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

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Background and Objectives. The two main complications of severe chronic neutropenia are fatal sepsis and myelodysplasia/acute leukemia (MDS/AL). Granulocyte colony-stimulating factor (G-CSF) therapy has significantly reduced the frequency and severity of infections, but its possible influence on the risk of malignancy is not known.

Design and Methods. The French Severe Chronic Neutropenia (SCN) Registry has prospectively collected data since 1994 on 231 patients with various forms of SCN, namely severe congenital neutropenia (n=101), cyclic neutropenia (n=60), glycogen storage disease type Ib (GSDIb) (n=15) and Shwachman-Diamond syndrome (SDS)(n=55). The median overall follow-up is 11.1 years. Parameters of exposure to G-CSF therapy, such as the time averaged dose, follow up after first use of G-CSF, and the cumulative dose, have been recorded.

Results. Eight septic deaths occurred, of which 6 among patients with severe congenital neutropenia and 2 in patients with cyclic neutropenia; none of these 8 patients was receiving G-CSF therapy. No septic deaths occurred during G-CSF therapy. Thirteen cases of MDS/AL were recorded. The cumulative incidence of MDS/AL was 2.7% (SD 1.3%) at 10 years and 8.1% (SD 2.7%) at 20 years.

Interpretation and Conclusions. Risk factors for MDS/AL were the diagnostic category, the severity of neutropenia, younger age at diagnosis, and strong exposure to G-CSF. MDS/AL only occurred in patients with severe congenital neutropenia and SDS. Owing to their particular susceptibility to infections, patients with severe congenital neutropenia had the strongest exposure to G-CSF; the risk of leukemia increased with the degree of G-CSF exposure in this subgroup.

Key words: Severe chronic neutropenia, myelodysplasia, Shwachman-Diamond syndrome, *ELA2* gene, leukemia, G-CSF, risk factors.

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Severe chronic neutropenia is a group of rare and heterogeneous disorders characterized by permanent or recurrent severe neutropenia, including adult-onset idiopathic neutropenia and congenital neutropenias such as severe congenital neutropenia (also known as Kostmann's syndrome), cyclic neutropenia, Shwachman-Diamond syndrome, and glycogen storage disease type Ib.[1-5] In severe congenital neutropenia, the myelopoietic disorder is usually diagnosed during the first months of life. These patients have frequent severe bacterial infections, resulting in high morbidity and mortality in early childhood. Granulocyte colony-stimulating factor (G-CSF), used for this indication since 1988, has significantly reduced the frequency and severity of infections. G-CSF therapy normalizes the absolute neutrophil count (ANC) in most cases, and improves quality of life in almost all forms of constitutional neutropenia.[6,7] Spontaneous hematologic malignancies were reported prior to the G- CSF era in patients with severe congenital neutropenia[8-10] and Shwachman-Diamond syndrome.[1] The possible influence of G-CSF therapy on the risk of malignancies was raised in the initial report on G-CSF use for this indication.[6] The *International Severe Chronic Neutropenia Registry* (ISCNR), based on a survey of 348 patients with congenital neutropenia monitored until the end of 1999, showed that the risk of hematologic malignancies was about 13% after 8 years of follow-up.[11] The duration of follow-up after initial G-CSF therapy was not identified as a risk factor, but the possible influence of other measures of G-CSF exposure, such as the dose per injection and the cumulative dose, was not examined. A recent Japanese survey showed an increased risk of myelodysplasia associated with G-CSF administration to patients with aplastic anemia.[12] More recently, G-CSF therapy was suspected of increasing the risk of secondary myeloid transformation in childhood acute lymphoblastic leukemia[13]. None of

these findings has been confirmed by other teams.[14,15] The *French Severe Chronic Neutropenia Registry* has recently attained sufficient follow-up (>10 years) to observe late and rare adverse events, such as secondary leukemia and myelodysplasia. Here, we analyze the preventive efficacy of G-CSF on severe infections in patients with congenital neutropenia, and risk factors for leukemic transformation.

Design and Methods

Organization of the Registry and data monitoring

The *French Severe Chronic Neutropenia Registry* was created in 1994 and is organized by an independent association (*Groupe d'Etude des Neutropénies*). A reference document approved by the *Société d'Hématologie et Immunologie Pédiatrique* (SHIP) defines the procedures for monitoring patients. The database was approved by the French computer watchdog commission (CNIL certificate #97.075). Patients' files are monitored by clinical research associates, who visit each center at least once a year. The following parameters are recorded: gender, age at diagnosis, severe bacterial or fungal infections (septicemia, cellulitis, pneumonia, osteitis, and liver abscess), complete blood cell counts, and differential bone marrow cell counts (smear method).

Inclusion and exclusion criteria

Patients are eligible for enrollment in the registry, with their informed consent, if they meet the following criteria: documented ANC less than 500 cells/mm^3 on at least three occasions during the three months prior to registration, or documented ANC less than 1000 cells/mm^3 with recurrent severe infections. A bone marrow smear study is required, except for patients with cyclic neutropenia, glycogen storage disease type Ib, and Shwachman-Diamond syndrome. Patients must be older than 3 months, except for those with a positive family history. Leukemia must be ruled out prior to G-CSF therapy and inclusion in the registry (normal bone marrow cytology and cytogenetics). A panel of cytologists review the bone marrow slides and validate the inclusion criteria for this analysis. Exclusion criteria consist of known drug-induced neutropenia, thrombocytopenia of less than 50,000 platelets/mm^3, and anemia less than 8 g/dL (except for patients with glycogen storage disease type Ib or Shwachman-Diamond syndrome), human immunodeficiency virus (HIV) seropositivity, known hematologic malignancy, previous chemotherapy, and neutropenia associated with B or T lymphocyte deficiency. Autoantibodies against neutrophils are sought in every case; patients with positive results are diagnosed as having autoimmune neutropenia and were not included in this analysis; patients with adult idiopathic neutropenia were likewise not included in this analysis.

Classification of neutropenia

For this analysis, congenital neutropenia was split into four diagnostic categories.

1) Severe congenital neutropenia was diagnosed in patients with no cyclic variations of ANC; most patients in this group have Kostmann's disease, with very early clinical onset. Various other disorders could be associated with the neutropenia, except for exocrine pancreatic deficiency and glycogenosis. Patients with Cohen's syndrome (n=2) (OMIM #216550) and an entity combining skin and urogenital malformations[16] were included in this latter group.
2) Cyclic neutropenia was diagnosed in patients in whom neutrophil counts oscillated with 21-day periodicity.
3) Glycogen storage disease type Ib was diagnosed if glucose-6-phosphatase translocase deficiency was detected by liver biopsy.
4) Shwachman-Diamond syndrome was diagnosed if exocrine pancreatic deficiency was associated with skeletal, skin or liver abnormalities, and if Pearson's syndrome could be ruled out. Patients with severe congenital neutropenia and cyclic neutropenia were screened for *ELA2* gene mutations as previously reported.[17]

Definition of hematologic events

Acute leukemia was defined using WHO criteria, i.e. at least 20% of blast cells on bone marrow smears. As dysplastic cytological abnormalities were nearly always present in this group of patients, myelodysplastic syndrome (MDS) was diagnosed if cytological abnormalities were associated with anemia (and/or thrombocytopenia) requiring blood transfusion, and if clonal cytogenetic abnormalities were present. G-CSF receptor mutations were screened for in 55 patients by means of single-strand conformational polymorphism (SSCP), as previously described,[18] and were confirmed by sequencing.

Definition of G-CSF exposure

To calculate G-CSF exposure, we assumed dose equivalence between filgrastim and lenograstim. As the unit dose and frequency of G-CSF injections are prescribed on an individual basis, each patient's overall treatment was estimated using several parameters. The following parameters were calculated for each period of G-CSF therapy in which both the dose and the frequency of injections were stable: (i) ddi = dose delivered per injection (μg/kg); (ii) ni = number of injections; (iii) cumulative dose = total dose received during the

Table 1. Baseline characteristics of the 231 patients with congenital neutropenia in the French registry.

Diagnosis	*n*	*Age (y) at diagnosis Median (min-max)*	*Sex ratio Male/female*	*ANC/mm³ at diagnosis[1] Median (min-max)*	*AMC/mm³ at diagnosis[1] Median (min-max)*	*Follow-up (y) Median (min-max)*
Severe congenital neutropenia	101	0.5 (birth-12.6)	42/59	316 (0-1412)[2]	774 (56-6975)	11 (0.13-49.5)
Cyclic neutropenia	60	1.5 (birth-33.4)	29/31	651 (0-4240)	534 (140-3059)	14 (0.1-54)
Glycogen storage disease type Ib	15	0.5 (birth-3.3)	9/6	747 (172-2788)	718 (0-1471)	19 (1.9-31.3)
Shwachman-Diamond syndrome	55	0.5 (birth-23)	34/21	864 (46-4020)	515 (15-1708)	8 (0.3-35.6)

[1]ANC: absolute neutrophil count, AMC: absolute monocyte count. All available complete blood counts prior to G-CSF therapy were taken into account, even if collected during an infectious episode. [2]The median ANC value was above 0.5×10⁹/L in 31 cases in this group. The diagnosis of severe congenital neutropenia was confirmed in all these cases because additional criteria were present, such as severe life-threatening infections, gingivitis or granulocyte maturation arrest on bone marrow smear. [3]Age at the last follow-up visit was below 0.25 years in one case of a familial history of dominant neutropenia with early death.

relevant period (μg/kg), calculated as ddi × ni; and (iv) duration of the relevant period in days. The total cumulative dose (μg) was calculated as the sum of cumulative doses for each treatment period, from the first day of G-CSF to the last day of follow-up. The cumulative duration of G-CSF therapy was calculated as the sum of all treatment periods, and was expressed in years. For each patient, total follow-up since the onset of G-CSF therapy was calculated from the first injection to the last day of follow-up (FU-G-CSF), and was expressed in years. The time-averaged dose (TAD, μg/kg/day) was calculated by dividing the cumulative dose by the cumulative duration of treatment. In the hypothetical case of a patient who received daily injections of 10 μg/kg/day G-CSF for one year, followed by a 2-year period without G-CSF, and then a 3-year period with 5 μg/kg G-CSF every other day, the characteristics of G-CSF exposure would be as follows: FU-G-CSF: 6 years; cumulative duration: 2.5 years, cumulative dose: 6387 μg/kg, and time-averaged dose: 7 μg/kg/day.

Statistical methods

Demographic, clinical, biological and therapeutic data were recorded using the Access database (Microsoft Corp.). Stata® version 7 software was used for all statistical analyses. Categorical data were compared using Fisher's exact test and quantitative data using the Mann-Whitney test. All tests were two-tailed. *p* values of less than 0.05 are considered to indicate statistical significance, unless otherwise stated. For survival analyses, the endpoints were death and onset of MDS/leukemia. The period taken into account was the interval between birth and either an event or the last examination when no event occurred. The median follow-up was estimated using Schemper's method.[19] The Kaplan-Meier method was used to estimate survival rates. Survival was compared between groups of subjects by means of the log-rank test.[20] Multivariate analysis was not used, owing to the small number of events.

Results

As of 31 March 2003, 231 patients with congenital neutropenia had been enrolled in the registry. The diagnoses were severe congenital neutropenia in 101 patients, cyclic neutropenia in 60 patients, glycogen storage disease type Ib in 15 patients and Shwachman-Diamond syndrome in 55 patients. Although all four diagnoses could be made at birth, the median age at diagnosis was 1.5 years for cyclic neutropenia and 0.5 years for the other three groups. The sex ratio was not significantly different from 1 in any of the four diagnostic groups. As expected, the median ANC before G-CSF therapy varied according to the diagnostic category, and also within each category. The lowest median ANC (316/mm³) was found in patients with severe congenital neutropenia; median values in the other three groups ranged from 651 to 864/mm³. The median ANC value in patients with severe congenital neutropenia was above 500/mm³ in 31 cases (Table 1). The diagnosis was nonetheless retained in all these patients, as they had additional criteria for severe congenital neutropenia, such as life-threatening infections, gingivitis, or granulocytic maturation arrest. The overall median follow-up at the cut-off date was 11.1 years (from 7.8 years in Shwachman-Diamond syndrome to 18.9 years in those with GSDIb). Sixty-nine patients had reached 18 years of age at the last visit.

G-CSF exposure

Eleven patients were included in clinical trials of filgrastim (n=3) or lenograstim (n=8),[21] which ended in 1993. G-CSF treatment was individually tailored, based essentially on the recurrence of severe infections and G-CSF tolerability (Table 2). Nearly all the patients with GSDIb (14/15) received G-CSF, and the treatment was always effective in this group. Only 15/55 SDS patients (27%) received G-CSF, and three of these patients were resistant, initially or during treatment.

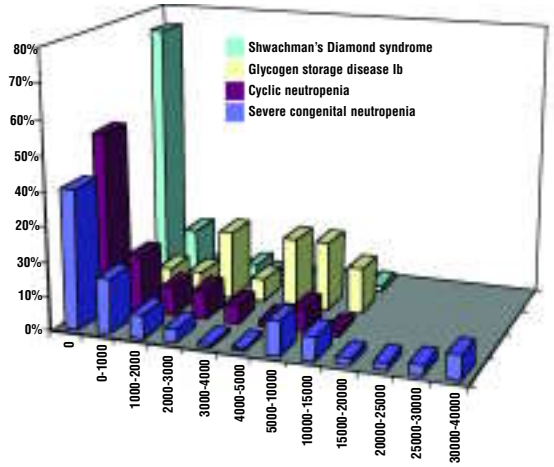

Figure 1. Distribution of cumulative G-CSF doses according to the diagnostic category.

Half the patients with severe congenital neutropenia (60/101) or cyclic neutropenia (29/60) received G-CSF; this treatment failed in three patients, all with severe congenital neutropenia. Patients with GSDIb had the longest median cumulative duration of G-CSF therapy (2.8 years), followed by patients with severe congenital neutropenia (1.6 years), cyclic neutropenia (1.1 years), and Shwachman-Diamond syndrome (0.36 years). Patients with severe congenital neutropenia received the highest time-averaged dose (median: 9.4 μg/kg/day), followed by patients with Shwachman-Diamond syndrome (6.9 μg/kg/day), cyclic neutropenia (5 μg/kg/day) and GSDIb (5 μg/kg/day). However, patients with severe congenital neutropenia and GSDIb had the highest total cumulative dose (5489 μg/kg and 4226 μg/kg, respectively). Figure 1 shows the distribution of total cumulative doses in each diagnostic category (note the heterogeneous distribution within each group). Only patients with severe congenital neutropenia were exposed to very high doses of G-CSF (above 15,000 μg/kg) (Figure 1, Table 2); 14% of patients in this category, and no patients in the other three diagnostic categories, received such doses.

Sepsis and other causes of death

The overall 10-year and 20-year survival rates were 95.3% (SD 1.5) and 87.3% (SD 3.0), respectively (Figure 2).

Causes of death were analyzed with regard to the diagnostic category and the circumstances (Table 3). Eight deaths were related to sepsis (six patients with severe congenital neutropenia, two with cyclic neutropenia). None of the patients who died of sepsis was receiving G-CSF. Two of these deaths occurred just prior to the advent of G-CSF, while the other six involved patients whose parents or physicians had chosen not to use G-CSF.

Nine deaths were related to MDS/AL; three occurred in patients with severe congenital neutropenia (including one death post-BMT) and six in patients with Shwachman-Diamond syndrome (including three post-BMT).

Three patients died of causes unrelated to sepsis or MDS/AL: two patients with severe congenital neutropenia died from complications of BMT (indicated for G-CSF failure and bone marrow failure in one case each), and one patient with Shwachman-Diamond syndrome died from cardiomyopathy. One of the patients who died after BMT (indicated for G-CSF failure) had had multiple life-threatening infections, including pulmonary aspergillosis.

Myelodysplasia and acute leukemia: occurrence and risk factors

Thirteen leukemic transformations had occurred by the time of analysis. The cumulative frequencies of MDS/AL were 2.7% (SD 1.3) at 10 years and 8.1% (SD 2.7) at 20 years (Figure 3A). The main cytological and cytogenetic characteristics of the hematologic malignancies are shown in Table 4. Seven cases were initially diagnosed as MDS, one as AML0, two as AML2, two as AML6 and one as ALL. Monosomy 7 was detected in six patients and isochromosome 7q in two patients. Age at leukemia onset ranged from 4.2 years to 32.4 years, and differed according to the diagnostic category: the median age at onset was 10.7 years and 19.1 years, respectively, in patients with severe congen-

Table 2. Description of G-CSF therapy by diagnostic category.

	No.	*Patients treated by G-CSF (n) %*	*G-CSF failure*	*Follow-up after onset of G-CSF Median (y) (min-max)*	*G-CSF therapy*		
					Total cumulative duration Median (y) (min-max)	*Total cumulative dose Median (μg/kg) (min-max)*	*Time-averaged dose Median (μg/kg/day) (min-max)*
Severe congenital neutropenia	101	60 (59%)	3	6.1 (0.3-11.5)	1.6 (0.1-11.5)	5489 (10-66147)	9 (2-37)
Cyclic neutropenia	60	29 (48%)	0	4.3 (0.2-11.9)	1.1 (0.1-5.4)	1942 (20-10006)	5 (1.4-10)
Glycogen storage disease type Ib	15	14 (93%)	0	5.7 (0.1-12.1)	2.8 (0.1-8.8)	4226 (24-14028)	5 (1-10)
Shwachman-Diamond syndrome	55	14 (25%)	3	2.8 (0.19-10.8)	0.4 (0.1-3.7)	1324 (15-12116)	6.9 (2.5-11)

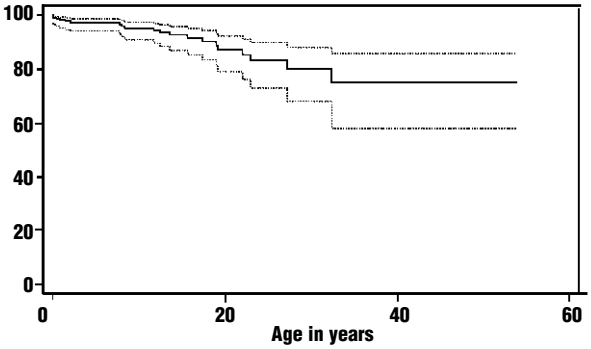

Figure 2. Overall survival among 231 patients with congenital neutropenia. The 95% pointwise confidence band is shown.

ital neutropenia and Shwachman-Diamond syndrome. The initial manifestation was isolated diabetes insipidus with pituitary nodules in two patients, both of whom had severe congenital neutropenia, MDS and monosomy 7. No malignancies occurred after the age of 12.5 years among patients with severe congenital neutropenia, while most malignancies occurred after the age of 20 years in patients with Shwachman-Diamond syndrome (Figure 3b). The median follow-up of these patients with MDS/AL ranges from 0.4 to 4.2 years. Four of the 13 patients are still alive.

Univariate analysis of risk factors for MDS/AL (Table 5) identified a low ANC count (p=0.04), early age at diagnosis (p= 0.04), strong exposure to G-CSF (time-averaged dose, p= 0.0005; cumulative dose, p= 0.01), and the type of neutropenia (p=0.006). Gender, granulopoiesis arrest, the number of severe infections, follow-up after the first G-CSF injection, and the cumulative duration of G-CSF therapy were not significant risk factors. Multivariate analysis was inappropriate, owing to the small number of events.

No cases of MDS/AL were observed among patients with GSDIb or cyclic neutropenia, while the risk was

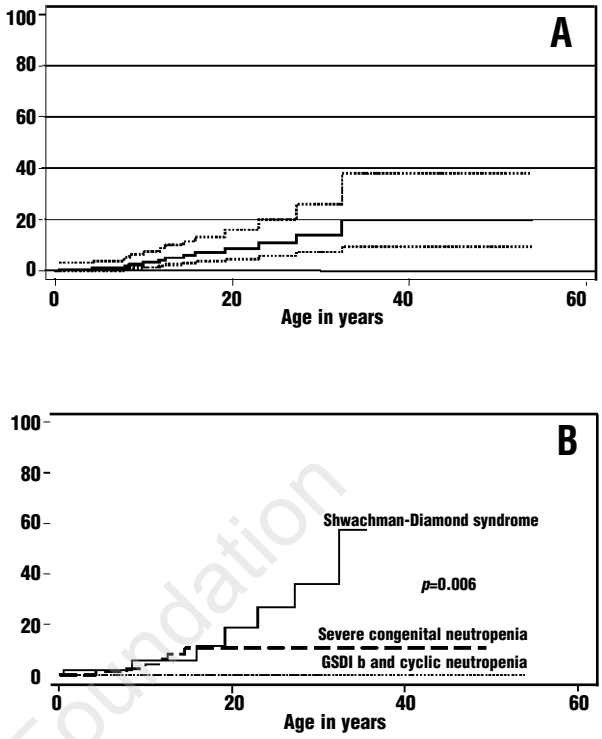

Figure 3. A. Overall cumulative risk of MDS/AL in 231 patients with congenital neutropenia. The 95% pointwise confidence band is shown. B. Cumulative risk of MDS/AL according to the diagnostic category.

10.8% at 20 years of age among patients with severe congenital neutropenia, and 18.8% and 36.1% at 20 and 30 years, respectively, among patients with Shwachman-Diamond syndrome (Figure 3B). There were thus three risk categories for MDS/AL. Patients with cyclic neutropenia or GSDIb had no apparent increase in the risk, patients with severe congenital neutropenia had an intermediate risk, and patients with Shwachman-Diamond syndrome were at high risk (p=0.0067, heterogeneity test) (Table 5).

Table 3. Causes of death among the 231 patients.

	No.	*Total no. of deaths*	*Deaths from sepsis*	*Deaths from other causes*	*Deaths from MDS/AL*
Severe congenital neutropenia	101	11 (10%)	6	2 G-CSF failure after BMT BM failure after BMT	3
Cyclic neutropenia	60	2 (3%)	2	0	0
Glycogen storage disease type Ib	15	0	0	0	0
Shwachman-Diamond syndrome	60	7 (13%)	0	1 Cardiomyopathy	6

Table 4. Main clinical and cytogenetic features of MDS/AL cases.

Diagnostic category	Patient No.	Interval between G-CSF onset and TA (y)	G-CSF cumulative dose (μg/kg)	ELA 2 gene encoding neutrophil elastase	G-CSF R gene mutation[1]	MDS/AL DX[2]	Age at MDS/AL (y)	Karyotype at MDS/AL	Therapy	Follow-up after MDS/AL (y)	Outcome (alive/cause of death)
SCN	133015213	4.4	28888	L92P	+	MDS	7.8	47, XY, -7, +21, +21[9]/46, XY[5]	CHT	0.9	AML
	233015168	3.7	17640	H24L	–	MDS	4.2	46,XX [2] / 45, XX, -7 [19].	CHT+BMT	1.9	Alive
	233015129	6.8	63476	G185R	+	ALL	11.7	48 XX, del (5 q21q34), +21, +22 (16)/46 XX [8]	CHT+BMT	1.4	Sepsis after BMT
	233015200	9.4	51852	G185R	+	MDS	9.8	45, XY, del(6)(q21),-7,+21, +21[9]/46, XY[5]	BMT	0.9	Alive
	233015163	0	0	NT	NT	AML2	12.5	45 XX; -19		0.8	AML
	233015194	0	0	NT	NT	AML2	12.5	t(8,21)	CHT+BMT	6.9	Alive
SDS	233015082	0	0	NT	NT	MDS	15.9	46 XY [7],46 XY, iso(7q)[13]	BMT	4.2	Alive
	233015023	0	0	NT	NT	AML0	22.9	46 XY [3], 44 XY, add(15 p), -20, -21, -22 + mar [20]	CHT	1.5	AML
	233015038	0	0	NT	NT	MDS	19.1	46, XY/45, XY,del(5)(q15q33), -7,+f /44, X,Y, der(3)t(3 ;6), del(5q), -6, -7	CHT+BMT	1.8	AML
	233015073	0	0	NT	NT	AML6	32.4	41-46, XY, -5, ,+der(3),+der(6),-7,-15,18 del(13)(q13q33),der(20)	CHT	1.2	AML
	233015081	7.2	2661	NT	NT	AML6	27.2	45, XX add(1)(p11), -7, add(14)(q32), add(21)(q22)	CHT+BMT	0.6	BMT-related
	233015253	0	0	NT	NT	MDS	8.34	47, XY, del(5q inter), add(9q), +11,add(17p), -20, +22	CHT+BMT	0.25	BMT-related
	233015208	0.4	1678	NT	NT	MDS	0.44	46, XY, iso(7q)[10], 46, XY[10]	Supportive care	0.4	MDS refractory cytopenia

G-CSF: granulocyte colony stimulating factor; G-CSF R: granulocyte colony stimulating factor receptor; MDS/AL: myelodysplasia/acute leukemia; BMT: bone marrow transplantation; [1]G-CSF receptor gene analysis performed at the time of transformation: + mutation - no mutation; [2]Cytology diagnosis according to the FAB classification SCN: severe congenital Neutropenia; SDS: Schwachmann-Diamond syndrome; DX: diagnosis, NT: not tested; CHT: chemotherapy.

It should be noted that severe congenital neutropenia is the diagnostic category associated with the most severe neutropenia, the highest frequency of severe infections, and the earliest age at diagnosis. As a result, these patients had the strongest G-CSF exposure. In addition, within this diagnostic category, most cases of MDS/AL occurred among the most heavily G-CSF-treated patients. Patients with severe congenital neutropenia and MDS/AL did not differ from their counterparts without MDS/AL according to follow-up at the cut-off date (median 10.7 years versus 11.3 years), initial hematologic features (ANC 313/mm^3 versus 289/mm^3) or follow-up after the first G-CSF injection (6.03 years versus 5.6 years). The only differences were in parameters reflecting clinical severity, such as the number of severe infections prior to G-CSF (median 2.5 versus 1.0) and the intensity of G-CSF exposure, i.e. the time-averaged dose (median 14 μg/kg versus 3 μg/kg), the cumulative duration of G-CSF therapy (median 4.7 years versus 0.06 years) and the median cumulative dose (23 264 μg/kg versus 140 μg/kg).

This difference in G-CSF exposure was also significant in the subset of 22 patients with severe congenital neutropenia and elastase mutations, who were the most severely ill and most heavily treated. The following elastase variants were found in patients with severe congenital neutropenia and MDS/AL: L92P and H24L in one case each, and G185R in two cases. G-CSF receptor studies were done in 55 patients with severe congenital neutropenia, including four patients who subsequently developed MDS/AL. No abnormalities were observed prior to the diagnosis of MDS/AL, while abnormalities were found at the time of malignant transformation in three of the four patients concerned (Table 4).

Discussion

Infections and MDS/AL are the main life-threatening

Table 5. Risk factors for progression to myelodysplasia and leukemia. Univariate analysis.

Variables	*n*	*Observed/expected*	*p*	
Sex				
Male	112	8/5.7	0.19	NS
Female	119	5/7.3		
Age at diagnosis				
0-3 months	76	6/3.4	0.043	
3 months - 1 year	54	5/2.6		
1 year-5 years	66	2/3.4		
> 5 years	35	0/3.5		
Diagnostic category				
Severe congenital neutropenia	101	6/5.1	0.0067	
Cyclic neutropenia	60	0/4.1		
GSDIb	15	0/1.1		
Shwachman Diamond syndrome	55	7/2.7		
Ela2 gene encoding neutrophil elastase*				
Mutated	22	4/1.5	0.01	
Not mutated	32	0/2.5		
Median ANC/mm^3 before GCSF therapy				
< 100	28	4/1.4	0.04	
100-300	32	1/2		
300-500	52	0/2.4		
≥ 500	119	8/7.2		
Bone marrow				
< 5	69	7/4.4	0.29	NS
Metamyelocytes + band cells + neutrophils (%)				
5-10	19	2/1.8		
10-20	21	2/1.2		
> 20	83	2/5.5		
Severe infections prior to G-CSF				
	113	2/4.9	0.34	NS
Number of distinct episodes				
1-2	47	3/2.2		
3-4-5	52	6/3.8		
More than 5	19	2/2.1		
Time-averaged G-CSF dose (µg/kg/day)				
0	114	7/6	0.00054	
0.1-5	62	1/4.7		
5-15	43	3/1.9		
>15	12	2/0.3		
Cumulative G-CSF dose (µg/kg)				
0	114	7/6	0.0131	
1-1000	34	0/1.9		
1000-10000	58	2/4		
>10000	25	4/1.1		
Cumulative duration of G-CSF therapy (y)				
0	114	7/6	0.34	NS
0-2 years	72	1/3.7		
2-4 years	24	2/1.6		
> 4	21	3/1.7		
Follow-up after onset of G-CSF (y)				
0	114	7/6	0.84	NS
0-2.5	35	1/1.2		
2.5-5	28	2/1.5		
> 5	54	3/4.3		

**Test performed only in severe congenital neutropenia.*

complications of congenital neutropenia. Long-term use of cytokines in constitutional neutropenia, which started in 1988, has virtually eliminated the infectious risk and transformed the quality of life of these patients. However, it has also raised questions as to the balance between the natural risk of severe infections and that of treatment-induced malignancy. Despite the heterogeneity of severe congenital neutropenia, there has been a clear increase in the total number of reports of MDS/AL between the pre-G-CSF era[8-10] (3 cases) and the 15 years of the G-CSF era[11,22-28] (more than 30 cases). Among patients with glycogen storage type Ib, one case, which did not fulfill WHO criteria for acute myeloid leukemia, was reported prior to the G-CSF era[29] and one case after.[30] No cases of MDS/AL have been reported in patients with cyclic neutropenia. Finally, among patients with Shwachman-Diamond syndrome, there were many reports prior to the G-CSF era, and also in patients not receiving G-CSF,[1-5] but no cases have been described in patients treated with G-CSF. Only large observational surveys of patients enrolled prior to the onset of malignancy and monitored prospectively would be able to show the possible link between G-CSF and MDS/AL in this setting, and the risk-benefit ratio of G-CSF therapy. Only the international severe chronic neutropenia registry created in 1993,[11,22] and the national registry established in France in 1994 meet these conditions, and both have recently attained sufficient follow-up (>10 years) to observe late or rare adverse events.

Furthermore, analysis of data on the risk of life-threatening infections is equally important. However, it is important to analyze recent data on this risk. A historical survey[31,32] showed that most patients with severe congenital neutropenia died from bacterial sepsis, while sepsis is less frequent in cyclic neutropenia,[33] SDS[34] and GSDIb.[35] We recorded 8 septic deaths among 231 patients. All septic deaths involved patients with severe congenital neutropenia (n=6) or cyclic neutropenia (n=2), while none occurred among patients with SDS or GSDIb. All the septic deaths in our cohort involved patients who were not receiving G-CSF therapy at the onset of infection. Although uncontrolled, this comparison suggests that G-CSF protects these patients from severe infections. Only one randomized study of mild infections occurring during a 3-month therapeutic window has demonstrated the value of G-CSF in severe chronic neutropenia.[7] In the latest ISCNR report, 21 septic deaths were recorded among 493 patients; G-CSF therapy at the time of death was not reported in detail,[11] but it could be inferred that none of the patients was receiving G-CSF at onset of the infection.

MDS/AL is the second most frequent life-threatening event in patients with congenital neutropenia. Several factors contribute to or are associated with leukemic transformation in this group of patients: our univariate analysis identified the diagnostic category, parameters reflecting the severity of neutropenia, and the degree of exposure to G-CSF. The diagnostic category was a major determinant. First, MSD/AL was only observed

in patients with severe congenital neutropenia or SDS.

Second, the risk of MDS/AL differed between patients with severe congenital neutropenia (20-year incidence 11%) and those with SDS (19%), as did age at onset (median 10.7 years and 19.1 years, respectively). Finally, the diagnostic category determined both the severity of neutropenia (particularly the risk of infection), and the intensity of G-CSF therapy.

The possible link between G-CSF therapy and MDS/AL onset is the most difficult question in this subset of patients. Patients with severe congenital neutropenia had the highest exposure to G-CSF in our study, in terms of both the time-averaged dose and the duration of treatment. Likewise, patients with severe congenital neutropenia and *ELA2* gene mutations had the earliest and most severe infections, and thus were most strongly exposed to G-CSF.[17] Patients with GSDIb were most likely to receive G-CSF, but cumulative exposure was lower than in patients with severe congenital neutropenia. Patients with cyclic neutropenia or SDS had the lowest exposure to G-CSF. Given that patients with severe congenital neutropenia were exposed to the highest cumulative dose of G-CSF, as a consequence of their poor infectious status, this group would be most likely to show any leukemogenic effect of G-CSF. Although two cases of leukemia were observed in patients not treated with G-CSF, the risk of leukemia increased significantly with the cumulative dose of G-CSF in this group. As exposure was far lower in the other diagnostic subgroups, it is difficult to conclude on whether their sensitivity to leukemic transformation, which is also related to the diagnostic category, and was especially high in patients with SDS, is different, or whether they simply did not reach the *threshold dose* of G-CSF required for leukemic transformation. The high incidence of MDS/AL observed in heavily G-CSF-treated patients could simply be an effect of the longer survival conferred by this therapy. Another possibility is that such patients at high risk of leukemia have a specific constitutional disorder responsible simultaneously, but independently, for susceptibility to leukemia, life-threatening infections, and heavy G-CSF requirements. It is difficult to conclude on the risk-benefit ratio of G-CSF in this setting, because of the large number of deaths by sepsis, which always involved patients who were not receiving G-CSF, and because the only alternative treatment is bone marrow transplantation.[36] Our results do not justify withholding G-CSF therapy from patients with congenital neutropenia on the basis of an increased risk of leukemia in some diagnostic subtypes, as this would expose them to an added risk of life-threatening infectious complications. Given the major initial benefit of G-CSF therapy, randomized clinical trials would be unjustified. Our results can therefore only be confirmed by other surveys based on similar parameters, and by additional follow-up. If the leukemogenic effect of high cumulative doses of G-CSF is confirmed, the indications for bone marrw transplantation in congenital neutropenia (currently recommended for G-CSF failure and leukemic transformation) should be extended to those patients requiring the highest doses of G-CSF. The threshold dose justifying this approach remains to be defined.

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