletion]	1	1
p.[Lys62X]+[missense mutation]	1	1
p.[Lys62X]+[undetermined mutation]	2	1
p.[Ala154Val]+[Ala154Val]	1	1
		93

**Table 3.**

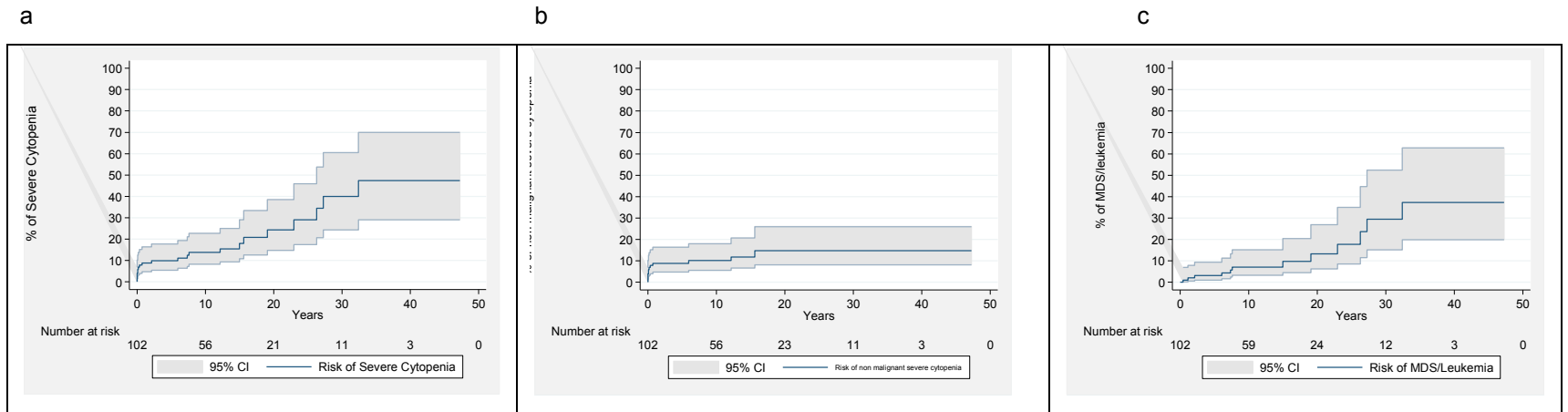
<b>Variables</b>	<b>Modality</b>	<b>No. at risk</b>	<b>Observed/Expected</b>	<b>Hazard ratio</b>	<b>p value</b>
Gender	Male	57	13/10.6	1.6	0.3
	Female	45	8/10.4		
Age at diagnostic	< 3 months	35	12/4.3	7.3	0.0001
	3 months – 3 years	49	6/11.5	.99	
	3 years	18	3/5.2	1	
Genotype	[Cys84fs]+[Lys62X]	62	13/14.4	0.75	0.41
	Others	38	8/6.5		
CBC at diagnosis: ANC < 0.5 G/L	Yes	23	8/4	2.54	0.02
	No	79	13/19		
CBC at diagnosis: Platelets < 100 G/L	Yes	11	7/1.9	5.9	<0.0001
	No	91	13/18		
CBC at diagnosis: Hemoglobin < 9g/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 < 0.5 G/L	Yes	19	9/2.6	5.5	<0.0001
	No	83	12/18.4		
Routine CBC Platelets < 100 G/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 <sup>3</sup> – 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	< 0.0001
	Intermediate risk	43	10/7.8	5.9	
	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 diagnostic age < 0.25 years and if at diagnosis CBC with a low value / Low risk if diagnostic age >= 0.25 years and if no low value at diagnosis CBC

\* Intrauterine growth retardation ( $\leq 3$  SD), severe developmental retardation, orthopedic complications have been assessed and did not provide any prognostic information.



**Figure 1.** Kaplan-Meier plot showing the risk of severe cytopenia. The events in figure 1a are any severe cytopenia, in figure 1b the non-malignant severe cytopenia and in figure 1c the malignant severe cytopenia (MDS/ leukemia). The time is expressed in years since birth.



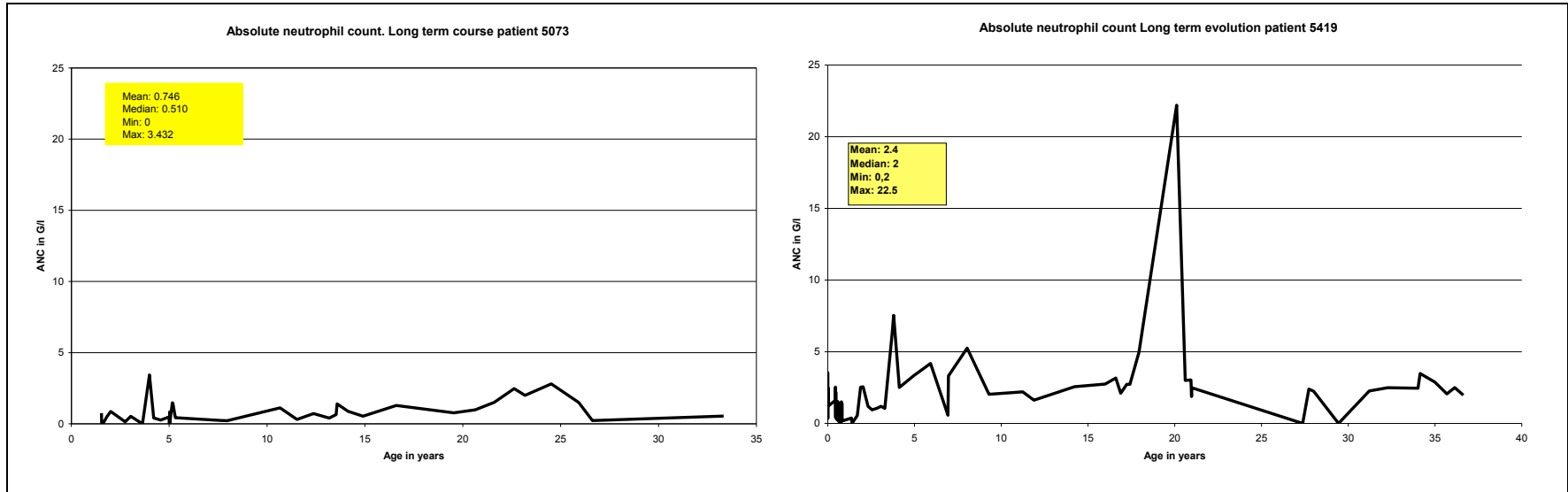
**Online Supplementary Table 1.** Main clinical features of the 10 pairs of siblings. All the patients carried the p.[Lys62X]+[Cys84fs] genotype, except for two families, one\* with the p.[C84fs]+[Pro6Leu] genotype and one\*\* with p.[Cys84fs]+[large deletion]. The concordance among siblings is shown in the right-hand column in terms of major hematological events and also morphologic and developmental features.

UPN	Sex	Age at diagnosis (y)	Age at last follow up / vital status + cause of death	Severe cytopenia (age)	Major intestinal complications	Gastro complications	Major developmental impairment	Bone complications	Heart abnormalities	Baseline ANC median G/l	Baseline platelets median G/l	Concordance for SC
5073	M	0.29	33.7 D (AML)	Yes clonal (MDS)	No	No	No	No	Yes (aortic coarctation)	0.510	152	No
5074	F	23	39 L – mother of 3 children	No (but isolated transient del 20q)	No	No	No	No	No	0.768	180	
5616	F	0.73	1.3 D (sepsis during measles)	No	Yes	No	No	No	No	0.215	54	Yes both with severe expression but AML in only one
5617	F	0.02	2.3 D (AML)	Yes AML like (2.1)	No	No	No	No	No	1.040	–	
5438*	M*	0.9	2.5 L	No	No	No	No	No	No	0.595	275	Yes
5439*	M*	0.9	2.3 L	No	No	No	No	No	No	0.660	258	
5171	M	2.2	11.9 L	No	No	No	No	No	No	0.574	261	No
5170	M	0.3	9.7 L	Yes SC not clonal	Yes	Yes	Yes	No	No	0.392	125	
5062	F	0.4	30L	No	No	No	No	No	No	0.886	166	Yes
5063	F	1.05	38 L – mother of two children	No	No	No	No	No	No	0.566	154	
5029	M	0.13	17 L	No	No	Yes	Yes	Yes	No	0.864	341	Yes
5030	M	0.54	12 L	No	No	No	No	No	No	0.784	191	
5769	F	0.22	0.7 D sepsis	No	No	No	No	No	No	0.694	401	No (severe expression in 2/3)
5570	F	0.06	1.1 D sepsis	Yes	No	Yes severe seizure	No	No	Cardiomyopathy	0.825	459	
5579	F	0.15	8.4 L	No	No	No	No	No	No	0.595	275	
5777	M	7.6	8.6 L	No	No	No	No	No	No	0.885	186	Yes
5778	M	4.5	5.6 L	No	No	No	No	No	No	1.577	244	

UPN: unique patient number; M: Male; F: Female; L: Living; D: Dead; ANC: Absolute Neutrophil Count; AML: Acute myeloid leukemia; SC: Severe cytopenia

\*monozygotic twins

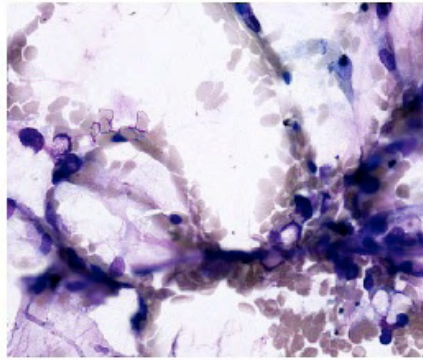
**Online supplementary figure 1.** Long term outcome of routine absolute neutrophils count in two unrelated patients bearing the same mutation p.Lys62X p.Cys84fs.



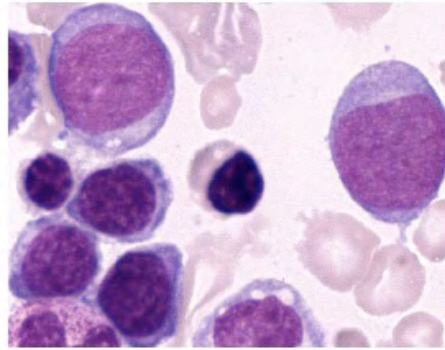
**Online supplementary figure 2.** a: Marrow smear in a patient with Shwachman-Diamond syndrome complicated by bone marrow aplasia: poor cellularity, fat cells and mast cells. b: Marrow smear in a patient with Shwachman-Diamond syndrome complicated by acute erythroid leukemia (FAB AML 6). c: Transient not clonal SC with granulopoiesis maturation arrest. d: Marrow smear in a patient with Shwachman-Diamond syndrome complicated by cytopenia and monosomy 7 (RAEB-1). Top panel: Dyserythropoiesis: karyorrhexis, multinuclearity, internuclear bridging, megaloblastoid changes. Lower panel: Dysgranulopoiesis: nuclear hypolobulation, cytoplasmic hypogranularity, dual nuclear and blast

e: Marrow smear in a patient with Shwachman-Diamond syndrome transient severe cytopenia with transient dysmyelopoietic features Up: dual nuclear Low: micromegakaryocytes with non-lobated nuclei. f: Neutrophils on marrows smears in 3 patients with Shwachman-Diamond syndrome and one control showing condensed chromatin and nuclear hyposegmentation and in one control. For each bone marrow, the proportion of neutrophils with condensed chromatin and nuclear hyposegmentation was counted . Top left: example in a patient with SC but non malignant (mean: 34%)

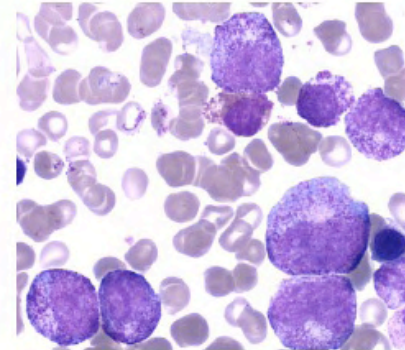
Top right: example in a patient without SC with cytogenetic abnormality (mean: 26%). Below and left: example in SDS without SC and without cytogenetic (mean: 20%). Below and right: without SDS (mean: 4%).



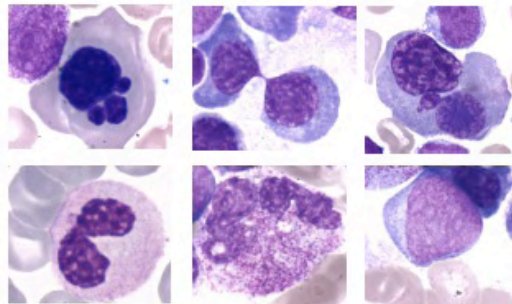
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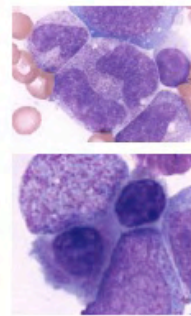
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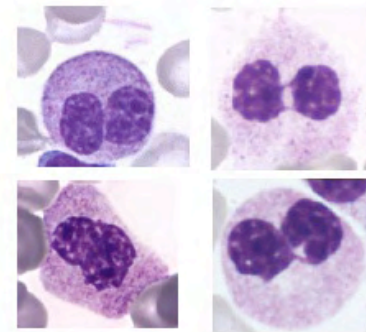
c:



d:



e:



f:

**Online supplementary figure 3.** Kaplan-Meier plot showing the overall survival of the cohort of 102 patients. The time is expressed in year since birth.

