Recurrent bacterial infections, but not fungal infections, characterise patients with \textit{ELANE}-related neutropenia: a French Severe Chronic Neutropenia Registry study

Gioacchino A. Rotulo,1,2 Geneviève Plat,3 Blandine Beaupain,1 Stéphane Blanche,4 Despina Moushous,1 Flore Sicre de Fontbrune,5 Thierry Leblanc,6 Cécile Renard,7 Vincent Barlogis,8 Marie-Gabrielle Vigue,9 Claire Freycon,10 Christophe Piguet,11 Marie-Gabrielle Vigue,9 Claire Fieschi,12 Wadih Abou-Chahla,13 Virginie Gandemer,14 Fanny Rialland,15 Frédéric Millot,16 Aude Marie-Cardine,17 Catherine Paillard,18 Pascal Levy,19 Nathalie Alajdiji,20 Martin Biosse-Duplan,21 Christine Bellamé-Chante-lot,19 Jean Donadieu1 and the French Severe Chronic Neutropenia Registry7

Among 143 patients with elastase, neutrophil-expressed (\textit{ELANE})-related neutropenia 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 analysed according to their natural occurrence without granulocyte-colony stimulating factor (G-CSF), on G-CSF, after myelodysplasia/acute leukaemia or after haematopoietic stem-cell transplantation. During the disease’s natural history period (without G-CSF; 1913 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 \textit{Staphylococcus aureus} (37-4%), \textit{Escherichia coli} (20%) and \textit{Pseudomonas aeruginosa} (16%), while fungal infections accounted for 1%. Profound neutropenia (<200/mm$^3$), high lymphocyte count (>3000/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 µg/kg/day. Infections had permanent consequences in 33% of patients, most frequently edentulism.

Keywords: severe congenital neutropenia, \textit{ELANE}-related neutropenia, opportunistic infections.
Introduction
Congenital neutropenia (CN) covers a group of diseases characterised by fewer neutrophils circulating in peripheral blood. The epidemiology of infections in patients with CN 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), characterised 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 as cyclic (CyN), characterised 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.
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, or targeted sequencing with a gene panel including ELANE. We numbered mutations as recommended by the Human Genome Variation Society ([http://www.hgvs.org/](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. 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.

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 µg/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 haematological complications, such as myelodysplasia syndrome or acute leukaemia (MDS/AL), had not undergone haematopoietic stem-cell transplantation (HSCT) or was not on G-CSF); ‘receiving G-CSF’; ‘post-HSCT’ 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 (Stata Corp., College Station, TX, USA) was used to compute all statistical analyses. Infection ratios were compared using the Kruskal–Wallis test. For survival analyses, the end-points 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, haematological 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, the median [interquartile range (IQR)] age at diagnosis was 0·3 [0·09–1·5] years. A total of 116 (81%) patients received G-CSF (81 with SCN, 35 with CyN) for a median cumulative duration of 3 years and 18 patients underwent HSCT.8 The median (IQR) follow-up per patient was 17·6 (9·2–30·5) years, for a total observation period of 3079 person-years, broken down per period as follows – natural history: 1913 person-years; on G-CSF: 1026 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-versus-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 1543 cells/mm³ and median absolute lymphocyte count (ALC) was 5175 cells/mm³. Among the 99 patients with assessable immunoglobulin (Ig) levels, only four (4·7%) had normal IgG levels according to age, while 81 (95%) were hypergammaglobulinotaemic.

The 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 (IQR) time-averaged dose was 5·9 (4·9–9·45) µg/kg/day, with a median (IQR) cumulative dose of 6987 (1986–16 578) µg/kg for the entire cohort up to the last follow-up visit.
Epidemiology of Infections in ELANE-related Neutropenia

| Variable                                      | All patients (n = 143) | SCN (n = 94) | CyN (n = 49) | $P^*$  |
|-----------------------------------------------|------------------------|-------------|------------|--------|
| **Demographic**                               |                        |             |            |        |
| Males/females, n                              | 71/72                  | 46/48       | 25/24      |        |
| Age at diagnosis, years, median (IQR)         | 0.3 (0.09–1.5)         | 0.2 (0.06–0.68) | 1 (0.23–6.8) | 0.0002 |
| Age at last follow-up, years, median (IQR)    | 17.6 (9.30–1)          | 14.5 (8.3–25.7) | 25.6 (13.34–2) | 0.003  |
| MDS(AL, n (%))                                | 6 (4.2)                | 6 (6.4%)    | 0          | 0.07   |
| HSCT, n (%)                                   | 18 (12.56)             | 18 (18/19)  | 0          | <0.0001|
| Deaths, n (%)                                 | 7 (5/4)                | 4 (4.3)     | 3 (6.1)    | NS     |
| Total follow-up periods, person-years          | 3079                   | 1797        | 1282       |        |
| Natural history                               | 1913                   | 905         | 1008       |        |
| Under G-CSF                                   | 1926                   | 753         | 273        |        |
| After HSCT                                    | 134                    | 134         | 0          |        |
| After MDS/AL                                   | 5–5                    | 5–5         | 0          |        |
| **Haematological values at diagnosis, median (IQR)** |                        |             |            |        |
| ANC, $/\mu m^3$, n (%)                         | 287 (102–705)          | 160 (64–408) | 755 (360–1265) | <0.0001|
| AMC, $/\mu m^3$                                | 1543 (936–2556)        | 1953 (1100–3008) | 1095 (738–2110) | 0.0011 |
| ALC, $/\mu m^3$                                | 5175 (3097–6840)       | 5581 (3747–6920) | 4440 (2750–6491) | 0.0521 |
| Haemoglobin, g/l                              | 111 (100–125)          | 106 (96–129) | 114 (105–125) | 0.0538 |
| Platelets, $\times 10^9$/mm$^3$               | 406 (284–522)          | 410 (295–527) | 403 (274–512) | NS     |
| ANC ($/\mu m^3$), n (%)                        | $<$200                 | 26 (18)     | 25 (26–6)  | 1 (2)  | <0.0001|
|                                              | 200–500                | 75 (53)     | 55 (58–5)  | 20 (41) |        |
|                                              | 500–1000               | 28 (19–6)   | 13 (13–8)  | 15 (30–6) |        |
|                                              | $>$1000                | 14 (9.8)    | 1 (1)      | 13 (26–5) |        |
| **Haematological values during follow-up, median (IQR)** |                        |             |            |        |
| FBCs assessable, n                            | 13 (6–28)              | 12 (6–25)   | 15 (9–34)  | NS     |
| ANC, $/\mu m^3$                                | 269 (126–564)          | 183 (91–305) | 595 (403–1000) | <0.0001|
| AMC, $/\mu m^3$                                | 1232 (799–1920)        | 1435 (1003–2157) | 824 (638–1351) | <0.0001|
| ALC, $/\mu m^3$                                | 3732 (2635–6000)       | 5219 (3023–6309) | 2904 (2398–4040) | 0.0002 |
| Haemoglobin, g/l                              | 113 (102–124)          | 106 (98–120) | 119 (112–127) | <0.0001|
| Platelets, $\times 10^9$/mm$^3$               | 369 (296–442)          | 390 (303–452) | 341 (294–416) | NS     |
| ANC ($/\mu m^3$), n (%)                        | $<$200                 | 30 (21)     | 44 (29–8)  | 15 (4)  | <0.0001|
|                                              | 200–500                | 59 (42)     | 13 (46–8)  | 11 (30–6) |        |
|                                              | 500–1000               | 24 (17)     | 28 (13–8)  | 28 (22–4) |        |
|                                              | $>$1000                | 30 (9–8)    | 9 (9–5)    | 21 (43)  |        |
| Differential bone-marrow count, n             | 111                    | 83          | 28         |        |
| Myeloblasts$^1$, %, median (IQR)              | 2 (1–3)                | 2 (1–3)     | 1·9 (0·25–2·75) | NS     |
| Promyelocytes and myelocytes$^2$, %, median (IQR) | 6·7 (3–9)              | 5·7 (3–8)   | 7 (2·3–13) | NS     |
| Metamyelocyte B and mature neutrophils$^4$, %, median (IQR) | 6·2 (0·5–9)            | 2·75 (0–7)  | 7 (2·5–19) | 0·003  |
| Myeloid maturation arrest$^5$, n (%)          | 91 (82)                | 73 (88)     | 18 (64–3)  | 0·008  |
| Immunoglobulin level, n                      | 99                     | 72          | 27         |        |
| IgG, g/l, median (IQR)                        | 14·41 (11–18–4)        | 13·8 (10·6–18·3) | 15·7 (12·2–19·1) | NS     |
| IgA, g/l, median (IQR)                        | 3·1·6–4–8              | 2·7 (1·3–4–7) | 3·7 (1·7–5–2) | NS     |
| IgM, g/l, median (IQR)                        | 1·32 (0·94–2·1)        | 1·3 (0·94–1·8) | 1·32 (1·2–1) | NS     |
| Lymphocyte subsets, n                         | 62                     | 47          | 15         |        |
| CD3$^+$, $/\mu m^3$, median (IQR)             | 2276 (1504–3157)       | 2353 (1569–3440) | 2080 (1306–2385) | NS     |
| CD4$^+$, $/\mu m^3$, median (IQR)             | 1321 (860–1971)        | 1368 (801–2132) | 1229 (869–1453) | NS     |
| CD8$^+$, $/\mu m^3$, median (IQR)             | 733 (489–1044)         | 776 (530–1044) | 688 (396–1100) | NS     |
| CD19$^+$, $/\mu m^3$, median (IQR)            | 591 (353–1403)         | 920 (456–1443) | 364 (288–512) | <0.0001|
| G-CSF, n                                      | 116                    | 82          | 35         |        |
| Failure, n                                    | 8                      | 8           | 0          |        |
| Time-averaged dose, $\mu g/kg/day$, median (IQR) | 5 (4.9–9.45)          | 5·9 (4·98–10·45) | 5 (4·5–7) | 0·0096 |
Molecular characteristics

The cohort includes 99 probands and 44 affected relatives (Table SI). A total of 51 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. Figure 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 pre-emptive intravenous antibiotic administration was always initiated promptly in this context. Bacteria were identified as responsible for 99% of those infections (Table II), with

| Variable                                      | All patients (n = 143) | SCN (n = 94) | CyN (n = 49) | P*   |
|-----------------------------------------------|------------------------|-------------|-------------|------|
| Total duration, days, median (IQR)            | 1129 (387–2135)        | 1443 (527–3052) | 687 (127–1442) | 0.0039 |
| Cumulative dose, µg/kg, median (IQR)          | 6987 (1986–16 578)     | 8658 (3945–22 194) | 3446 (635–8704) | 0.0010 |

ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; CD, cluster of differentiation; FBCs, full blood counts; G-CSF, granulocyte-colony stimulating factor; HSCT, haematopoietic stem-cell transplantation; Ig, immunoglobulin; IQR, interquartile range; MDS/AL, myelodysplasia syndrome/acute leukaemia; NS, not significant.

*SCN versus CyN.
1During routine follow-up, haematological parameters fluctuated over time, with no detectable regular variation, in patients with SCN or CyN.
2Normal 0-3–4%.
3Normal 12–25%.
4Normal 33–48%.
5Mature metamyelocytes <10%; normal 0%.

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![Fig 1. Distribution of the time-averaged granulocyte-colony stimulating factor (G-CSF) doses* given to the 143 patients with ELANE-related neutropenia. *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 (µg/kg); (B) ni, number of injections; (C) cumulative dose, total dose received during the relevant period (mg/kg) = ddi × ni; and (D) duration of the relevant period in days. The total cumulative dose (µg/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 (µg/kg/day) was calculated by dividing the cumulative dose by the cumulative duration of treatment.](image-url)
Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa being the most frequently isolated. The different types of microorganisms by infection site are reported in Figure S2.

It should be emphasised 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 Figure 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

| Parameter | All patients (n = 143) | SCN (n = 94) | CyN (n = 49) |
|-----------|------------------------|-------------|-------------|
| Severe infections |                         |             |             |
| Patients with at least one episode | 98 (68.5) | 72 (76.6) | 26 (53.1) |
| Total distinct episodes, n | 302 | 242 | 60 |
| Septicaemia | 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 one 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 one 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†) |             |             |             |
| Staphylococcus aureus | 44 (38.3) | 30 (34.5) | 14 (50.0) |
| Staphylococcus epidermidis | 6 (5.2) | 4 (4.6) | 2 (7.1) |
| Streptococcus | 9 (7.8) | 6 (6.9) | 3 (10.7) |
| Streptococcus pneumoniae | 7 (6.1) | 6 (6.9) | 1 (3.6) |
| Pseudomonas aeruginosa | 18 (15.7) | 14 (16.1) | 4 (17.9) |
| Escherichia coli | 25 (21.7) | 22 (25.3) | 3 (10.7) |
| Proteus | 7 (6.1) | 7 (8.0) | 0 (0) |
| Other gram-negative | 6 (5.2) | 6 (6.9) | 0 (0) |
| Klebsiella pneumoniae | 3 (2.6) | 3 (3.4) | 0 (0) |
| Non-typhoidal Salmonella | 2 (1.7) | 0 (0) | 2 (7.1) |
| Hemophilus | 0 (0) | 0 (0) | 0 (0) |
| Candida albicans‡ | 1 (0.9) | 1 (1.1) | 0 (0) |

FVO, fever of unknown origin.

*% of total number of episodes, N.

†% of all documented episodes, N.

‡Fungus.

Table II. Types of infections developed by the patients with ELANE mutations.
infection, 209 episodes, 21.7%), adenitis (68 episodes, 7.1%) and urinary infections (10 episodes, 1.0%), with 200 (20.8%) less well-characterised episodes with either fever of unknown origin or mild bronchitis.

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

During the natural history period, infection-ratio distributions among patients were asymmetric, with annual medians (IQRs) of 0.14 (0.04–0.96) for severe infections (Fig 2A), 0.93 (0.2–2.42) for mild infections and 0.2 (0.01–0.72) for 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.

A total of 47 (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 edentulous before the age of 30 years for 17 patients), chronic respiratory failure with bronchiectasis for 10, 13 became deaf secondary to otitis, five had aesthetic sequelae, including two with facial necrosis, two experienced soft tissue loss-of-substance and one each had 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. There was no late sequela-rate difference between the CyN and SCN subgroups.

**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 patients with CyN, 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 median (range) severe infection ratio of 1.66 (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. Among 16 patients who survived the initial post-HSCT period, with neutrophil recovery, the median (range) ratio of serious infections per year was 0 (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*-related neutropenia. To analyse that variability, we first considered some basic information, e.g. blood counts, genotype, immunological features and the diagnoses of CyN or SCN (Fig 2E). To analyse 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 ($P = 0.07$).

Uni- and multivariate analyses, using the first severe infection as an event, are summarised 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 patients with *ELANE*-related neutropenia, the severe infection ratio appeared to be strongly associated with age, for patients with CyN and SCN. 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**

In the present study, we described a cohort of neutropenic patients bearing *ELANE* variants, enabling epidemiological
analysis of their infectious episodes. Since their initial description in 1999,\textsuperscript{9,10} ELANE mutations have been reported in ~500 patients worldwide, mainly in two large series of 188\textsuperscript{11} and 307 patients,\textsuperscript{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-related neutropenia carry a remarkably high risk of infections. In

Fig 2. (A–F) Severe infections and oral infections in the 143 patients with ELANE-related neutropenia: distributions of the severe and oral infection ratios calculated by dividing the number of episodes per ‘natural history’ duration. (A, B) Severe and oral infection ratios, respectively, varied very widely among patients. (C, D) Kaplan–Meier curves of the estimated risks of severe or oral infections, respectively, as the first event, plotted versus age at occurrence. (E, F) The risks of severe and oral infections, respectively, according to the diagnostic categories: cyclic (CyN) or severe chronic neutropenia (SCN).
Table III. Impact of granulocyte-colony stimulating factor (G-CSF) on the ratio of infections.*

| Variable | Severe infection | Mild infection | Oral infections |
|----------|-----------------|---------------|----------------|
|          | Natural history | G-CSF         | p†             | Natural history | G-CSF         | p†             | Natural history | G-CSF         | p†             |
| At baseline |                 |               |                |                |               |                |                |               |                |
| Never G-CSF, n = 27 | 0 (0-0.06) | 0.12 (0.05–0.55) | 0.03 (0-0.5) | 0.35 (0-01–0.75) |
| All G-CSF recipients, n = 116 | 0.22 (0-04–1-23) | 1.2 (0.47–3.65) |               |                |                |                |                |                |
| Natural history versus G-CSF |           |               |                |                |                |                |                |                |
| All G-CSF recipients, n = 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, n = 85 | 0.15 (0-0.65) | 0 (0-0-25) | 0.0015 | 0.91 (0.32–0.91) | 0.77 (0.3–1.3) | NS | 0.3 (0-0.4–0.72) | 0.09 (0-0.32) | 0.0003 |
| ≥10, n = 23 | 0.88 (0.16–2.49) | 0.14 (0-05–57) | 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, n = 8 | 1.95 (1.2–5.6) | 0 (0-0-71) | 0.04 | 5.5 (4.3–8) | 3.9 (2–2.8) | NS | 0.2 (0-1.16) | 0 (0-0-18) | NS |
| By diagnosis |                 |               |                |                |                |                |                |                |
| SCN, n = 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, n = 35 | 0.05 (0-0-14) | 0 (0-0-11) | NS | 0.7 (0-0–1–14) | 0.62 (0.28–2.8) | NS | 0.3 (0-1-2) | 0 (0-0-35) | 0.0003 |

CyN, cyclic neutropenia; G-CSF, granulocyte-colony stimulating factor; NS, not significant; SCN, severe chronic neutropenia.

*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 versus on-G-CSF period ratio.
‡Time-averaged dose in µg/kg/day.
addition to mild infections, which can be non-specific (e.g. 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

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

| Parameter                                      | Univariate   |   | Multivariate |   |
|------------------------------------------------|--------------|---|--------------|---|
| Diagnosis (SCN vs. CyN)                       | 2.3 (1.5–3.7) | <0.0001 | 1.7 (1.5–3.7) | 0.03 |
| ANC (<200 vs. >200/mm³)                       | 1.7 (1.2–2.6) | 0.007 | 1.34 (0.86–2) | 0.2 |
| AMC (>2000 vs. <2000/mm³)                     | 1.5 (1.1–2.3) | 0.07 | 1.06 (0.8–1.6) | 0.8 |
| ALC (>3000 vs. <3000/mm³)                     | 3.2 (1.8–5.8) | <0.001 | 2.7 (1.5–5.1) | 0.02 |
| p.Gly214Arg variant (yes vs. no)              | 2.29 (0.92–5.69) | 0.07 | 1.3 (0.5–3.5) | 0.5 |
| Hypergammaglobulinaemia                       | 0.76 (0.26–2.1) | 0.76 |             |   |
| Sex (male versus female)                      | 0.91 (0.61–1.36) | 0.65 |             |   |

Only factors identified as significant in univariate analyses at the \( P = 0.1 \) threshold were included in the multivariate analysis. ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; CI, confidence interval; CyN, cyclic neutropenia; HR, hazard ratio; SCN, severe chronic neutropenia.

Fig 3. Severe infection ratios (number of severe infections/follow-up period) according to age. The age is divided in eight intervals (0–3, 3–12 months; 1–2, 2–5, 5–10, 10–15, 15–20 and >20 years) and the total number of severe infections observed during each interval is divided by the observation period [natural history period without granulocyte-colony stimulating factor (G-CSF)], calculated for all patients (■) together and separately for those with severe chronic neutropenia (SCN; ▲) or cyclic neutropenia (CyN; ♦). 

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indirect and direct methods, were extremely rare [one of 302 episodes (0.3%)]. Some findings, suggestive of fungal infections, like round-shaped lung lesions or liver pseudo-tumour, were finally diagnosed as bacterial infections (Figure 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, characterised 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 defence against Aspergillus is a multistep process.¹⁶ Phagocyte recruitment represents only part of this immunity. Indeed, in ELANE-related neutropenia, several redundant systems of anti-Aspergillus immunity remain active. Monocytes and macrophages are still present, and nicotinamide adenine dinucleotide phosphate (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 patients with ELANE-related neutropenia 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-related neutropenia. Their frequency, observed in patients with CyN and SCN, is close to that found for other phagocyte disorders, e.g. leucocyte-adhesion deficiency²¹ and Papillon–Lefebvre syndrome.²² The high oral infection ratio for chronic periodontal disease highlights the chronic inflammation typical of ELANE-related neutropenia.²³

The second part of our present analysis evaluated the risk factors for severe infections. Genetic analyses identified 53 distinct ELANE 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 In the present study, only the p.Gly214Arg variant was associated with a very high risk of infections in univariate analysis; but it is quite rare (seven cases, 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 categorisation fails to summarise all the prognostic information; e.g. 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 precarious, high infection risk suggests that the maturation of adaptive B-cell immunity²⁶ and T-cell and innate immunities²⁷,²⁸ later in life can partially compensate for the ELANE dysfunction and neutropenia.

Concerning high risk of infections in patients with ELANE-related neutropenia, 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 present 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-related 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’ with the severe infection ratios of G-CSF-treated patients being far higher than those not treated. As demonstrated in the literature, G-CSF can 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 sepsis;³¹ and, moreover, although not within the scope of the present study, the greater the risk of leukaemic transformation.⁷,³¹ Such patients would probably benefit most from HSCT, which contains the risks of leukaemia and severe infections, thereby lowering the sequela rate.⁸

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**Author contributions**

The original study design was conceived by Jean Donadieu, Geneviève Plat and Gioacchino A. Rotulo. The FSCNR, coordinated by Jean Donadieu and Blandine Beaufain, is responsible for data management. All the authors contributed to the writing and revision of the manuscript and approved the final version.

**Conflict of interest**

None of the authors have any conflict of interest to disclose with regards to this study.

**Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Distributions of the ELANE mutations observed in the 143 patients: three in-frame, five initiation, 85 missense, 32 splicing defects and 18 truncated. Variant nomenclature using the reference sequence NM_001972.2.

**Fig S1.** Examples of infections in patients with ELANE-related neutropenia.

**Fig S2.** Aetiologies of most common severe infections. The results are expressed in percentage of microorganisms, if documented.

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**Appendix 1 French Severe Chronic Neutropenia Registry**

Elodie Gouache, Fanny A. Laurent, Yves Bertrand, Pierre-Simon Rohrlich, Eric Jeziorksi, Liana Carausu, Marc Michel, Valérie L. T. Te, Philippe Bensaid, Loïc de Pontual, Louis Terriou, Nicolas Schleinitz, Karima Yakouben, Nizar Mahlouji, Felipe Suarez, Xavier Delbrel, Dalila Adjoud, Jean-Louis Stephan, Guy Leverger, Fanny Fouyssac, Alice Garnier, Chiara Sileo, Aline Moignet, Nathalie Cheikh, Damien Bodet, Eric Dore, Florent Neumann, Fabrice Jardin.