Chronic non-bacterial osteomyelitis: another disease associated with MEFV gene mutations

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ABSTRACT

Objective. *Chronic non-bacterial osteomyelitis (CNO) is an autoinflammatory bone disease of unknown aetiology. The relationship between CNO and familial Mediterranean fever (FMF) is not clearly documented so far. This cross-sectional study aims to evaluate the clinical and laboratory characteristics of a cohort of CNO patients within the context of its relationship with FMF and MEFV gene mutations.*

Methods. *Demographic and clinical data were extracted from electronic medical records of patients with CNO. The MEFV gene analysis was performed for all patients.*

Results. *A total number of 18 patients with CNO with a median follow-up of 36.50 (13.00-84.00) months were included in the study. Five patients (27.8%) were found to have at least one exon 10 mutations (four with M694V and one with M680I). Four of them (22.2%) had homozygous or compound heterozygous mutations of the MEFV gene. Two patients had a previous diagnosis of FMF and developed CNO while FMF was under control. Patients with MEFV mutations had an earlier onset of CNO, higher acute phase reactants, lower haemoglobin concentrations, and a higher number of bone lesions at disease onset with a persistent course of disease more frequently.*

Conclusion. *Our results demonstrated an increased frequency of MEFV gene mutations in CNO and a more severe disease phenotype of CNO in patients with MEFV gene mutations. Physicians practicing in regions where FMF is prevalent should be aware of this relationship and ask about the symptoms of FMF in detail in patients with CNO. Moreover, FMF should be included in CNO-associated conditions.*

Introduction

Chronic non-bacterial osteomyelitis (CNO) is an autoinflammatory bone disease primarily of children and young adults and it is characterised by sterile inflammatory bone lesions. Its multifocal form with frequent relapses is called chronic recurrent multifocal osteomyelitis (CRMO (1)). The aetiology of the disease is still not clear; however, some susceptibility genes and dysregulation in the innate immune system have been shown (2-4). Persistent or relapsing and remitting focal bone pain, absence of constitutional symptoms, multifocal bone lesions on imaging with typical lesion characteristics on typical locations, and a personal and/or family history of CNO-associated disorders suggest a diagnosis of CNO although it remains a diagnosis of exclusion (5, 6). Supporting its genetic and pathogenic bases, CNO has been frequently associated with a wide spectrum of inflammatory disorders, including but not limited to inflammatory bowel disease (IBD), arthritis, and skin diseases (7).

Familial Mediterranean fever (FMF) is a well-defined hereditary autoinflammatory disease typically presented with recurrent and self-resolving episodes of fever and serositis (8). The responsible gene called the MEditerranean FeVer (MEFV) gene is located on chromosome 16p and encodes a protein known as pyrin. The mutated pyrin causes augmented inflammation with excessive IL-1β and NF-κβ secretion that induces a pro-inflammatory condition both in patients with FMF and in asymptomatic carriers of MEFV mutations (9). This pathogenetic principle of the disease causes persistent subclinical inflammation in a substantial portion of FMF patients, facilitates the coexistence of other inflammatory diseases, and further, the MEFV gene

carriers to be associated with totally different inflammatory diseases other than FMF (9-11). Several diseases such as vasculitis, juvenile idiopathic arthritis (JIA), and IBD have been associated with FMF but CNO is not one of those (10). Moreover, although bone involvement is a widening spectrum of several hereditary autoinflammatory diseases, the relation of CNO with FMF is not clearly reported (12). Beginning from 2010, a few case reports suggested a link between CNO and FMF/MEFV gene mutations and the use of colchicine as a treatment option for CNO (13-16). After persistent and multifocal episodes of CNO have developed in two of our patients with FMF, we aimed to evaluate clinical characteristics, treatment modalities, and response to therapies in a cohort of CNO patients within the context of its relationship with FMF and MEFV gene mutations.

Materials and methods

Patients with a diagnosis of CNO followed-up at the Department of Paediatric Rheumatology of Ankara University School of Medicine were included in the study. Demographic and clinical findings, results of blood tests, bone biopsies, bone marrow aspirations, and microbiological cultures, radiological findings, and treatment modalities were recorded from the electronic medical records of patients. The diagnosis of CNO was based on clinical symptoms and imaging features after the exclusion of CNO-mimicking diseases, such as infection and malignancy. Patients with a possible diagnosis of CNO or with a follow-up less than 6 months were excluded.

Regional magnetic resonance imaging (MRI), whole-body MRI (WBMRI), and bone scintigraphy, if WBMRI was not available, were used to assess bone lesions. Whole-body MRI was performed with 1.5 Tesla MR system (Achieva, Philips Healthcare, Best, The Netherlands). The coronal plane was the principal plane by its extensive coverage and detailed evaluation of the axial skeleton. Sagittal and axial plane images were added when necessary. The WBMRI protocol included pre-contrast sagittal spin-echo T1-weighted (T1W), coronal gradient-echo T1W, coronal short tau inversion recovery (STIR), axial plane diffusion-weighted single-shot echo-planar imaging (b-values of 0 and 1000 s/mm^2). Contrast-enhanced coronal and sagittal plane T1W images with a slice thickness of 3 to 6 mm were obtained in patients with abnormal signal changes in the pre-contrast MR series. Regional MRI protocol was composed of pre-contrast T1W three plane imaging, coronal STIR, axial fat-saturated T2-weighted (T2W), axial diffusion-weighted single-shot echo-planar imaging, and post-contrast three plane imaging with fat saturation. Radiologic images were evaluated by only one paediatric radiologist (SF) experienced in CNO.

To classify patients, the clinical scoring system by Jansson *et al*. was used that had a positive predictive value over 97% for the diagnosis of CNO if the clinical CNO score was ≥39 (5). Treatment responses were evaluated according to the clinical symptoms and imaging findings on MRI. Patients with only one episode during the follow-up were accepted as "nonrecurrent" whereas those with relapses as "recurrent". Patients who did not experience clinical and radiological remission despite therapies for 6 months were classified as "persistent". Solitary lesions on WBMRI were categorised as "unifocal" and multiple lesions as "multifocal". Clinical remission was defined as not having any symptoms for the last 6 months and radiological remission as without any active lesions on WBMRI. Patients with a previous diagnosis of FMF according to Yalçınkaya criteria had already had the MEFV gene analysis before the diagnosis of CNO (17). For others, the MEFV gene analysis was performed. At least six mutations in the MEFV gene including p.M694V, p.M694I, p.M680I, p.V726A, p.E148Q, and p.K695R were analysed by direct sequencing of the PCR-amplified fragments for exon 10 mutations and by PCR-restriction fragment length polymorphism protocol for exon 2 mutations.

The study was approved by the Ethics Committee of Ankara University (#I4-232-20). Signed informed consent was obtained from the patients and their parents.

Statistical analysis

The IBM SPSS Statistics 21.0 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp) was used to perform statistical analysis. Results were described with mean ± standard deviation (SD) or median (minimum-maximum) for continuous variables and with frequency (n) and percentage (%) for categorical variables. The Kolmogorov-Smirnov test was used to examine the data for normal distribution. One-way analysis of variance (ANOVA) was used for homogeneity of the variables, Student's T-test was used for parametric data. The Mann-Whitney U, ANOVA, and Spearman's correlation were used for non-parametric data. The correlation was considered meaningful if the correlation coefficient (r_s) was >0.40. The statistical significance level was accepted as a p-value <0.05.

Results

Patient characteristics, laboratory findings, and treatment modalities

A total number of 18 patients with CNO were included in the study. The demographic, clinical, and laboratory characteristics of the patients with CNO are presented in Table I. All patients had bone pain with a median diagnostic delay time of 11.00 (2.00-48.00) months. One patient had been treated with a diagnosis of JIA three years before the onset of CNO. Two patients had a previous diagnosis of FMF and one of them also had IBD. None had a family history of CNO or CNO-associated diseases whereas two patients had a family history of FMF.

Four patients had persisting anaemia compatible with anaemia of chronic disease. Fourteen patients had increased C-reactive protein (CRP) and 10 had increased erythrocyte sedimentation rate (ESR) at the time of diagnosis. One patient received antibiotics because of the suspected bacterial osteomyelitis before the diagnosis of CNO. Twelve patients had bone biopsy/bone marrow aspiration because most of

Table I. Demographic, clinical and laboratory characteristics of patients with chronic non-bacterial osteomyelitis*.

Characteristics	Patients (n=18)
Female:Male	5 (27.8%):13 (72.2%)
Age at disease onset, years	8.50 (1.50-16.00)
Age at diagnosis, years	10.50 (3.50-16.17)
Symptoms	
Bone pain	18 (100%)
Point tenderness	10 (55.6%)
Arthritis	6 (33.3%)
Sacroiliac joint pain	5 (27.8%)
Local warmth	3 (16.7%)
Fever	3 (16.7%)
Acne	1 (5.6%)
Bone biopsy and/or bone marrow aspiration	12 (66.7%)
Normal histopathologic findings	6 (50%)
Presence of inflammation	5 (41.6%)
Presence of fibrosis	1 (8.3%)
Laboratory findings at disease onset	
White blood cell count, $*10^9$/L	10,107 ± 2,326
Neutrophil count, $*10^9$/L	6,216 ± 2,082
Platelet count, $*10^9$/L	433,388 ± 168,480
Haemoglobin concentration, gr/dL	12.32 ± 1.68
C-reactive protein, mg/L	27.93 ± 31.63
Erythrocyte sedimentation rate, mm/h	18.61 ± 11.55
ANA positivity	4 (22.2 %)
HLA-B27 positivity	1 (5.6 %)
MRI findings at disease onset	
Increased signal intensity on STIR	18 (100%)
Lesions on long bones	10 (55.6%)
Metaphyseal	9 (50%)
Metaphysiodiaphyseal	1 (5.6%)
Vertebral lesions	10 (55.6%)
Vertebral compression	2 (11.2 %)
Synovial thickening and joint effusion	8 (44.4%)
Sacroiliitis	8 (44.4%)
Clavicular lesions	4 (22.2%)
Radiographic sclerotic lesions	7 (38.9%)
Radiographic osteolytic lesions	4 (22.2%)
Clinical CNO score	42.50 (26.00-54.00)
Treatments for CNO	
Naproxen	18 (100%)
TNFi	7 (38.9%)
Etanercept	3 (16.7%)
Adalimumab	4 (22.2%)
Corticosteroids	5 (27.8%)
Colchicine	5 (27.8%)
Methotrexate	2 (11.2%)
Biphosphonates	2 (11.2%)

ANA: anti-nuclear antibody; CNO: chronic non-bacterial osteomyelitis; FMF: familial Mediterranean fever; HLA: human leukocyte antigen; MRI: magnetic resonance imaging; STIR: short tau version recovery; TNFi: tumour necrosis factor-α inhibitor.
*mean ± SD, median (min-max), or n (%).

them were referred to our clinic after the exclusion of malignancy. Imaging findings of all patients reported from our centre were compatible with CNO and the majority of patients (88.9%) had a clinical CNO score over 39 at disease onset.

All patients had multifocal bone lesions on MRI with a median lesion number of 6.50 (3.00–17.00) and 15 patients (83.3%) had symmetric lesions. Overall, 17 patients (94.4%) had lower extremity involvement and 3 patients (16.7%) had upper extremity involvement. None of the lesions on long bones were isolated diaphyseal or epiphyseal lesions. One patient with sacroiliitis had HLA-B27 positivity. No significant correlation between the age of disease onset, laboratory parameters, number of bone lesions, and clinical CNO scores was found (r_s <0.40; p>0.05).

The median follow-up was 36.50 (13.00-84.00) months. All patients received naproxen at 15-20 mg/kg/day twice daily. Oral corticosteroids up to 6 weeks were used in five patients as a transitional drug to other agents. A total of nine patients (50%) received bisphosphonate, methotrexate, tumour necrosis factor-α inhibitor (TNFi), or a combination of these. Clinical remission was achieved in half of the study population (n:9) only with naproxen. The disease course was persistent in 9 (50%) and recurrent in 6 patients (33.3%).

MEFV gene analysis and comparison of patients with and without MEFV gene mutations

Five patients out of 18 CNO patients (27.8%) were found to have MEFV mutations including four (22.2%) with homozygous or compound heterozygous mutations. Two patients developed CNO 2-4 years after the diagnosis of FMF while FMF was under control on a regular dose of colchicine at 1 mg/m^2/day. Patient 1 was concurrently diagnosed with FMF and IBD at the age of 18 months. Although he did not have frequent FMF attacks in the first year after the diagnosis, he experienced chronic disease anaemia and poor growth with continuous subclinical inflammation and anakinra was used for 2 years in addition to other therapies. His symptoms of CNO was started 6 months after the cessation of anakinra. Patient 2 was diagnosed with CNO after complaining of persistent back and leg pain two years after FMF onset. Both patients' symptoms relieved after the first month of TNFi. Patient 1 experienced clinical and radiological remission at the first year of TNFi. The other three patients found to have MEFV gene mutations did not have any typical clinical symptoms of FMF. Patient 3 was treated with TNFi for 18 months and his treatment was ceased after having both clinical and radiological remission. However, his

Table II. Characteristics of patients with chronic nonbacterial osteomyelitis and MEFV gene mutations.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	Male	Male	Male	Male	Male
Age at CNO onset, years	4.5	5	10	1.5	2
Disease course of CNO	Persistent multifocal	Persistent multifocal	Persistent multifocal	Persistent multifocal	Persistent multifocal
Age at FMF onset, years	1.5	3	-	-	-
Clinical findings of FMF	Recurrent fever, abdominal pain	Recurrent fever, abdominal pain	-	-	-
MEFV gene mutation	M694V/M694V	M694V/V726A	M694V/V726A	M694V/-	M680I/V726A
Family history of FMF	+	-	-	-	-
Inflammatory diseases other than CNO	FMF, IBD	FMF	Acne fulminans	-	-
Location of inflammatory bone lesions	Lower extremity, pelvis, occipital condyles, vertebrae	Lower extremity, pelvis, vertebrae	Lower extremity, pelvis, vertebrae, clavicula	Lower extremity, upper extremity, vertebrae	Pelvis, vertebrae
Fever at CNO onset	-	-	+	+	-
Increased acute phase reactants at CNO onset	+	+	+	+	+
Sacroiliitis	-	+	+	-	+
Treatment before CNO diagnosis	Colchicine, oral corticosteroid, mesalazine, azathioprine, anakinra	Colchicine	-	-	-
Treatment after CNO diagnosis	Colchicine, naproxen, TNFi	Colchicine, naproxen, TNFi	Naproxen, oral corticosteroid, TNFi, colchicine	Naproxen, biphosphonate, TNFi, colchicine	Naproxen, biphosphonate, TNFi, colchicine

CNO: chronic non-bacterial osteomyelitis; FMF: familial Mediterranean fever; IBD: inflammatory bowel disease; MEFV: Mediterranean FeVer; TNFi: tumour necrosis factor-α inhibitor.

symptoms recurred after one month. After his MEFV gene mutation was documented, both TNFi and colchicine were initiated concurrently, and he was followed with clinical remission for the last six months. After combined initiation of colchicine and TNFi for patients 4 and 5, they had been followed with clinical remission. Patient and disease characteristics of CNO patients with MEFV gene mutations are shown in Table II.

Overall, the total allelic frequency of MEFV genes in the study population was 25%. The frequency of the M694V allele was 13.9%. Patients with MEFV mutations had a significantly earlier onset of CNO, lower haemoglobin concentrations, higher CRP and ESR levels, and a higher number of bone lesions at disease onset than the patients without a mutation. Although there was 2.4 times increased risk of sacroiliitis in patients with MEFV mutations, the statistical difference was not significant (p=0.410; OR=2.400; 95% confidence interval (CI)=0.210–19.784). All patients with MEFV mutations had a persistent disease course that was significantly more prevalent than it was observed in patients without a mutation (p=0.009; OR=0.308; 95% CI=0.136–0.695). Tumour necrosis factor-α inhibitors were used significantly more common in patients with a mutation (p=0.001; OR=0.154; 95% CI=0.043–0.550). A comparison of disease characteristics of patients with and without MEFV gene mutations is presented in Table III.

Discussion

In this study, we evaluated the frequency of MEFV gene mutations in a cohort of patients with CNO and found that 28% of the patients had at least one and 22% had two mutations. These frequencies were higher than the prevalence of FMF and the carrier rates of MEFV gene mutations reported from Turkey (18, 19). Moreover, patients with MEFV mutations had an earlier onset of CNO with higher acute phase reactants and a higher number of bone lesions and they showed a persistent course more frequently suggesting a more severe disease phenotype of CNO.

Familial Mediterranean fever is thought of as the best understood of the hereditary periodic fever syndromes; however, it is a heterogeneous disease representing different clinical manifestations and associated with several other inflammatory conditions (10). Although the disease has been increasingly reported from all over the world, it is still most prevalent in the Mediterranean and Middle Eastern regions, particularly in Turks, Armenians, Jews, and Arabs (20). There are many genetic and epigenetic factors affect-

Table III. Comparison of disease characteristics of CNO in patients with and without MEFV gene mutations*.

	Patients with MEFV gene mutations (n=5)	Patients without MEFV gene mutations (n=13)	*p*-value
Age at disease onset, years	4.60 ± 3.38	9.57 ± 3.72	**0.020**
Laboratory findings at disease onset			
White blood cell count, $*10^9$/L	9,858 ± 1,347	10,203 ± 2,650	0.787
Platelet count, $*10^9$/L	480,600 ± 196,849	415,230 ± 161,278	0.478
Haemoglobin concentration, gr/dL	10.92 ± 1.62	12.86 ± 1.42	**0.024**
Mean corpuscular volume, fL	70.56 ± 7.05	78.26 ± 4.78	**0.018**
Red-cell distribution width, %	14.54 ± 2.09	13.87 ± 1.13	0.403
C-reactive protein, mg/L	65.74 ± 38.30	13.40 ± 10.21	**0.000**
Erythrocyte sedimentation rate, mm/h	27.80 ± 14.49	15.07 ± 8.39	**0.032**
Number of bone lesions on MRI at disease onset	11.60 ± 4.50	6.53 ± 4.05	**0.035**
Sacroiliitis on MRI	3 (60%)	5 (38.5%)	0.410
Clinical CNO score	43.80 ± 9.44	43.76 ± 7.79	0.994
The use of TNFi	5 (100%)	2 (15.3%)	**0.001**
Persistent course of the disease	5 (100%)	4 (30.8%)	**0.009**

CNO: chronic nonbacterial osteomyelitis; FMF: familial Mediterranean fever; MEFV: Mediterranean FeVer; MRI: magnetic resonance imaging; TNFi: tumour necrosis factor-α inhibitor
* mean ± standard deviation (SD) or n (%).

ing phenotypic presentations. Some patients with FMF, majority carrying M694V mutations, had an earlier onset of disease, a relatively more severe disease phenotype, persistent subclinical inflammation, and a higher association with other inflammatory diseases (9, 21, 22). Likewise, asymptomatic MEFV carriers with high-risk mutations showed an increase in acute phase reactants suggesting a proinflammatory condition among these people (9). The proinflammatory state both in FMF patients and asymptomatic MEFV carriers was related to the expression of other inflammatory diseases. Vasculitis, JIA, spondyloarthritis, and IBD were the most commonly associated diseases among paediatric patients with FMF (10, 23-25). In the current study, two patients already had the diagnosis of FMF before the manifestations of CNO. Three asymptomatic patients were additionally found to have MEFV mutations. Overall, this frequency was higher than the prevalence and the carrier rates of MEFV mutations in population-based field studies from Turkey (0.1% and 14.8%, respectively (18, 19)). All patients with MEFV mutations were found to have exon 10 mutations (four with M694V and one with M680I) that were known to be associated with a severe phenotype of FMF (26). Moreover, the M694V allele frequency (13.9%) and total allelic frequency (25%) in our CNO cohort were significantly higher in the study population compared to the Turkish population (4% and 8.4%, respectively (19)).

Although the actual incidence and prevalence of CNO are still not known, the increasing availability and tendency to perform MRI and the heightened state of awareness about the disease lead more patients to be diagnosed. Overall, the prevalence of CNO has been estimated at between 1/160,000 to 1/2,000,000 (27). Its pathogenesis remains unclear; however, studies show that it is an autoinflammatory disease like FMF. Mainly, IL-1β seems to drive the disease (28). Additionally, IL-1, IL-6, and TNF-α were found increased whereas a decreased IL-10 secretion was identified (29). Autoantibodies generally not detected or found in low titers with a nonspecific profile and the lack of antigen-specific T cells endorse this hypothesis (29). Evidence supports that CNO might have a genetic basis especially after the identification of familial clustering cases with CNO and CNO-associated diseases. Whole exome sequencing revealed FBLIM1 and FGR gene mutations in a small portion of CNO patients as susceptibility genes (4, 30). Additionally, two monogenic diseases are defined as syndromic CNOs. Majeed syndrome caused by homozygous LPIN2 mutations presents with early-onset CNO associated with congenital dyserythropoietic anaemia (31). Secondly, the deficiency of IL-1 receptor antagonist (DIRA) has a neonatal onset of CNO, periostitis, and pustulosis (32).

In 2010, the first case report of a CRMO patient without typical FMF symptoms but carrying MEFV gene mutation was released from Japan and the patient benefitted from colchicine treatment (13). Afterward, four other cases with concurrent CNO and FMF originated from Arabic, Jewish, and Turkish descents were reported (14-16). Although a study from Iran claimed that there was no significant relationship between MEFV genes and CNO, recently, an increased frequency of FMF among CNO patients was reported (33, 34). Cicek *et al*. reported FMF as the most common concomitant disease with a frequency of 34.8% in patients with CNO. They did not find any differences in clinical and laboratory findings of CNO patients with or without concomitant diseases (34). On the other hand, the comparison of the disease characteristics of CNO between patients with and without FMF was not defined in the study. In our study, patients with MEFV gene mutations had a significantly earlier onset of CNO with higher acute phase reactants, lower haemoglobin concentrations, and a higher number of bone lesions. Moreover, they had a persistent disease course more commonly than patients without MEFV mutations that caused to add biologics to treatments of all patients with mutations. Our results demonstrating an increased frequency of MEFV gene mutations in CNO and a more severe disease phenotype of CNO in patients with MEFV gene mutations support the possible relationship between CNO and MEFV gene mutations. We think that because of the infrequency of CNO and the limited awareness about the disease, this relationship might not have been realised until recently. Due to the presence of few paediatric rheumatologists in our country, probably, the patients with CNO had been treated with other diagnoses. Likewise, we suggest that there should be more cases with CNO

in regions where FMF is prevalent as it can be in our country. We believe that particularly in these regions, the symptoms of FMF should be questioned in detail in patients with CNO. In the presence of FMF symptoms or early-onset CNO with a severe disease phenotype, MEFV gene mutations should be performed.

Limitations of the current study are the limited number of patients with CNO restricted to a group of Turkish children from a single centre and their relatively short follow-up. Multicentric international studies are needed to confirm our findings. Moreover, the additional analysis of other candidate genes associated with CNO might have been beneficial. Despite these limitations, the current study demonstrated an increased frequency of MEFV gene mutations among patients with CNO and compared the differences between CNO patients with and without MEFV gene mutations for the first time.

In conclusion, CNO appears as a disease associated with MEFV gene mutations. Moreover, the presence of MEFV mutations seems to affect the disease phenotype of CNO. Physicians practicing in regions where FMF is prevalent should be aware that patients with FMF or asymptomatic MEFV carriers may present with CNO and these patients may have a severe disease phenotype of CNO. Lastly, we suggest that FMF should be included in CNO-associated conditions.

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