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Evaluation of bone mineral density in patients with severe congenital neutropenia: Experience of six centers in Turkey

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Abstract

Aims: Severe congenital neutropenia (SCN) is a rare hematological disorder characterized by a marked reduction in circulating neutrophils and recurrent infections. This study aimed to evaluate bone metabolism in patients with SCN via biochemical and bone mineral density (BMD) measurements.

Methods: This study included 76 patients who were diagnosed with SCN at six tertiary immunology centers. Demographic, clinical, laboratory, and BMD findings were retrospectively analyzed and measured via dual-energy X-ray absorptiometry (DXA).

Results: The study included 76 patients, 39 (51.3%) females and 37 (48.7%) males. The mean age was 206.9 ± 105.1 months, with a median age at diagnosis of 18.5 months (range: 1–264) and a follow-up period of 120 months (range: 2–324). Parental consanguinity was present in 57 patients (75%). Among patients evaluated by DXA, 30 (39.5%) had low BMD, with a mean BMD score of 0.63 ± 0.20 and a Z-score of -2.88 ± 0.90. Osteoporosis was detected in 11 adult patients (29.7%). The most common genetic mutations were *HAX1* in 46 patients (60.5%) and *ELANE* in 15 patients (19.7%). No significant difference in BMD was observed between patients with *HAX1* and *ELANE* mutations (p=0.60).

Granulocyte colony-stimulating factor (G-CSF) was administered to 73 patients. The median G-CSF dose was 5 (2–12) µg/kg/day in the normal BMD group and 5 (2–10) µg/kg/day in the low BMD group. No significant relationship was found between the duration or dose of G-CSF and BMD (p=0.76, p=0.38). Additionally, 13 patients received vitamin D, four received calcium, and one was treated with calcitonin and alendronate.

Conclusions: Our findings indicate that patients with SCN have a high prevalence of bone mineral loss. The underlying pathophysiology and potential effects of recombinant G-CSF treatment on increased bone mineral loss remain controversial and warrant further investigation.

Keywords: Severe congenital neutropenia, bone mineral density, dual-energy X-ray absorptiometry

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Introduction

Severe congenital neutropenias (SCN) are a form of inborn error immunity characterized by severe chronic neutropenia with an absolute neutrophil count less than 500 cells/μL due to a defect in the differentiation of neutrophilic granulocytes associated with various genetic abnormalities [1-5]. The prevalence of SCN is estimated at 1–8.5 cases per million [6-8]. The International Union of Immunological Societies Expert Committee has defined 20 subtypes of congenital neutropenia according to their genetic causes in classifying immune deficiencies [9].The most common causes of SCN are autosomal dominant mutations in *ELANE*, which encodes neutrophil elastase, and autosomal recessive mutations in *HAX1*, which encodes a multifunctional protein associated with mitochondrial integrity and the CSF3 signaling pathway [1]. Other genetic mutations include *GFI-1, WASP, G6PC3, CSF3R, CLPB, VPS13B* and *JAGN1* [1,10].

Patients with SCN are prone to recurrent and life-threatening bacterial and fungal infections of the respiratory tract, gastrointestinal tract, skin, and mucosa. Severe bacterial infections often occur in the first year of life, and omphalitis, which begins shortly after birth, may be the first symptom [1,3,10]. Bone marrow examination revealed maturation arrest of myelopoiesis at the promyelocyte level [1,5,11]. The introduction of recombinant granulocyte colony-stimulating factor (G-CSF) in the treatment of SCN has led to a significant increase in neutrophil counts, reducing both the frequency and severity of infections and markedly improving quality of life [12,13].

Bone mineral density (BMD) measurements indicate that early-onset osteopenia/osteoporosis/low BMD are significant comorbidities in approximately 40% of patients with SCN. The underlying mechanisms remain unclear. While G-CSF appears to increase the risk of abnormal bone density, it is unclear whether this is due to the treatment itself or the underlying genetic defects [1,14].

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In this study, we aimed to describe changes in BMD in patients with SCN, mainly *HAX-1* and *ELANE* mutations, treated with G-CSF, as a high frequency of decreased BMD has been described in patients with SCN.

Method

Patient selection and laboratory

A total of 76 patients diagnosed with SCN, followed up at six tertiary immunology centers, and evaluated for bone metabolism were included in the study. Clinical parameters (age, sex, age at diagnosis, follow-up period, body mass index (BMI), and treatment details) and laboratory findings (complete blood count, blood biochemistry—including serum calcium, phosphate, magnesium, alkaline phosphatase, creatinine, protein, alanine aminotransferase, and aspartate aminotransferase levels—along with 25-hydroxy vitamin D and parathyroid hormone levels) were retrospectively analyzed from medical records.

Measurement of BMD

The BMD was measured via dual-energy X-ray absorptiometry (DXA) at the lumbar vertebrae (L1–L4) and expressed as grams of mineral per square centimeter (bone mineral content (BMC) , g/cm^2). The World Health Organization and the International Osteoporosis Foundation recommend DXA as the reference technique for diagnosing osteoporosis, with T- and Z-scores used to evaluate BMD [15]. The Z-score is recommended for diagnosing osteoporosis in premenopausal women, men under 50 years of age, and children. The Z-score represents the number of standard deviations (SDs) by which a patient's BMC deviates from the average BMC of individuals of the same age and sex.

Definition of osteoporosis in adults

A Z-score of -2.0 SD or lower is classified as 'lower bone mass than expected for chronological age', whereas a score above -2.0 SD is considered 'normal bone mass for chronological age' [15,16]. According to the World Health Organization, osteoporosis is a BMD 2.5 SD or greater below the young adult reference population average [16,17].

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Definition of osteoporosis in the pediatric population

The International Society for Clinical Densitometry published 'The Official Pediatric Positions', updated in 2019 [18]. The use of DXA assessment in children and adolescents with diseases that may affect the skeleton was explained as follows [18]:

1) DXA measurement is part of a comprehensive skeletal health assessment in patients at high risk of fractures.
2) Patients with primary bone disease or at risk of secondary bone disease should undergo DXA if they are likely to benefit from interventions to reduce their risk of clinically significant fractures and if the results influence management decisions.
3) The definition of osteoporosis and fracture prediction is as follows: In the absence of local disease or high-energy trauma, evidence of one or more vertebral compression fractures indicates osteoporosis.
4) In the absence of vertebral compression fractures, osteoporosis is diagnosed on the basis of a history of clinically significant fractures. Clinically significant fractures are defined as one or more long bone fractures by the age of ten or three or more long bone fractures by the age of nineteen.

Evaluation of BMD

Patients were divided into three age groups: 0–120 months (childhood), 120–216 months (adolescence), and >216 months (adulthood).

A Z-score of ≤ -2.0 was interpreted as 'lower than expected for age', a Z-score of > -2.0 was considered 'within the expected limits for age', and a Z-score of ≤ -2.5 SD was classified as osteoporosis in adults.

“Low BMD” was accepted when the Z-score was ≤ -2.0 corrected for age and sex in children. “Osteoporosis” was accepted when evidence of 1 or more vertebral compression fractures or a history of clinically significant fractures with or without low DXA Z-score.

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Ethical statement

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study was reviewed and approved by the Bursa Uludag University Faculty of Medicine Medical Research Ethics Committee with decision number 2024-1/25.

Statistics

Statistical analyses were performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA), with a two-sided significance level set at $\alpha = 0.05$. The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables with a normal distribution are presented as mean ± standard deviation (SD), whereas non-normally distributed variables are expressed as median (minimum–maximum). Categorical variables are presented as frequencies and percentages. For comparisons between two independent groups, the independent samples t-test was used for normally distributed continuous variables, and the Mann–Whitney U test was applied for non-normally distributed variables. Categorical variables were compared using the Pearson chi-square test or Fisher's exact test, as appropriate. Logistic regression analysis was performed to evaluate the independent effects of multiple predictor variables on categorical dependent outcomes.

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Results

A total of 76 patients were included in the study: 39 (51.3) females and 37 (48.7%) males. The mean age of the patients was 206.9 ± 105.1 months, the median age at diagnosis was 18.5 (1–264) months, and the follow-up period under the G-CSF treatment was 120 (2–324) months. Seventy-two of the patients had genetic mutations associated with congenital neutropenia (Table 1). The most common genetic mutations were *HAX1* in 46 (60.5%) patients and *ELANE* gene mutations in 15 (19.7%) patients. In contrast, other mutations were *G6PC3, SLC37A4, JAGN1, CSF3R, CLPB,* and *VPS13B* in order of frequency, and no mutation was detected in 4 patients (Figure 1).

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The diagnosis for 4 patients without detected pathogenic mutations was made clinically based on evaluations of persistent severe neutropenia and bone marrow maturation arrest.

Consanguineous marriages were present in 57 patients (75%). The median BMI for all patients was 18 (range: 11.6–43). Z-scores revealed that the BMD was low in 30 patients (39.5%) and normal in 46 patients (60.5%). The mean BMD score on DXA was 0.63 ± 0.20 in patients with low BMD, with a Z-score of -2.88 ± 0.90. In contrast, patients with a normal BMD had a mean BMD of 0.81 ± 0.30 and a Z-score of -0.26 ± 1.3 (Table 2).

Patients with a Z-score ≤ -2.5 were grouped as severely low BMD and further evaluated by comparing them with patients with normal BMD (Table 3).

Osteoporosis was detected in 11 adult patients (36.6%) in the low BMD group. No patients had a history of pathological fractures. Because no pathological fractures were present, no pediatric patient was diagnosed with osteoporosis. All patients diagnosed with osteoporosis were adults.

Sex, age, age at diagnosis, follow-up duration, consanguineous marriage, and BMI had no statistically significant effect on BMD. No statistically significant difference was observed in the effect of the most common genetic mutations, *HAX1* and *ELANE*, on BMD **(**p=0.60) (Table 1).

G-CSF was administered to 73 patients (96%)**.** The median G-CSF dose was 5 (2–12) µg/kg/day in the group with normal BMD and 5 (2–10) µg/kg/day in the group with low BMD. The mean duration of G-CSF was 107.6 ± 85.4 months in patients with normal BMD and 124.5 ± 72.1 months in those with low BMD. Neither the G-CSF dose (p=0.76) nor the treatment duration (p=0.38) had a statistically significant effect on BMD **(**Table 2**).**

Logistic regression analysis was performed for the effects of genetic mutation, sex, age, consanguineous marriage, disease follow-up period, G-CSF dose and treatment duration, absolute neutrophil count, and BMI (kg/m2) parameters on BMD, and no independently significant influencing factors were detected (Table 4). Other treatments included vitamin D (13 patients), oral calcium (4 patients), calcitonin (1 patient), and alendronate (2

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patients)**.** Among the laboratory parameters, the neutrophil count and the calcium, phosphorus, magnesium, alkaline phosphatase, 25-hydroxy vitamin D, and parathyroid hormone levels had no statistically significant effects on BMD **(**Table 1**).**

Discussion

Osteoporosis is an important comorbidity in patients with SCN, but the pathogenesis of bone mineral loss remains unclear [19,20].

Elhasid et al. suggested that low levels of OPG (which plays a role in regulating osteoclastogenesis) and a high RANKL/OPG ratio are involved in the pathogenesis of osteoporosis [21]. Furthermore, studies have suggested that long-term activation of cytokines such as IL-1β and TNF-α contributes to the pathogenesis of bone resorption by inducing bone turnover [22,23]. The majority of patients in our study (80.3%) had genetic mutations in *HAX1* (60.5%) or *ELANE* (19.7%). The *HAX1* gene encodes a multifunctional regulatory protein that is widely expressed across various cell types and that interacts closely with multiple cellular proteins. However, the pathogenesis of bone metabolism disorders associated with mutations in the *HAX1* gene has not yet been fully defined [24,25]. Mutations in the *ELANE* gene are cyclic, and the SCN, which is limited to myeloid progenitor cells, has been defined. In the review of patients with *ELANE* gene mutations, 467 had SCN, and 90 had cyclic neutropenia. Xiao et al. [26] demonstrated that osteoporosis was a clinical finding in 9 patients with SCN due to *ELANE* mutation. However, no information has been provided regarding its effect on pathogenesis. When we examined the effect of the underlying genetic mutation on osteopenia/osteoporosis/low BMD, no statistically significant effect of *HAX1* and *ELANE* gene mutations, which constitute the majority of our patients in our study, on BMD was observed.

Radiological evidence of osteoporosis in 12 of 32 patients with SCN (38%) was first reported in 1993 [27]. Yakisan et al. [14] reported evidence of osteopenia/osteoporosis in 15 out of 30 patients with SCN. The Severe

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Chronic Neutropenia International Registry (SCNIR) reported osteoporosis/osteopenia in 19 of 197 (9.6%) SCN patients treated with filgrastim compared with 17% of neutropenic patients (93 congenital, 13 cyclic, 21 idiopathic) in a separate evaluation of the same registry. Most cases are asymptomatic and are based on abnormal bone density measurements [28,29].

DiMeglio et al. [30] reported on BMD reports collected through the SCNIR in 128 patients, including 40 with SCN, 28 with cyclic neutropenia, 3 with autoimmune neutropenia, and 57 with idiopathic neutropenia. During the BMD evaluation period, patients had been receiving G-CSF for a mean duration of 8.8 years (range: 0.1–19.9 years). Among the children, 17.5% had low BMD with respect to age (Z-score < –2.0). In adults, the mean BMD T score was –1.1 ± 1.4, with 46% meeting the T-score criteria for osteopenia (≤ –1.0) and 9% meeting the criteria for osteoporosis (< –2.5). In a report by Bonilla et al. [31], osteopenia/osteoporosis was documented in 15 (28%) of 54 neutropenic patients (congenital n = 44, cyclic n = 10) who received G-CSF treatment for 4–6 years. In a study by Borzutzky et al., low bone BMC values were reported in six of nine patients diagnosed with SCN and in two of nine children with congenital neutropenia of various etiologies (three with cyclic neutropenia, two with Shwachman–Diamond syndrome, two with SCN, and two with idiopathic chronic neutropenia) in a report by Fewtrell et al. [32,33]. Our study revealed low BMD in 30 of 76 patients (39.5%) on the basis of the Z-score. The mean BMD score in patients with low mineral density was 0.63±0.20, whereas the Z-score on DXA was -2.88±0.90. Osteoporosis was detected in 11 adult patients (36.6%). In our study, some children were not diagnosed with osteoporosis despite having low BMD due to the absence of pathological fractures. The observed low BMD was considered a clinically relevant condition, and dietary changes, calcium, and vitamin D supplementation were recommended for the children. Bisphosphonate therapy was not considered due to the absence of pathological fractures.

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Papadaki et al. [34] reported osteoporosis in 7 (15.6%) and osteopenia in 20 (44.4%) of 45 adult patients diagnosed with chronic idiopathic neutropenia who were not receiving G-CSF. These authors reported that BMD values were significantly lower in neutropenic patients than in control patients and that femoral BMD values decreased in correlation with the severity of neutropenia. In our study, the mean absolute neutrophil count in the low BMD group was lower than that in the normal BMD group, but no statistically significant difference was found between the two groups.

G-CSF treatment effectively reduces the frequency and severity of severe and fatal bacterial and fungal infections in patients with severe neutropenia. However, G-CSF also has adverse effects. According to the SCNIR, G-CSF not only increases the risk of progression to myelodysplastic syndrome and AML but is also associated with complications such as headache, musculoskeletal symptoms, hematuria, injection site reactions, rashes, bone pain, splenomegaly, thrombocytopenia, and osteoporosis/low BMD [28,35,36]. Other theoretical reasons why congenital neutropenia or G-CSF treatment might lead to osteopenia/osteoporosis/low BMD include increased production of phagocytic mononuclear cells and circulating macrophages due to the underlying mutation or G-CSF treatment [13,37] , G-CSF-treated mononuclear cells have increased osteoclastogenic potential compared with untreated cells [38], G-CSF-stimulated neutrophils produce cytokines that can induce bone resorption [39], and G-CSF-treated mice exhibit a significant increase in osteoclast numbers [19]. Given the rarity of SCN, limited data are available on the relationship between BMD Z-scores and disease characteristics, such as neutropenia genotypes and the duration of G-CSF treatment [32]. In many studies, no correlation was found between the dose or duration of G-CSF use and the development of osteopenia or osteoporosis in patients receiving G-CSF due to neutropenia [13,28,29,32,33,40]. In our study, the median G-CSF dose was similar between the groups with normal and low BMD, and there was no statistically significant difference in BMD.

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Although the duration of G-CSF treatment was longer in the group with low BMD than in the group with normal BMD, it had no statistically significant effect on BMD.

In the study conducted by Yakisan et al. [14], two out of 30 patients with SCN exhibited pathological fractures. In the case report by Simon et al. [41], a patient with SCN developed a vertebral fracture due to osteoporosis in the third month of G-CSF treatment. Similarly, in the case report by Bishop et al. [42], a patient with SCN experienced a fracture during the third year of G-CSF treatment. In our study, 19 of the 30 patients with osteoporosis had no history of pathological fractures. Decreased bone mass (osteopenia/osteoporosis/low BMD) has been reported in SCN patients before starting G-CSF [14,31]. In our study, no patients were found to have low BMD before G-CSF. This may be because most patients were asymptomatic and had not undergone DXA evaluation prior to treatment.

Our study also revealed that patient sex, age, age at diagnosis, follow-up duration, consanguinity, and BMI had no statistically significant effect on BMD. The effects of laboratory findings, such as the neutrophil count and the serum levels of urea, creatine, AST, ALT, calcium, phosphorus, magnesium, alkaline phosphatase, 25-hydroxy vitamin D, and parathyroid hormone, on BMD were not statistically significant. Although some parameters in Table 2 showed numerical differences between groups, these did not reach statistical significance. Given the rarity of the disease and the limited sample size, the study may have been underpowered to detect modest differences. Accordingly, these observations should be interpreted cautiously and considered hypothesis-generating.

In light of these considerations, our study demonstrated a high prevalence of low BMD in patients with SCN, similar to the findings of previous studies. Our study is one of the most comprehensive studies evaluating BMD in patients with SCN with HAX1 and ELANE deficiency. The study's limitations include the inability to contain the desired number of patients due to the

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rarity of the disease and the lower prevalence of ELANE deficiency in our cohort.

In our study, we were unable to perform bone density measurements before initiating G-CSF treatment; therefore, it was not possible to definitively assess the osteopenia-inducing effects of the disease or treatment. In the discussion, we argued that low BMD is a multifactorial process resulting from the complex interplay between disease-specific mechanisms such as chronic inflammation, immune dysregulation, and genetic predispositions, and the potential effects of G-CSF treatment. Therefore, international monitoring protocols are needed to standardize and prospectively assess bone health from the time of diagnosis in patients with SCN. Future studies, including cohort designs examining early pro-inflammatory cytokine profiles, translational studies investigating the cellular and molecular effects of G-CSF on bone cells, and functional consequences of genetic mutations on bone-myeloid cell interactions, will contribute to a better understanding of the pathophysiology of low BMD in SCN.

Several limitations should be acknowledged. The retrospective design of this study limits causal inference, and the multicenter setting may have introduced heterogeneity in clinical practice and BMD assessment. In addition, the rarity of SCN resulted in a relatively small sample size, potentially limiting statistical power and subgroup analyses. Despite these limitations, the study provides valuable multicenter data highlighting bone health as an important yet underrecognized aspect of SCN and underscores the importance of routine bone health assessment in clinical management.

Conclusion

The pathophysiology of osteopenia/osteoporosis/low BMD in patients with SCN remains unclear. Since clinical symptoms of osteopenia/osteoporosis/low BMD, such as fractures, are infrequent in patients with SCN, the significance of changes in BMD and a possible relationship with long-term G-CSF are still unclear. Therefore, the true

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incidence remains unknown. Some patients with SCN present with osteopenia/low BMD even before the initiation of G-CSF therapy. A significant proportion of patients receiving long-term G-CSF have evidence of osteopenia/osteoporosis/low BMD, which is often detected by radiographic examinations or more quantitatively by DXA, and the risk of abnormal bone density tends to increase with G-CSF treatment. However, whether this trend is due to treatment or the underlying bone marrow disorder is unclear. We do not yet have sufficient data to determine whether this adverse effect increases or decreases over time. The fact that some studies revealed osteopenia years later in patients with normal initial assessments reinforces the importance of reevaluating BMD. These findings suggest that the bone mineral status is continuously deteriorating. The issue of osteoporosis/low BMD in the SCN should be addressed prospectively and quantitatively, including bone metabolism parameters before and during G-CSF treatment. We recommend annual monitoring of bone mineral content in patients with SCN.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability

The data that support the study findings are available from the corresponding author upon reasonable request.

Authors' contributions

All the authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by YK, DCA, DYK,

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AM, EKA, HK, CC, NK, KID, AOO, MY, YDO and SSK. The first draft of the manuscript was written by YK and SSK, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

Ethical Approval

Ethical approval was obtained from the Bursa Uludag University Faculty of Medicine Medical Research Ethics Committee for the study (2024–1/25).

Consent to Publish

The participants gave permission to publish their data.

Consent to Participate

Informed consent was obtained from the participants.

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Figure 1: Distribution of patients with SCN according to genetic mutations

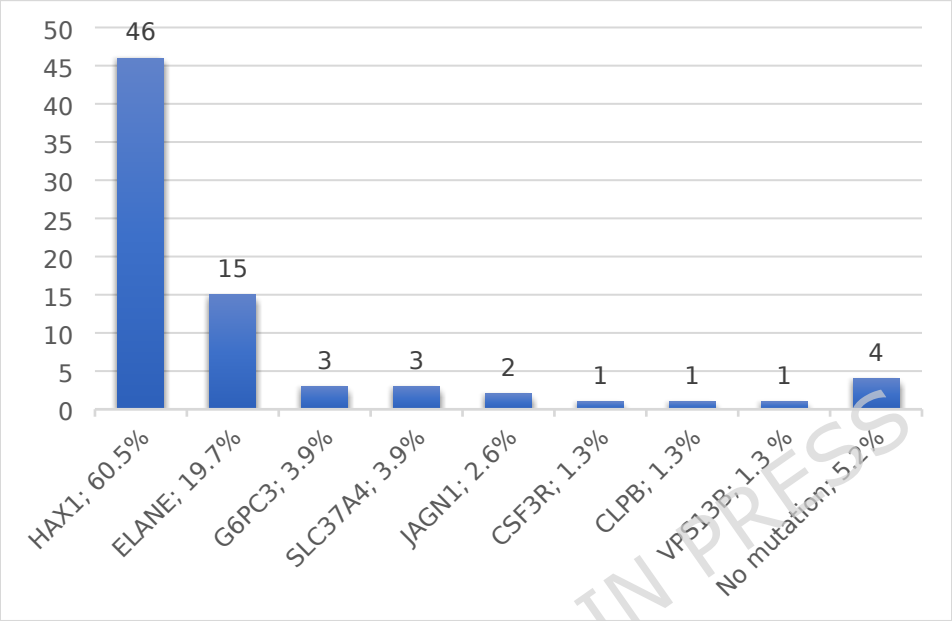


Table 1. Baseline demographic, clinical, and laboratory characteristics of the entire cohort (n = 76)

	All Patients (n=76)
Gender (n) (%)	
Female	39 (51.3)
Male	37 (48.7)
Age (month), mean ± std. deviation	
0-120	67.2 ± 24.7
120.1-216	165.3 ± 26.4
>216	298.3 ± 54.5
Age Groups, month (n) (%)	
0-120	18 (23.7)
120.1-216	21 (27.6)
>216	37 (48.7)
Age of diagnosis (month), mean ± std. deviation	47 ± 59.5

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Disease follow-up period (month) mean ± std. deviation	117.3 ± 83
Genetic mutation (n) (%) *HAX1* *ELANE* *G6PC3* *SLC37A4* *JAGN1* *CSF3R* *CLPB* *VPS13B* No mutation	 46 (60.5) 15 (19.7) 3 (3.9) 3 (3.9) 2 (2.6) 1 (1.3) 1 (1.3) 1 (1.3) 4 (5.2)
Consanguineous marriage n (%)	57 (75)
G-CSF doses (microgram/kg/day) median (min–max)	5 (2-12)
G-CSF treatment duration (months) mean ± std. deviation	115.2 ± 81.6
BMI (kg/m2) median (min–max)	18 (11.7-43)
BMD (g/cm^2) mean ± std. deviation	0.74±0.29
Z-score , mean ± std. deviation	-1.11± 1.74
DEXA (n) (%) Normal BMD Low BMD	 46 (60.5) 30 (39.5)
Absolute neutrophil count (cells/mm^3) median (min–max)	900 (0-6110)
Calcium (8.5-10.2 mg/dl) mean ± std. deviation	9.65 ± 0.35
Phosphorus (2.3-4.7 mg/dl) mean ± std. deviation	4.3± 1.16
Magnesium (1.7-2.2 mg/dl) mean ± std. deviation	2.0± 0.37
Alkaline phosphatase (40-150 u/L) mean ± std. deviation)	147.7± 73.6
25-hydroxyvitamin D (> 20 ng/ml) mean ± std. deviation	22.6± 13.9

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Parathyroid hormone (1-67 pg/ml) mean ± std. deviation	40.1± 35.5

BMD: Bone mineral density

BMI: body mass index, **Min:** minimum, **Max:** minimum

DXA: Dual-energy X-ray absorptiometry

Table 2. Demographic, clinical, and laboratory characteristics of patients according to DXA-derived bone mineral density status

	Normal BMD (n=46)	**Low BMD (n=30)**	**p**
Gender (n) (%)			
Female	21 (53.8)	18 (46.2)	0.22
Male	25 (67.6)	12 (32.4)	
Age (month), mean ± std. deviation	197.1 ± 104.9	221.8 ±105.3	0.32
Age Groups, month (n) (%)			
0-120	11 (14.5)	7 (9.2)	
120.1-216	16 (21.1)	5 (6.6)	0.17
>216	19 (25)	18 (23.7)	
Age of diagnosis (month), mean ± std. deviation	45.3± 61.2	49.5 ± 57.6	0.76
Disease follow-up period (month) mean ± std. deviation	118.9 ± 88.4	133.7±68.7	0.44
Genetic mutation (n) (%)			
HAX1	31 (40.8)	15 (19.7)	0.60
ELANE	9 (11.8)	6 (7.9)	
Others			
G6PC3	1 (1.3)	2 (2.6)	
SLC37A4	0 (0)	3 (3.9)	
JAGN1	2 (2.6)	0 (0)	N/A
CSF3R	1 (1.4)	0 (0)	
CLPB	0 (0)	1 (1.3)	
VPS13B	0 (0)	1 (1.3)	
No mutation	2 (2.6)	2 (2.6)	

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Consanguineous marriage n (%)	36 (47.4)	21 (27.6)	0.41
G-CSF doses (microgram/kg/day) median (min–max)	5 (2-12)	5 (2-10)	0.76
G-CSF treatment duration (months) mean ± std. deviation	107.6 ± 85.4	124.5 ± 72.1	0.38
BMI (kg/m2) median (min–max)	17.9 (11.6-43)	20.3 (12.8-39.5)	0.61
BMD (g/cm^2) mean ± std. deviation	0.81±0.30	0.63±0.20	0.006
Z-score , mean ± std. deviation	-0.25± 1.33	-2.88 ± 0.90	<0.01
Absolute neutrophil count (cells/mm^3) median (min–max)	895 (20-4240)	620 (0-6110)	0.06
Calcium (8.5-10.2 mg/dl) mean ± std. deviation	9.56 ± 0.43	9.75± 0.50	0.08
Phosphorus (2.3-4.7 mg/dl) mean ± std. deviation	4.26± 1.20	4.14 ± 0.86	0.66
Magnesium (1.7-2.2 mg/dl) mean ± std. deviation	2.01± 0.45	2.0± 0.18	0.90
Alkaline phosphatase (40-150 u/L) mean ± std. deviation)	142.9± 70.8	155.5± 78.4	0.48
25-hydroxyvitamin D (> 20 ng/ml) mean ± std. deviation	24.3± 15.5	19.8± 10.5	0.19
Parathyroid hormone (1-67 pg/ml) mean ± std. deviation	39.7± 37.3	40.6± 32.9	0.92

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BMD: Bone mineral density

BMI: body mass index, **Min:** minimum, **Max:** minimum

DXA: Dual-energy X-ray absorptiometry

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	Normal BMD (n=46)	**Severely Low BMD (n=19)**	**p**
Gender (n) (%) Female Male	 21 (67.7) 25 (73.5)	 10 (32.3) 9 (26.5)	0.60
Age (month), mean ± std. deviation	197.1 ± 104.9	226.1 ±110.3	0.77
Age Groups, month (n) (%) 0-120 120.1-216 >216	 11 (23.9) 16 (34.8) 19 (41.3)	 4 (21.1) 4 (21.1) 11 (57.9)	0.43
Age of diagnosis (month), mean ± std. deviation	45.3± 61.2	53.2± 65.2	0.90
Disease follow-up period (month) mean ± std. deviation	118.9 ± 88.4	126.7 ± 63.5	0.40
Genetic mutation (n) (%) *HAX1* *ELANE* Others *G6PC3* *SLC37A4* *JAGN1* *CSF3R* *CLPB* *VPS13B* No mutation	 31 (67.3) 9 (19.5) 1 (2.2) 0 (0) 2 (4.4) 1 (2.2) 0 (0) 0 (0) 2 (4.4)	 9 (47.3) 4 (21) 2 (10.5) 1 (5.3) 0 (0) 0 (0) 1 (5.3) 1 (5.3) 1 (5.3)	0.53 N/A
Consanguineous marriage n (%)	36 (78.3)	12 (63.2)	0.17
G-CSF doses (microgram/kg/day) median (min–max)	5 (2-12)	5 (2-10)	0.36
G-CSF treatment duration (months) mean ± std. deviation	107.6 ± 85.4	127.8 ± 768.9	0.37
BMI (kg/m2) median (min–max)	17.9 (11.6-43)	19.4 (12.8-39.5)	0.85
BMD (g/cm^2) median (min–max)	0.77 (0.38-1.68)	0.64 (0.24-0.84)	0.01

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Z-score , mean ± std. deviation	-0.25± 1.33	-3.17 ± 0.59	<0.01
Absolute neutrophil count (cells/mm^3) median (min–max)	895 (20-4240)	1000 (0-5310)	0.10
Calcium (8.5-10.2 mg/dl) mean ± std. deviation	9.56 ± 0.43	9.73± 0.43	0.45
Phosphorus (2.3-4.7 mg/dl) mean ± std. deviation	4.26± 1.20	4.14 ± 0.83	0.52
Magnesium (1.7-2.2 mg/dl) mean ± std. deviation	2.01± 0.45	1.94± 0.55	0.78
Alkaline phosphatase (40-150 u/L) mean ± std. deviation)	142.9± 70.8	127.4± 53.7	0.72
25-hydroxyvitamin D (> 20 ng/ml) mean ± std. deviation	24.3± 15.5	21.5± 11.8	0.62
Parathyroid hormone (1-67 pg/ml) mean ± std. deviation	39.7± 37.3	45.3± 36.8	0.60

Table 3. Demographic, clinical, and laboratory characteristics of patients with severely low bone mineral density (Z-score ≤ −2.5)

BMD: Bone mineral density

BMI: body mass index, **Min:** minimum, **Max:** minimum

DXA: Dual-energy X-ray absorptiometry

Table 4: Logistic regression analysis was performed for the effects on BMD

Variables	B	p	95% C.I.for EXP(B)	
			Lower	Upper
Mutation	1,024	,294	,412	18,818
Gender	-1,231	,057	,082	1,038
Age (month)	,009	,091	,999	1,020
Consanguineous marriage	-,411	,675	,097	4,519
Disease follow-up period (months)	,002	,790	,985	1,020
G-CSF doses (microgram/kg/day)	-,153	,362	,618	1,192
G-CSF treatment duration (months)	-,002	,852	,981	1,016
ANS	,000	,101	1,000	1,001
BMI (kg/m2)	-,111	,195	,757	1,058

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BMI: Body mass index

G-CSF: granulocyte colony-stimulating factor

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