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Recurrent bacterial infections, but not fungal infections, characterize patients with ELANE neutropenia: a French Registry study

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Abstract

Among 143 ELANE-neutropenia patients enrolled in the French Severe Chronic Neutropenia Registry, 94 were classified as having severe chronic neutropenia (SCN) and 49 with cyclic neutropenia (CyN). Their infectious episodes were classified as severe, mild or oral, and analyzed according to their natural occurrence without G-CSF, on G-CSF, after myelodysplasia/acute leukemia or after HSCT. During the disease's natural history period (without G-CSF; 1,913 person-years), 302, 957 and 754 severe, mild and oral infectious events, respectively, occurred. Among severe infections, cellulitis (48%) and pneumonia (38%) were the most common. Only 38% of episodes were microbiologically documented. The most frequent pathogens were *Staphylococcus aureus* (37.4%), *Escherichia coli* (20%) and *Pseudomonas aeruginosa* (16%), while fungal infections accounted for 1%. Profound neutropenia ($<100/\text{mm}^3$), high lymphocyte count ($>3000/\text{mm}^3$) and neutropenia subtype were associated with high risk of infection. Only the p.Gly214Arg variant (5% of the patients) was associated with infections but not the overall genotype. The first year of life was associated with the highest infection risk throughout life. G-CSF therapy achieved lower ratios of serious or oral infectious event numbers per period but was less protective for patients requiring $>10 \mu\text{g}/\text{kg}/\text{day}$. Infections had permanent consequences in 33% of patients, most frequently edentulism.

Keywords: Severe congenital neutropenia; ELANE neutropenia; opportunistic infections

Running title: Epidemiology of infections in ELANE neutropenia

Introduction

Congenital neutropenia (CN) covers a group of diseases characterized by fewer neutrophils circulating in peripheral blood. The epidemiology of infections in CN patients is poorly documented. It is commonly thought that the type and occurrence of infections in CN closely resemble those observed in patients with chemotherapy-induced neutropenia.¹ But the genetic disorder spectrum leading to CN is highly heterogeneous and, to date, about 30 identified genes may be implicated in neutropenia, with variable risks of infections.² Among the various inherited-neutropenia subtypes, those with an elastase, neutrophil-expressed (*ELANE*) gene variant are the most frequent, and are typically classified as cyclic (CyN), characterized by oscillation of the absolute neutrophil count (ANC), or as permanent neutropenia, called severe CN (SCN). No lymphocyte-function or innate immune-system defect, or additional organ dysfunctions are commonly found in *ELANE*-related neutropenia, which represents a model of “pure” neutropenia and, thus, may help study the risk of ANC-associated infections.

Patients and methods

Registry organization and data monitoring

The French Severe Chronic Neutropenia Registry (FSCNR) was created in 1993 and has been thoroughly described elsewhere.³ The database was approved by the French computer watchdog commission (CNIL certificate no. 97.075). Clinical research associates regularly monitor patient files at each site. CyN was defined as multiple documented ANCs >500 cells/mm³, with intermittent ANC variation prior to granulocyte-colony stimulating factor (G-CSF) therapy, while SCN is defined as ANC persistently <500 cells/mm³ in almost all blood counts before G-CSF administration. As of 31 December 2020, 147 patients with *ELANE*-related neutropenia among 1,122 patients with chronic neutropenia had been enrolled in the FSCNR, including four not analyzed in this study, because of missing clinical data.

Genetic analysis of ELANE variants

The patients or their parents gave written informed consent for genetic testing. Genomic DNA was extracted from blood cells using standard procedures. The coding sequence and exon–intron boundaries of the *ELANE* gene were subjected to Sanger sequencing, as described previously,⁴ or targeted sequencing with a gene panel including *ELANE*. We numbered mutations as recommended by the Human Genome Variation Society

(<http://www.hgvs.org/>), using the reference sequence NM_001972.2. We classified variants according to American College of Medical Genetics and Genomics (ACMG) guidelines.⁵ Only pathogenic (class 5) and probably pathogenic *ELANE* variants (class 4) were retained.

Definitions of infections

We considered three types of infections. First, severe infections are potentially lethal in the absence of medical interventions and include sepsis, defined as fever and documented bloodstream infection, and deep infections of any type (pneumonitis, liver abscess, cellulitis, omphalitis, colitis, etc.). Mild infections, such as pharyngitis, otitis, furunculosis, paronychia, gastroenteritis and urinary infections, represent the second type. The third group comprised oral infections, e.g., mouth ulcers, gingivitis and periodontitis. Long-term and permanent sequelae of infections included tooth loss, necrosis, deafness, blindness and/or respiratory failure. For each episode, the patient's medical chart was consulted to establish whether the infection was of unknown origin or caused by identified microorganisms. Fungal infections were classified according to recent guidelines.⁶

Definition of G-CSF exposure

Because treating physicians individually prescribed the unit dose of G-CSF injections and their frequency, each patient's overall treatment was estimated using several parameters (total cumulative dose in $\mu\text{g}/\text{kg}$, cumulative G-CSF-administration duration expressed in years, and the time-averaged dose, as previously described.⁷

Study design and statistical methods

Each infectious episode was assigned to the treatment period, as defined below. Each patient's follow-up was divided into four periods: "natural history" [corresponding to the time during which the patient had no hematological complications, like myelodysplasia syndrome or acute leukemia (MDS/AL), had not undergone hematopoietic stem-cell transplantation (HSCT) or was not on G-CSF]; receiving G-CSF; post-HSCT period; or after MDS/AL. The infection ratios were calculated by dividing the numbers of episodes (severe, mild or oral infection considered separately) by the duration of each of the four periods. Stata® version 15 software was used to compute all statistical analyses. Infection ratios were compared using the Kruskal–Wallis test. For survival analyses, the endpoints were death, the first severe infection and the first oral infection. Between-group survival rates were compared with the log-rank test, and a Cox model was

built for multivariate analysis.

Results

Clinical, hematological and immunological characteristics

Among the 143 patients (71 males, 72 females) enrolled in this study (**Table I**), 94 (66%) had SCN and 49 (34%) had CyN. For the entire cohort, median age at diagnosis was 0.3 (interquartile range (IQR) 0.09–1.5) years. One hundred and sixteen (81%) patients received G-CSF (81 with SCN, 35 with CyN) for a median cumulative duration of 3 years. Eighteen patients underwent HSCT.⁸ Median follow-up per patient was 17.6 (IQR 9.2–30.5) years, for a total observation period of 3,079 person-years, broken down per period as follows: natural history: 1,913 person-years; on G-CSF: 1,026 person-years; post-HSCT: 134 person-years; and post-MDS/AL: 6 person-years). Seven (5%) cohort patients died: three of sepsis, one of anal squamous cell carcinoma and two each of MDS/AL or post-HSCT grade-IV graft-vs.-host disease (GVHD). The death rate did not differ significantly between CyN and SCN subgroups. At diagnosis, the median ANC was 287 cells/mm³, the median absolute monocyte count (AMC) was 1,543 cells/mm³ and median absolute lymphocyte count (ALC) was 5,175 cells/mm³. Among the 99 patients with assessable immunoglobulin levels, only four (4.7%) had normal IgG levels according to age, while 81 (95%) were hypergammaglobulinemic.

G-CSF regimens were individually tailored based on the recurrence of severe infections and G-CSF tolerance. G-CSF doses varied widely, as it can be indicated on demand (starting as of infection onset) or be prescribed preventively, as illustrated by the distribution of time-averaged doses for the 116 treated patients (**Fig 1**). For G-CSF, the median time-averaged dose was 5.6 (IQR 5–10.5) $\mu\text{g}/\text{kg}/\text{day}$, with the median cumulative dose of 6,887 (IQR 5–117,650) $\mu\text{g}/\text{kg}$ for the entire cohort up to the last follow-up visit.

Molecular characteristics

The cohort includes 99 probands and 44 affected relatives (**Table SI**). Fifty-one different variants were identified: 31 missense variants, nine truncated variants (nonsense and frameshift), five splice defects, three in-frame deletions, and three variants affecting the initiating codon. Among them, 17 were novel variants.

Types of infections

During the natural history period, 98 patients developed 302 severe infectious episodes (**Table II**), while 45 patients had none until the last follow-up. Only one infection site was involved during 273 episodes, two sites were affected during 21 episodes, three sites during four episodes and four sites during one episode. All severe infections were treated in-hospital, with 17 requiring intensive care unit admission. Lastly, three deaths were attributed to infections. **Fig S1** provides some vignettes of such life-threatening events. Only 38.1% (115/302 episodes) of the severe infectious events had a documented microorganism, probably because preemptive intravenous antibiotic administration was always initiated promptly in this context. Bacteria were identified as responsible for 99% of those infections (**Table II**), with *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* being the most frequently isolated. The different types of microorganisms by infection site are reported in **Fig S2**.

It should be emphasized that invasive fungal infections were extremely rare, with only one episode of *Candida albicans* sepsis, representing only 1% of all pathogens identified. Among the seven pneumonitides, with lung lobar condensation and central necrosis, *Aspergillus* invasive infections were suspected but never demonstrated, despite a panel of direct-detection methods (see **Fig S1D**).

All patients developed mild infections, with 962 episodes recorded, mostly ear, nose and throat (475 episodes, 49.4%), skin (furunculosis, paronychia or any superficial cutaneous infection, 209 episodes, 21.7%), adenitis (68 episodes, 7.1%) and urinary infections (10 episodes, 1.0%), with 200 (20.8%) less well-characterized episodes with either fever of unknown origin or mild bronchitis).

Lastly, 759 oral infectious episodes, frequently mixed, were observed with 660 mouth ulcers, 342 gingivitis and 45 chronic periodontitis.

During the natural history period, infection-ratio distributions among patients were asymmetric, with annual medians of 0.14 (IQR 0.04–0.96) severe infections (**Fig 2A**), 0.93 (IQR 0.2–2.42) mild infections and 0.2 (0.01–0.72) oral infections (**Fig 2B**). By 1 year of age, 45.6% of the patients had already experienced a first infectious episode, 56% by 5 years and 75% by 50 years (**Fig 2C**). The first-episode incidence of oral infections was 14% by the age of 1 year, 59% by 5 years and 96% by 50 years (**Fig 2D**). The first oral infectious episode appears to have been associated with tooth eruption. But neither the first-episode incidence (**Fig 2F**) nor the ratios of oral infection differed significantly between CyN and SCN.

Forty-seven (33%) patients developed long-term complications of infections. Such definitive sequelae can be associated in each patient with tooth loss (partial for 18 patients and complete edentulism before age of 30 years for 17 patients), chronic respiratory failure with bronchiectasis for 10, 13 became deaf secondary to otitis, five had esthetic sequelae, including two with facial necrosis, two experienced soft tissue loss-of-substance and one each suffered unilateral blindness secondary to eye infection or finger amputation. Lastly, two developed terminal end-stage renal disease, for which no clear explanations were found, other than chronic inflammation related to recurrent infections. No late sequela-rate difference between CyN and SCN subgroups was observed.

G-CSF impact

Among 143 patients, 27 had never received G-CSF as of the last follow-up. These patients seemed to have less frequent severe, mild or oral infections, compared to the 116 patients prescribed G-CSF (**Table III**). Overall, comparing the natural history and on-G-CSF periods, respectively, treated patients had significantly lower severe infection (median 0.22 vs. 0.04) and oral infection ratios (0.35 vs. 0.07), expressed in events/year, while their mild infection ratio was only slightly and non-significantly lower. The percentage decrease was smaller when a higher G-CSF dose was needed. Among CyN patients, the G-CSF effect was significant only for oral infections. Notably, 17 patients never had oral infections and, among them, six underwent HSCT exceedingly early in life after a severe infection and ineffective G-CSF challenge, while 12 had received long-term low-dose G-CSF, and only five patients never received G-CSF and never had oral infections.

Post-MDS/AL period

Among the six patients who developed MDS/AL, five later underwent HSCT and the last, who did not, died of refractory AL. Patients may have received chemotherapy or not and all received high-dose G-CSF prior to HSCT. During the cumulative follow-up of 6 years, eight severe infections occurred, yielding a severe infection ratio of 1.66 [range 0–2].

Post-HSCT period

Among the 18 patients who underwent HSCT, two died of short-term grade-4 GVHD: one associated with *P. aeruginosa* sepsis, despite full engraftment, and the other with microangiopathy and kidney failure, as reported elsewhere.⁸ Among 16 patients who survived the initial post-HSCT period, with neutrophil

recovery, the median ratio of serious infections per year was 0 (range 0–0.15) and no oral infections were observed after HSCT.

Risk factors for severe infection

Susceptibility to infections varied very widely for patients with ELANE neutropenia. To analyze that variability, we first considered some basic information, like blood counts, genotype, immunological features and the diagnoses of CyN or SCN (**fig 2E**). To analyze the impact of genotype, we grouped patients according to the exon location of the variant, to its truncated characteristic (Yes/No) and, lastly, according to the presence of the p.Gly214Arg mutation. Only the latter was associated with a trend towards higher risk of infection ($P = 0.07$).

Univariate and multivariate analyses, using the first severe infection as an event, are summarized in **Table IV**. Among seven variables studied, four had deleterious prognostic values, i.e., the diagnostic subtype (SCN vs. CyN), profound neutropenia (<200 cells/mm³), high ALC (>3000 cells/mm³) and high AMC (>2000 cells/mm³). The multivariate model retained only the diagnosis subcategories, ANC and ALC as being independently associated with the risk of severe infections. In addition to that risk, a constant in the life of ELANE patients, the severe infection ratio appeared to be strongly associated with age, for CyN and SCN patients. Among those with ELANE SCN, 72/94 (77%) developed at least one severe infectious episode throughout the entire follow-up period, compared to 53% (26/49) of the CyN subgroup. The severe infection ratio, quite high during the first year of life, decreased up to 6 years of age and remained stable thereafter (**Fig 3**).

Discussion

We described a cohort of neutropenic patients bearing *ELANE* variants, enabling epidemiological analysis of their infectious episodes. Since their initial description in 1999,^{9,10} *ELANE* mutations have been reported in about 500 patients worldwide, mainly in two large series of 188¹¹ and 307 patients,¹² and more limited series.

The neutropenia–infection link is considered a dogma. However, the type of infections occurring in this population is not well-defined. As expected, patients with ELANE neutropenia carry a remarkably high risk of infections. In addition to mild infections, which can be non-specific (like otitis, pharyngitis, gastroenteritis, etc.), we observed high rates of severe and potentially lethal pneumonitis, cellulitis, liver abscess or colitis. Among the mild infections, skin infections like furunculosis, paronychia or chalazion were

very frequent and quite specific to neutropenia. Meningitis was never seen, and osteitis or arthritis remained exceptional. Lastly, oral infections (mouth ulcers, gingivitis, chronic periodontitis) were also very common. The pathogens identified were also specific: almost exclusively bacterial infections; no severe viral or mycobacterial infections were documented. More surprisingly, fungal infections, actively sought by indirect and direct methods, were extremely rare [1/302 episodes (0.3%)]. Some findings, suggestive of fungal infections, like round-shaped lung lesions or liver pseudo-tumor, were finally diagnosed as bacterial infections (**Fig S1**). Those observations agree with the literature, as we found only one reported fungal infection in this context.¹³ That finding constitutes a very clear difference with post-chemotherapy infections¹⁴ and chronic granulomatous disease, characterized by defective neutrophil oxidative function without a quantitative neutrophil deficiency, for which the fungal infection frequency approaches 50%.¹⁵

The explanation of that phenomenon remains elusive. Animal models have shown that defense against *Aspergillus* is a multistep process.¹⁶ Phagocyte recruitment represents only part of this immunity. Indeed, in ELANE neutropenia, several redundant systems of anti-*Aspergillus* immunity remain active. Monocytes and macrophages are still present, and NADPH oxidative functions and proteolytic activities in monocytes and the few circulating neutrophils remain normal. Although the neutropenia in patients with mutated *ELANE* is not perfectly elucidated, the neutrophil-elastase function is not abolished and the associated neutropenia is commonly interpreted as resulting from defective intracellular granule trafficking, with an excessive, unfolded protein response but not an enzyme deficiency.¹⁷⁻²⁰ That difference distinguishes ELANE-neutropenia patients from those with chronic granulomatous disease, who are also at high risk of fungal infections and have either a broad deficit in all types of phagocytes or dysfunctional neutrophils with a NADPH oxidative deficit, whereas post-chemotherapy neutropenia, the most profound, is commonly associated with both neutrophils and monocytes.

Oral infections causing periodontitis and edentulism represent another specific characteristic of the epidemiology of infections in ELANE neutropenia. Their frequency, observed in CyN and SCN patients, is close to that found for other phagocyte disorders, like leukocyte-adhesion deficiency²¹ and Papillon-Lefebvre syndrome.²² The high oral infection ratio for chronic periodontal disease highlights the chronic inflammation typical of ELANE neutropenia.²³

The second part of our analysis evaluated the risk factors for severe infections. Genetic analyses

identified 53 distinct *ELANE*-gene variants in our cohort. An attempt to link genotype with the risk of infections was unsuccessful, regardless of the genotype group, the exon number or the functional consequences of the variant. This clinical finding appeared to be in contradiction with the recent observation of association between genotype and progenitor cells culture.²⁴ Herein, only the p.Gly214Arg variant was associated with a very high risk of infections in univariate analysis; but it is quite rare ($n = 7$; 5%) and it was not retained by the multivariate analysis, in contrast with previously published data suggesting that it was specific to a high risk of infection.²⁵

The CyN and SCN subcategories appeared to offer a clear distinction concerning the risk of infection. But such categorization fails to summarize all the prognostic information; for example, low ANC (<200/mm³) and high ALC are associated with a high risk of infections. Notably, those features are also associated with a high G-CSF requirement (>10 µg/day), which can be considered a surrogate marker of severity. Regardless of the prognostic markers of severe infections identified that represent a given patient's constant predicament throughout life, the patient's age is also an important factor. Young age, specifically <1 year, is associated with a high risk of severe infections and certainly warrants specific close clinical monitoring. This very precocious, high infection risk suggests that the maturation of adaptive B-cell immunity²⁶ and T-cell and innate immunities^{27;28} later in life are able to partially compensate for the *ELANE* dysfunction and neutropenia.

Concerning *ELANE* neutropenia patients' high risk of infections, active infection prevention is a major aim. Prophylactic antibiotics is a very common approach to managing immune deficiency.²⁹ Regarding the observed aetiology of severe infections, our data support the usefulness of co-trimoxazole, as for chronic granulomatous disease, because it may be beneficial and should be prescribed more frequently. On the contrary, no indication exists for anti-fungal prophylaxis. G-CSF and HSCT remain the two other major options to prevent infections in *ELANE* neutropenia. G-CSF is the key drug for its management but not all patients need to be treated with it, as highlighted by the 'natural history' severe infection ratios of G-CSF-treated patients being far higher than those not treated. As demonstrated in the literature, G-CSF is able to significantly increase the ANC.³⁰ Surprisingly, the benefit, in terms of lower infection rate, is less effective for the patients who required higher G-CSF doses (time-averaged dose >10 µg/kg). Indeed, those patients had less obvious prevention of severe infections, which confirms the paradox, observed previously: the more

G-CSF is needed, the greater the risk of dying from sepsis³¹; and, moreover, although not within the scope of this study, the greater the risk of leukemic transformation.^{7;31} Such patients would probably benefit most from HSCT, which contained the risks of leukemia and severe infections, thereby lowering the sequela rate.⁸

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Authorship

The original study design was conceived by J Donadieu, G Plat and GA Rotulo. The FSCNR, coordinated by J Donadieu and B Beaupain, is responsible for data management. All the authors contributed to the writing and revision of the manuscript, and approved the final version.

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Figure Legends

Fig 1. Distribution of the time-averaged G-CSF doses* given to the 143 ELANE-related neutropenia patients. *Because the G-CSF unit dose and frequency of injections are individually prescribed, each patient's overall treatment was estimated using several parameters. The following parameters were calculated for each G-CSF-therapy period, during which both the dose and the frequency of injections were stable: a) ddi = dose delivered per injection ($\mu\text{g}/\text{kg}$); b) ni = number of injections; c) cumulative dose = total dose received during the relevant period (mg/kg) = $ddi \times ni$; and d) duration of the relevant period in days. The total cumulative dose ($\mu\text{g}/\text{kg}$) 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 is 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, and is expressed in years. The time-averaged dose ($\mu\text{g}/\text{kg}/\text{day}$) was calculated by dividing the cumulative dose by the cumulative duration of treatment.

Fig 2. A to F: Severe infections and oral infections in the 143 ELANE neutropenia patients: distributions of the severe and oral infection ratios calculated by dividing the number of episodes per 'natural history' duration. (A and B) Severe and oral infection ratios, respectively, varied very widely among patients. (C and D) Kaplan–Meier curves of the estimated risks of severe or oral infections, respectively, as the first event, plotted vs. age at occurrence. (E and F) The risks of severe and oral infections, respectively, according to the diagnostic categories: cyclic (CyN) or severe chronic neutropenia (SCN).

Fig 3. Severe infection ratios (number of severe infections/follow-up period) according to age. The age is divided in 8 intervals (0–3 months, 3–12 months, 1–2 years, 2–5 years, 5–10 years, 10–15 years, 15–20 years and >20 years) and the total number of severe infections observed during each interval is divided by the observation period (natural history period without G-CSF), calculated for

all patients (■) together and separately for those with severe chronic neutropenia (SNC; ▲) patients or cyclic neutropenia (CyN; ◆).

Fig 1. Time-averaged dose in $\mu\text{g}/\text{kg}/\text{day}$

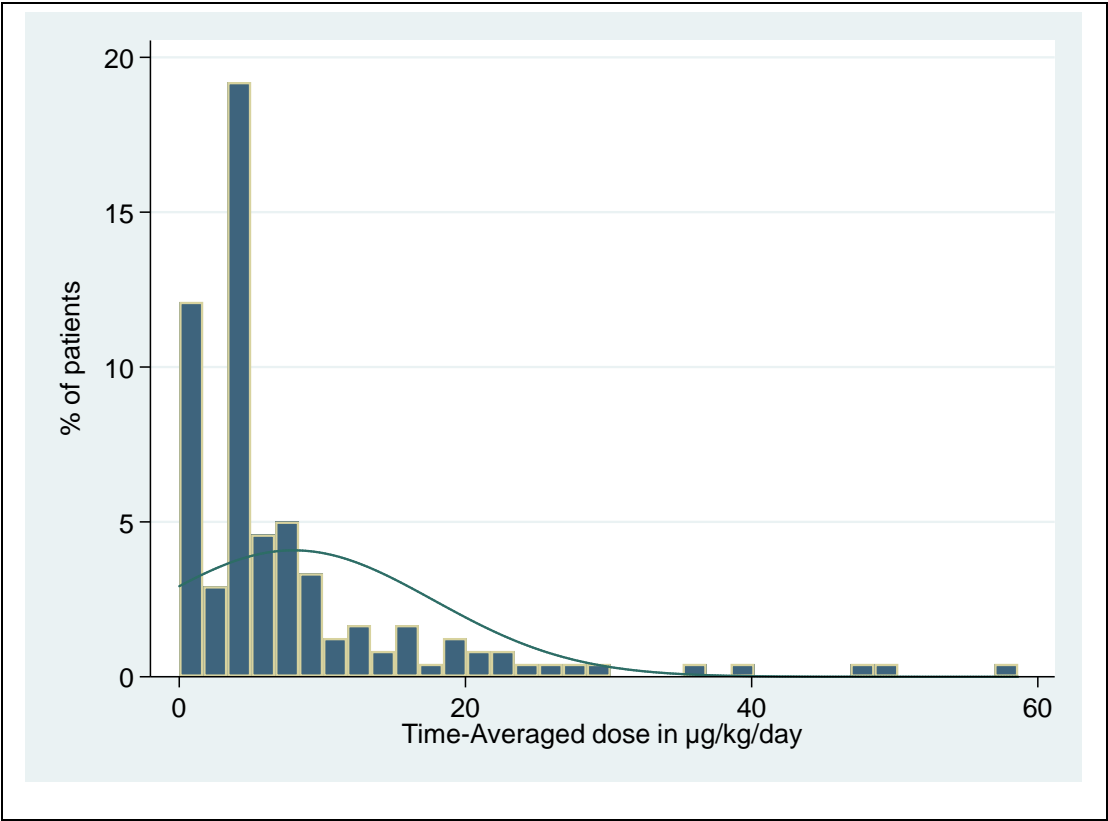
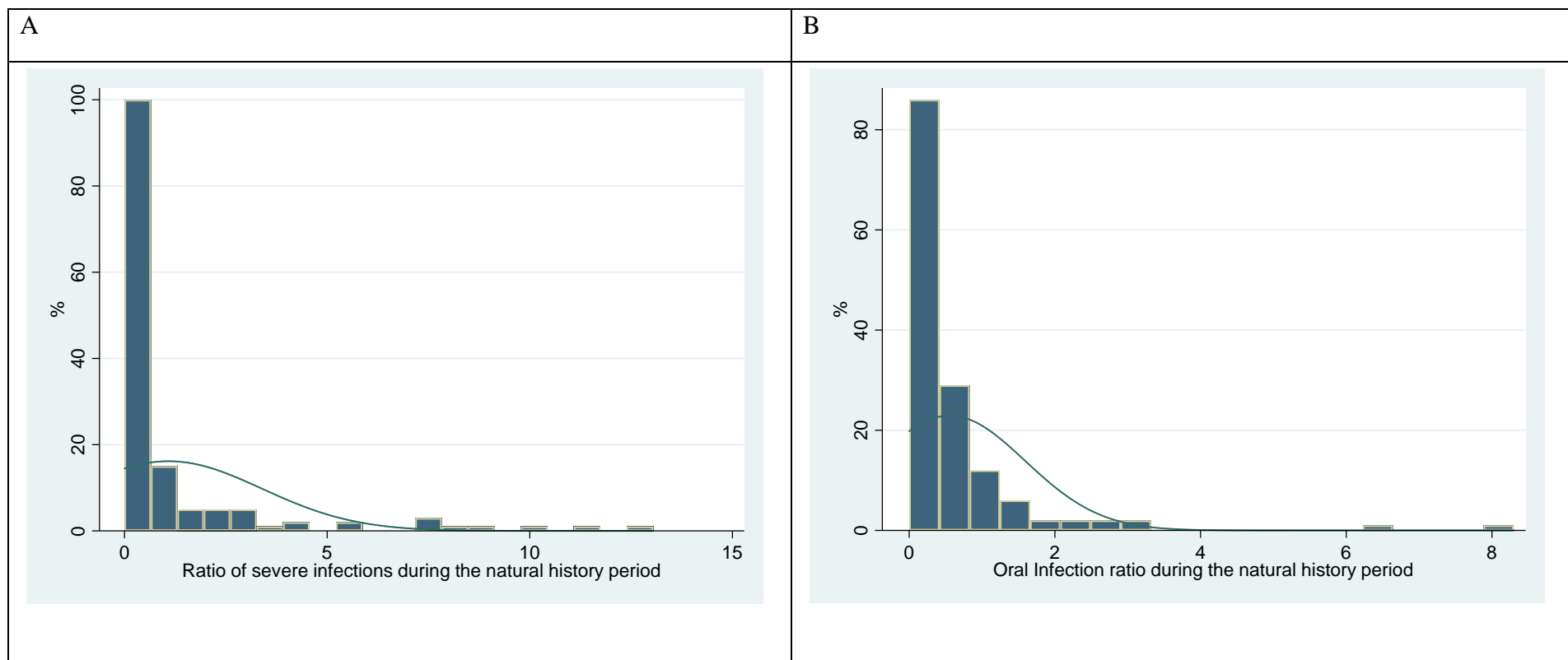
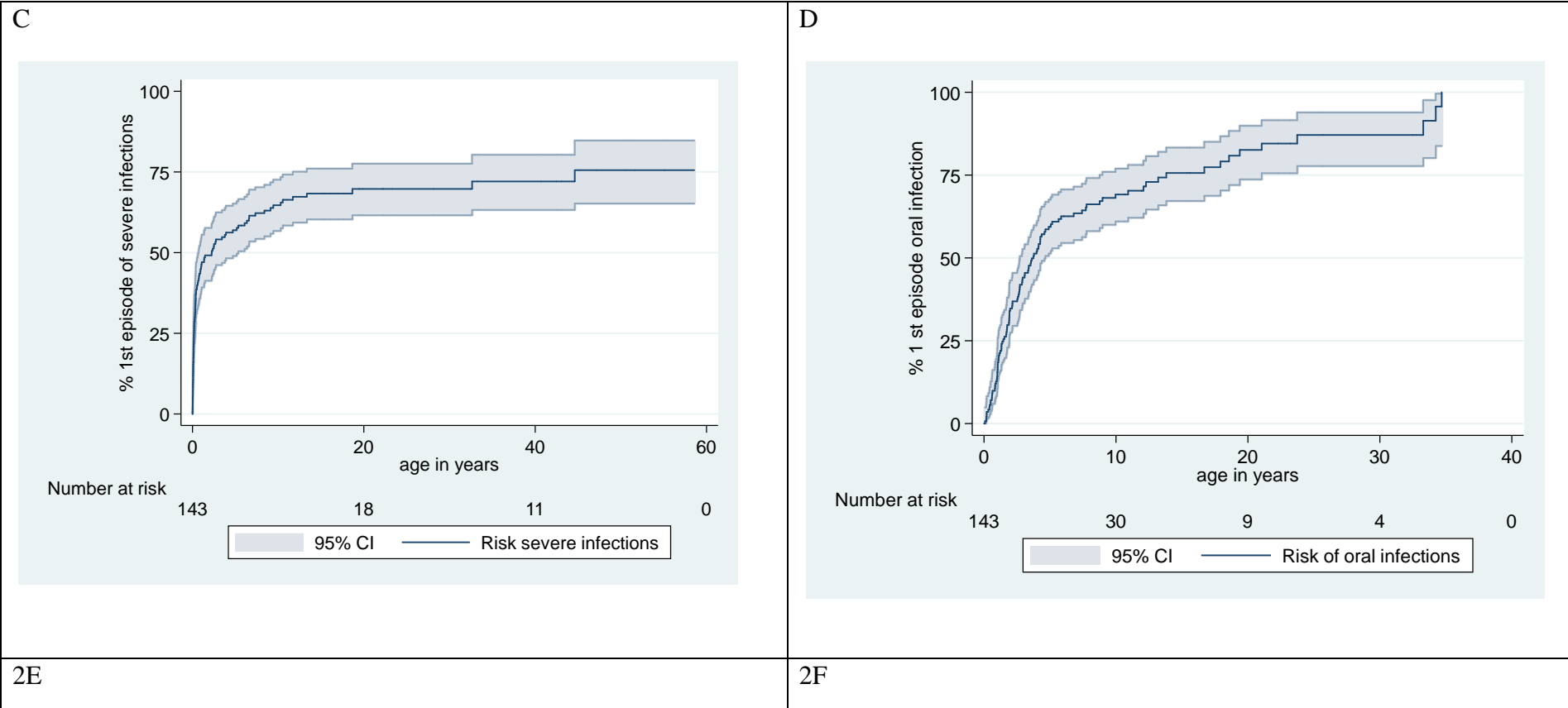


Fig 2.





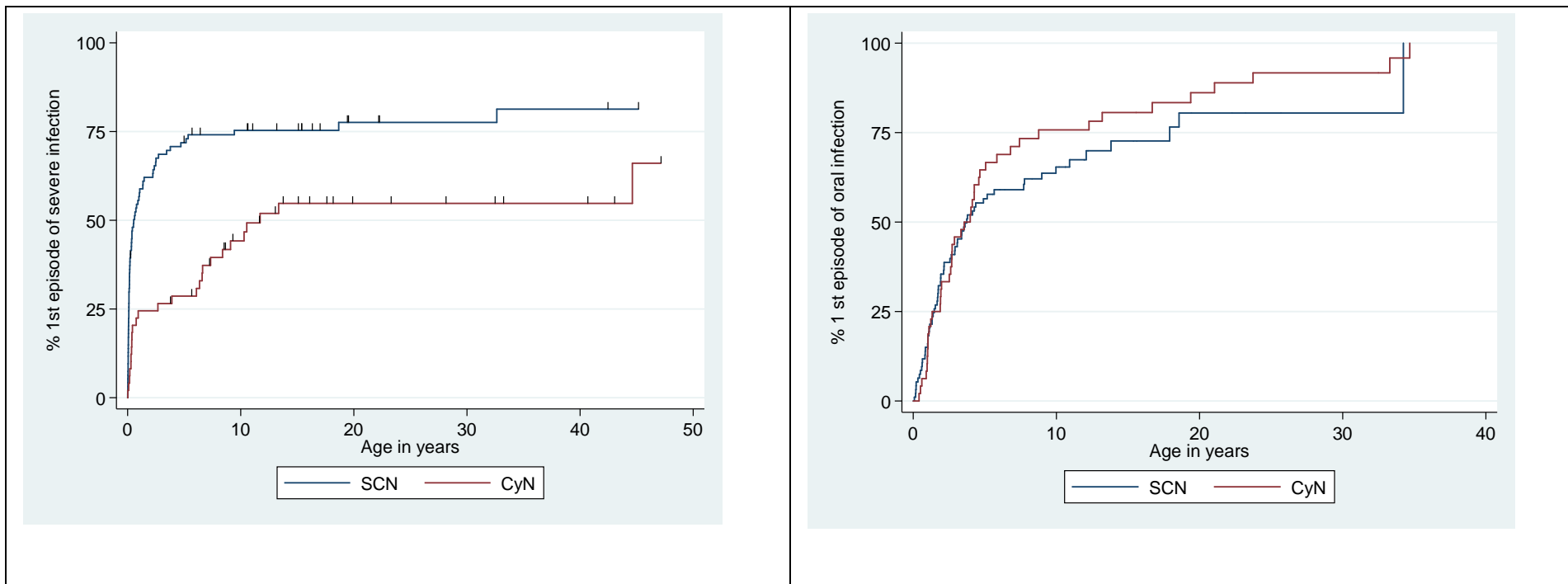


Fig 3.

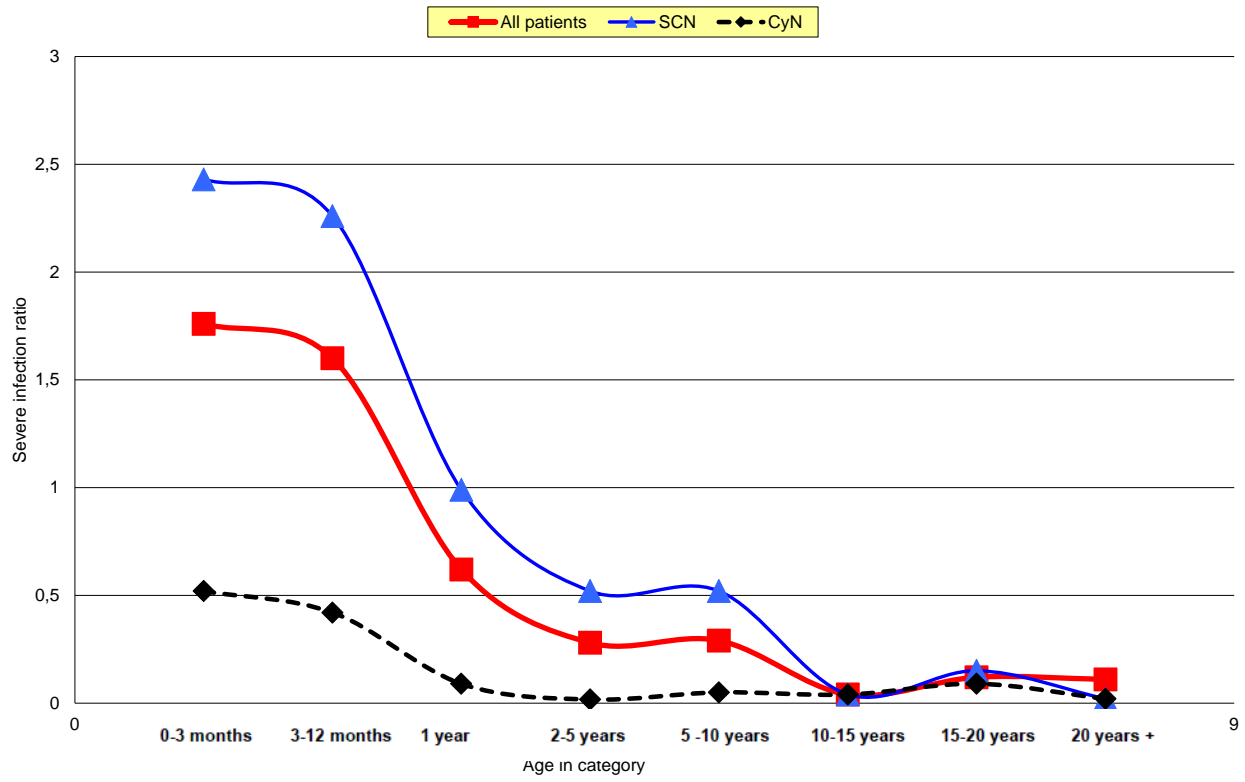


Table 1. Demographic, hematological and immunological description of the entire cohort, and according the cyclic (CyN) or severe chronic neutropenia (SCN) diagnosis.

Parameter	All patients (<i>n</i> = 143)	SCN (<i>n</i> = 94)	CyN (<i>n</i> = 49)	<i>P</i> *
Demographic				
Males/females, <i>n</i>	71/72	46/48	25/24	
Age at diagnosis, years	0.3 (0.09–1.5)	0.2 (0.06–0.68)	1 (0.23–6.8)	0.0002
Age at last follow-up	17.6 (9–30.1)	14.5 (8.3–25.7)	25.6 (13–34.2)	0.003
MDS/AL	6 (4.2%)	6 (6.4%)	0	0.07
HSCT	18 (12.5/6%)	18 (18/19.1%)	0	<0.0001
Deaths	7 (5/4.9%)	4 (4.3%)	3 (6.1%)	NS
Total follow-up periods, person-years	3079	1797	1282	
Natural history	1913	905	1009	
Under G-CSF	1926	753	274	
After HSCT	134	134	0	
After MDS/AL	5.5	5.5	0	
Hematological values at diagnosis				
ANC/mm ³	287 (102–705)	160 (64–408)	755 (360–1265)	<0.0001
AMC/mm ³	1543 (936–2556)	1953 (1100–3008)	1095 (738–2110)	0.0011
ALC/mm ³	5175 (3097–6840)	5581 (3747–6920)	4440 (2750–6491)	0.0521
Hemoglobin g/dL	11.1 (10–12.5)	10.6 (9.6–12.9)	11.4 (10.5–12.5)	0.0538
Platelets ×10 ³ /mm ³	406 (284–522)	410 (295–527)	403 (274–512)	NS
ANC mm ³ : <200 /200–500/500–1000/ >1000	26 (18%)/75 (53%)/28 (19.6%)/14 (9.8%)	25 (26.6%)/55 (58.5%)/13 (13.8%)/1 (1%)	1 (2%)/20 (41%)/15 (30.6%)/13 (26.5%)	<0.001
Hematological values during follow-up†				
CBCs assessable	13 (6–28)	12 (6–25)	15 (9–34)	NS
ANC/mm ³	269 (126–564)	183 (91–305)	595 (403–1000)	<0.0001
AMC/mm ³	1232 (791–1920)	1435 (1003–2157)	824 (638–1351)	<0.0001
ALC/mm ³	3732 (2635–6000)	5219 (3023–6309)	2904 (2398–4040)	0.0002
Hemoglobin g/dL	11.3 (10.2–12.4)	10.6 (9.8–12)	11.9 (11.2–12.7)	<0.0001
Platelets ×10 ³ /mm ³	369 (296–442)	390 (303–452)	341 (294–416)	NS
ANC/mm ³ : <200 /200–500/500–1000/ >1000	30 (21%)/59 (42%)/24 (17%)/30 (9.8%)	44 (29.8%)/13 (46.8%)/28 (13.8%)/9 (9.5%)	15 (4%)/11 (30.6%)/28 (22.4%)/21 (43%)	<0.0001
Differential bone-marrow count, <i>n</i>				
Myeloblasts††	111	83	28	
Myeloblasts††	2% (1–3%)	2% (1–3%)	1.9% (0.25–2.75%)	NS
Promyelocytes & myelocytes§	6.7% (3–9%)	5.7% (3–8%)	7% (2–13%)	NS
Metamyelocyte B & mature neutrophils	6.2% (0.5–9%)	2.75% (0–7%)	7% (2.5–19%)	0.003
Myeloid maturation arrest¶	91 (82%)	73 (88%)	18 (64.3%)	0.008
Immunoglobulin level, <i>n</i>				
IgG g/L	99	72	27	
IgG g/L	14.41 (11–18.4)	13.8 (10.6–18.3)	15.7 (12.2–19.1)	NS
IgA g/L	3 (1.6–4.8)	2.7 (1.3–4.7)	3.7 (1.7–5.2)	NS
IgM g/L	1.32 (0.94–2.1)	1.3 (0.94–1.8)	1.32 (1–2.1)	NS
Lymphocyte subsets, <i>n</i>				
CD3+/mm ³	62	47	15	
CD3+/mm ³	2276 (1504–3157)	2353(1569–3440)	2080 (1306–2385)	NS
CD4+/mm ³	1321 (860–1971)	1368 (801–2132)	1229 (869–1455)	NS
CD8+/mm ³	733 (489–1044)	776 (530–1044)	688 (396–1100)	NS
CD19+/mm ³	591 (353–1403)	920 (456–1443)	364 (288–512)	<0.0001
G-CSF, <i>n</i>				
Failure, <i>n</i>	116	82	35	
Failure, <i>n</i>	8	8	0	
Time-averaged dose, µg/kg/day	5 (4.9–9.45)	5.9 (4.98–10.45)	5 (4–5.7)	0.0096
Total duration, days	1129 (387–2135)	1443 (527–3052)	687 (127–1442)	0.0039
Cumulative dose, µg/kg	6987 (1986–16578)	8658 (3945–22194)	3446 (635–8704)	0.0010

ANC: absolute neutrophil count; AMC: absolute monocyte count; ALC: absolute lymphocyte count; CBCs, complete blood counts; MDS/AL, myelodysplasia syndrome/acute leukemia. Values are expressed as *n* (%) or median (interquartile range), unless stated otherwise.

*SCN vs. CyN. †During routine follow-up, hematological parameters fluctuated over time, with no detectable regular variation, in patients with SCN or CyN. ‡Normal 0.3–4%. §Normal 12–25%. || Normal 33–48%. ¶Mature metamyelocytes <10%; normal 0%.

Table II. Types of infections developed by the patients with *ELANE* mutations.

Parameter	All Patients (n = 143)		SCN (n = 94)		CyN (n = 49)	
	n	%	n	%	n	%
Severe infections						
Patients with at least 1 episode	98	68.5%	72	76.6%	26	53.1%
Total distinct episodes, n	302		242		60	
Septicemia	28	9.3%	21	8.7%	7	11.7%
Isolated bloodstream infections	15	5.0%	12	5%	3	5%
Cellulitis	143	47.4%	107	44.2%	36	60%
Pneumonitis	104	34.4%	94	38.8%	10	16.7%
Mastoiditis	14	4.6%	11	4.5%	3	5%
Omphalitis	12	4%	10	4.1%	2	3.3%
Liver abscess	6	2%	6	2.5%	0	0%
Osteitis and/or arthritis	4	1.3%	1	0.4%	3	5%
Appendicitis, or peritonitis colitis	6	2%	3	1.2%	3	5%
Pyelonephritis	13	4.3%	10	4.1%	3	5%
Mild infections						
Patients with at least 1 episode	143	100%	94	100%	49	100%
Total distinct episodes, n	962		558		404	
Ear Nose throat	475	49.4%	252	45.2%	223	55.2%
Skin (furunculosis, paronychia ...)	209	21.7%	142	25.4%	67	16.6%
Urinary tract	10	1%	5	0.9%	5	1.2%
Lymph node	68	7.1%	40	7.2%	28	6.9%
Others (bronchitis/FUO)	200	20.8%	119	21.3%	81	20%
Oral infections						
Patients with at least 1 episode	107	74.8%	63	67.0%	44	89.8%
Total distinct episodes, n	759		278		481	
Gingivitis	342	45.3%	172	61.9%	170	35.3%
Mouth ulcers	660	87.4%	215	77.3%	445	92.5%
Periodontitis	45	6%	20	7.2%	25	5.2%
Severe infection pathogen						
Documented episodes, n (% N*)	115	38.3%	87	37.2%	28	46.7%
Germs isolated from severe infection n (% N†)						
<i>Staphylococcus aureus</i>	44	38.3%	30	34.5%	14	50.0%
<i>Staphylococcus epidermidis</i>	6	5.2%	4	4.6%	2	7.1%
<i>Streptococcus</i>	9	7.8%	6	6.9%	3	10.7%
<i>Streptococcus pneumoniae</i>	7	6.1%	6	6.9%	1	3.6%
<i>Pseudomonas aeruginosa</i>	18	15.7%	14	16.1%	4	17.9%
<i>Escherichia coli</i>	25	21.7%	22	25.3%	3	10.7%
<i>Proteus</i>	7	6.1%	7	8.0%	0	0.0%
Other Gram-negative	6	5.2%	6	6.9%	0	0.0%
<i>Klebsiella pneumoniae</i>	3	2.6%	3	3.4%	0	0.0%
Non-typhoidal <i>Salmonella</i>	2	1.7%	0	0%	2	7.1%
<i>Hemophilus</i>	0	0.0%	0	0%	0	0%
<i>Candida albicans</i> ‡	1	0.9%	1	1.1%	0	0%

FUO, fever of unknown origin.

*% of total number of episodes, N.

† % of all documented episodes, N.

‡Fungus.

Table III. Impact of G-CSF on the ratio of infections*.

Parameter	Severe infection			Mild infection			Oral infections		
	Natural history	G-CSF	<i>P</i> †	Natural history	G-CSF	<i>P</i> †	Natural history	G-CSF	<i>P</i> †
At baseline									
Never G-CSF, <i>n</i> = 27	0 (0–0.06)			0.12 (0.05–0.55)			0.03 (0–0.5)		
All G-CSF recipients, <i>n</i> = 116	0.22 (0.04–1.23)			1.2 (0.47–3.65)			0.35 (0.01–0.75)		
Natural history vs G-CSF									
All G-CSF recipients, <i>n</i> = 116	0.22 (0.04–1.23)	0.04 (0–0.28)	<0.001	1.2 (0.47–3.65)	1 (0.42–2.6)	NS	0.35 (0.01–0.75)	0.07 (0–0.35)	<0.001
By G-CSF dose‡									
<10, <i>n</i> = 85	0.15 (0–0.65)	0 (0–0.25)	0.0015	0.91 (0.32–0.91)	0.77 (0.3–1.7)	NS	0.3 (0.04–0.72)	0.09 (0–0.32)	0.0003
≥10, <i>n</i> = 23	0.88 (0.16–2.49)	0.14 (0–0.57)	0.0039	1.4 (0.8–2.66)	1.45 (0.5–2.4)	NS	0.4 (0–0.92)	0.06 (0–0.4)	0.0436
Refractory, <i>n</i> = 8	1.95 (1–2.56)	0 (0–0.71)	0.04	5.5 (4.3–8)	3.9 (2.2–8.2)	NS	0.2 (0–1.16)	0 (0–0.18)	NS
By diagnosis									
SCN, <i>n</i> = 81	0.61 (0.15–1.98)	0.10 (0–0.37)	0.001	1.85 (0.61–4.4)	1 (0.43–2.46)	0.0637	0.37 (0–0.74)	0.1 (0–0.34)	0.0064
CyN, <i>n</i> = 35	0.05 (0–0.14)	0 (0–0.11)	NS	0.7 (0.18–1.4)	0.62 (0.28–2.8)	NS	0.3 (0.12–1)	0 (0–0.35)	0.0003

NS, not significant.

*The infection ratios were calculated by dividing the number of episodes by the duration of each period (natural history/G-CSF therapy period). Results are expressed as median (interquartile range) episodes/year.

† Kruskal–Wallis *P*: natural history vs on-G-CSF period ratio.

‡ Time-averaged dose in µg/kg/day.

Table IV. Univariate and multivariate analyses of developing a first severe infectious episode.

Parameter	Univariate		Multivariate	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Diagnosis (SCN vs CyN)	2.3 (1.5-3.7)	<.0001	1.7 (1.5-3.7)	.03
ANC (<200 vs >200/mm ³)	1.7 (1.2-2.6)	.007	1.34 (0.86-2)	.2
AMC (>2000 vs <2000/mm ³)	1.5 (1.1-2.3)	.07	1.06 (0.8-1.6)	.8
ALC (>3000 vs <3000/mm ³)	3.2 (1.8-5.8)	<.001	2.7 (1.5-5.1)	.02
p.Gly214Arg variant (Yes vs No)	2.29 (0.92-5.69)	.07	1.3 (0.5-3.5)	.5
Hypergammaglobulinemia	0.76 (0.26-2.1)	.76		
Sex (male vs female)	0.91 (0.61-1.36)	.65		

HR, hazard ratio, CI, confidence interval.

Only factors identified as significant in univariate analyses at the *P* = 0.1 threshold were included in the multivariate analysis.