Pediatric Rheumatology

RESEARCH ARTICLE

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Monocyte STAT1 phosphorylation and treatment response of JAK inhibitors in chronic nonbacterial osteomyelitis

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Abstract

Background Chronic nonbacterial osteomyelitis (CNO) is a rare autoinflammatory disease of unknown cause, predominantly affecting teens and young adults. The early diagnosis and management are challenging due to the lack of reliable diagnostic markers and the occasional intractable cases despite conventional anti-inflammatory treatments. Janus kinase (JAK) inhibitors have recently shown potential utility; however, reports on their use for pediatric patients with CNO remain limited, and no established biomarkers exist to monitor disease activity. We aimed to investigate the pathophysiology of CNO and explore the rapid testing methods for accurate diagnosis and also assessing the disease activity.

Methods We assessed intracellular phosphorylation of signal transducer and activator of transcription 1 (pSTAT1) in peripheral blood monocytes or T cells following interferon-gamma (IFNγ) stimulation, using flow cytometry in 9 patients under 15 years old with CNO. The pSTAT1 expression levels were compared with those in patients with STAT1-gain of function (STAT1-GOF) mutations (n = 5), other autoinflammatory diseases (n = 7), and healthy controls. Clinical and immunological data were monitored in 4 patients with intractable CNO treated with adjunctive JAK inhibitors, focusing on scoring scales, imaging data, lymphocyte subsets, cytokine profiles, and pSTAT1 levels.

Results Monocyte pSTAT1 expression after IFNγ stimulation was elevated at diagnosis or during active CNO, similar to levels observed in STAT1-GOF cases. The pSTAT1 levels in CNO patients were significantly higher than those in other autoinflammatory diseases ($p = 0.024$) or controls ($p < 0.001$). Notably, pSTAT1 levels in CNO monocytes fluctuated with disease activity, decreasing in 5 patients during clinical remission following conventional therapies ($p = 0.016$). In four intractable cases, pSTAT1 levels remained high despite conventional treatments but significantly decreased after initiating JAK inhibitors ($p = 0.036$). This reduction correlated with improved patient pain visual analog scale ($p = 0.008$), CNO clinical disease activity score ($p = 0.029$), and better bone and joint imaging, though cytokine levels remained unchanged.

Conclusions The monocyte pSTAT1 levels after IFNγ stimulation reflect the activity of CNO, indicating the diagnostic utility as well as the monitoring effect of disease control. Adjunctive JAK inhibitors successfully controlled inflammation in treatment-resistant cases. Rapid pSTAT1 testing may help reduce osteo-articular complications, although the long-term adverse effects and resistance should be further investigated.

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Keywords Autoinflammation, CNO, STAT1 phosphorylation, JAK inhibitors

Introduction

Chronic nonbacterial osteomyelitis (CNO), also known as chronic recurrent multifocal osteomyelitis (CRMO), is a rare autoinflammatory disease mostly occurring in childhood [1]. Bone pain is the most common initial symptom in afebrile children with CNO, typically occurring without skin lesions or rash. The skin lesions appear in some cases as a diagnostic clue, but the disease often follows a waxing and waning course of osteoarticular and a minority of dermatological manifestations. The full-brown condition characterized by chronic and relapsing inflammation of the synovium, skin, and axial or multiple bones leads to the final diagnosis of synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO) syndrome in adult patients [2]. The pathophysiology of CNO or SAPHO syndrome has not been fully understood, although interferon-gamma (IFNγ) signaling pathway plays a pivotal role in regulating osteoclast differentiation and bone resorption [3]. Previous research has evaluated the role of abnormalities in IFNγ signaling including in patients with STAT1 gain-of-function (STAT1-GOF) for bone complications [4]. This bone and joint disease was not common in patients with STAT1-GOF [5, 6] but suggested that IFNγ and its reactivity of immunocytes are essential for bone homeostasis.

Patients with CNO are initially treated with nonsteroidal anti-inflammatory drugs (NSAIDs). For cases resistant to NSAIDs, additional therapies such as conventional disease-modifying antirheumatic drugs (cDMARDs), bisphosphonates, and biological DMARDs including tumor necrosis factor (TNF) inhibitors are used to control disease activity following consensus treatment plans [7]. Because of varied presentation and severity of the disease, more information is needed for the pathophysiology and treatment for intractable cases. Janus kinase (JAK) inhibitors play a crucial role in the regulation of immune response and inflammation [8]. There is growing evidence for the therapeutic utility of JAK inhibitors in patients with arthritis or inflammatory bowel diseases [9]. Reports of successful cases involving the use of JAK inhibitors for the treatment of CNO and SAPHO syndrome also have been increasing in recent years [1, 10–17]. However, no specific biomarkers for the disease activity of CNO have been identified.

In the process searching for diagnostic markers, we found that STAT1 phosphorylation (pSTAT1) in IFNγ-stimulated monocytes was enhanced in patients at presentation of CNO. During the treatment course, the monocyte pSTAT1 levels came to decrease in all responders to conventional anti-inflammatory drugs. When additional JAK inhibitors were given to 4 treatment-resistant cases, all of them obtained clinical resolution with reduced pSTAT1 levels as expected from the functional role of STAT1 in the JAK signaling pathway [18, 19]. We present herewith case-series of intractable CNO and discuss about the clinical utility of rapid pSTAT1 testing for the diagnosis and assessment of treatment response.

Methods

Subjects

This study included patients aged < 15 years who received the diagnosis of CNO or transferred for disease control from May 2019 to May 2024 at Kyushu University Hospital, Fukuoka, Japan. All 9 patients received the diagnosis of CNO based on imaging and biopsy findings and some also presented with inflammatory bowel disease or skin manifestations of palmoplantar pustulosis, psoriasis vulgaris, and acne resembling SAPHO syndrome during follow-up [20, 21]. Healthy and disease controls were enrolled after the approval of Institutional Review Board (#531–03). All the guardians and patients submitted written informed consent and healthy controls volunteered for this study. The participants were all Japanese, including 9 patients with CNO (median [range] age at the study, 12.6 [5.4–15.2] years old; male/female ratio, 2/7), 3 patients with STAT1-GOF as positive controls for the analysis (age, 9.7 [0.9–10] years old; male/female ratio, 1/2), 7 patients with autoinflammatory diseases (age, 17.8 [7.2–23.1] years old; male/female ratio, 3/4), and 15 healthy controls (age, 30.4 [0.5–54.3] years old; male/female ratio, 16/0). Autoinflammatory diseases other than CNO were systemic juvenile idiopathic arthritis, familial Mediterranean fever, and cryopyrin-associated periodic syndrome. Clinical and laboratory profiles of all participants at the time of the pSTAT1 analysis are shown in Supplementary Table 1. All patients had active disease at the time of study, prior to the initial treatment or during the refractory and/or relapsing courses. During the study period, 5 patients with CNO (P5-P9) responded to NSAIDs alone or additional salazosulfapyridine. Four others did not respond to the combination with cDMARDs and biologics including TNF inhibitors and/or interleukin (IL)-6 receptor antibodies. Bisphosphonates were not used for treatment due to safety concerns in children with osteogenesis imperfecta and osteoporosis as detailed by Marini et al. in 2009 [22] and their

off-label use in Japan. Refractory patients then received JAK inhibitors (baricitinib, upadacitinib, or tofacitinib) after approval by the hospital regulatory committee and consent from guardians and/or patients.

Flow cytometric analyses

Flow cytometry expression was analyzed using an EC800 cell analyzer (Sony Corporation, Tokyo, Japan). Forward and side scatter gates were used to capture the cell populations, including lymphocytes and monocytes. Data were analyzed using the Kaluza software program (Beckman Coulter, Brea, CA, USA). Peripheral blood mononuclear cells were stained with the following antibodies: anti-CD3 antibody (UCHT1, Beckman Coulter, Miami, FL, USA; and UCHT1, BioLegend, San Diego, CA, USA), anti-CD4 (13B8.2, Beckman Coulter; and OKT4, BioLegend), anti-CD8 (RPA-T8, BioLegend; and B9.11, Beckman Coulter), anti-CD45RA (ALB11, Beckman Coulter), anti-CD45RO (UCHL1, Beckman Coulter), anti-CD19 (HIB19, BioLegend), anti-CD56 (N901, Beckman Coulter), anti-TCRαβ (IP26, BioLegend), anti-TCRγδ (IMMU510, Beckman Coulter), anti-CD38 (T16, Beckman Coulter), anti-CD138 (MI15, BioLegend), anti-CD20 (2H7, BioLegend), anti-CD27 (1A4CD27, Beckman Coulter), anti-IgD (IA6-2, BioLegend), and anti-CD14 (RMO52, Beckman Coulter).

Peripheral blood samples (100 µL per well) were stimulated with recombinant human IFNγ (500 unit/mL; Roche Diagnostics GmbH, Mannheim, Germany) at 37 °C for 15 min, as previously reported [23]. Cells were washed, fixed, permeabilized (PerFix EXPOSE; Beckman Coulter), and were stained with anti-Stat1 antibody (pY701; 4a, BD Biosciences, Franklin Lakes, NJ, USA), anti-CD3 antibody (UCHT1), anti-CD14 antibody (RMO52, Beckman Coulter), and anti-mouse IgG2a isotype antibody (7T4-1F5, Beckman Coulter).

Cytokine assays

Serum concentrations of IL-12p70, TNF, IL-10, IL-1β, IL-6, and IL-8 were measured using a BD™ Cytometric Bead Array Human Inflammatory Kit (BD Biosciences). Cytokine levels were analyzed using an EC800 Analyzer. The detection limit was 20 pg/mL.

Functional measures

Treatment efficacy was evaluated using the CNO clinical disease activity score (CDAS) and the Japanese Childhood Health Assessment Questionnaire (CHAQ) score [24, 25]. A CDAS consists of clinical measures such as the patient pain visual analog scale (VAS), patient/parent global assessment VAS, and total number of clinically active CNO lesions [24]. The VAS score is a measurement of the mean pain intensity or self-perceived disease activity over the last week and is scored on a 0- to 10-cm scale with severity [26]. The functional ability component of the Japanese CHAQ questionnaire assessed 36 items across 8 functional areas, producing a total score from 0 to 3, with higher scores denoting poorer functional ability.

Statistical analyses

The Wilcoxon test and *chi*-square test were used to analyze the continuous and categorical variables, respectively, with $p < 0.05$ considered significant. Each analysis was performed using the JMP Pro software program (ver. 17; SAS Institute, Cary, NC, USA).

Results

CNO monocytes but not T cells showed increased pSTAT1 expression after IFNγ stimulation

The pSTAT1 expression was upregulated in $CD14^{+}$monocytes but not $CD3^{+}$T cells after IFNγ stimulation at diagnosis or active phase of CNO as shown in the representative data of Fig. 1. The monocyte pSTAT1 levels at active CNO were similar to those of STAT1-GOF, and higher than those of other autoinflammatory diseases ($p = 0.024$) or controls ($p < 0.001$) (Fig. 2, Supplementary Fig. 1). By contrast, pSTAT1 levels in IFNγ-stimulated T cells of CNO showed similar levels of other autoinflammatory disease or controls, lower than the levels of those of STAT1-GOF as expected [23]. When 5 patients with CNO (P5-P9) responded to NSAIDs alone with or without salazosulfapyridine, increased pSTAT1 levels in monocytes but not T cells after IFNγ-stimulation declined with clinical remission ($p = 0.016$, Fig. 3). These results indicate that IFNγ-stimulated monocyte-limited high pSTAT1 expression is a diagnostic marker of active CNO to differentiate from STAT1-GOF or other inflammatory diseases and healthy controls.

Treatment response of JAK inhibitors in intractable cases

Four patients with CNO resistant to conventional therapy are summarized in Supplementary Table 2.

> Patient 1. A 13-year-old boy had recurred bone fractures in the lower limbs since age 4 years. As part of the evaluation for increased bone fragility, the patient underwent a comprehensive assessment for bone metabolic diseases in collaboration with a pediatric endocrinologist. Laboratory tests revealed no abnormalities in markers associated with metabolic bone disease. Furthermore, there was no evidence of vitamin C deficiency, and the patient did not exhibit any dietary habits that would lead to nutritional defi-

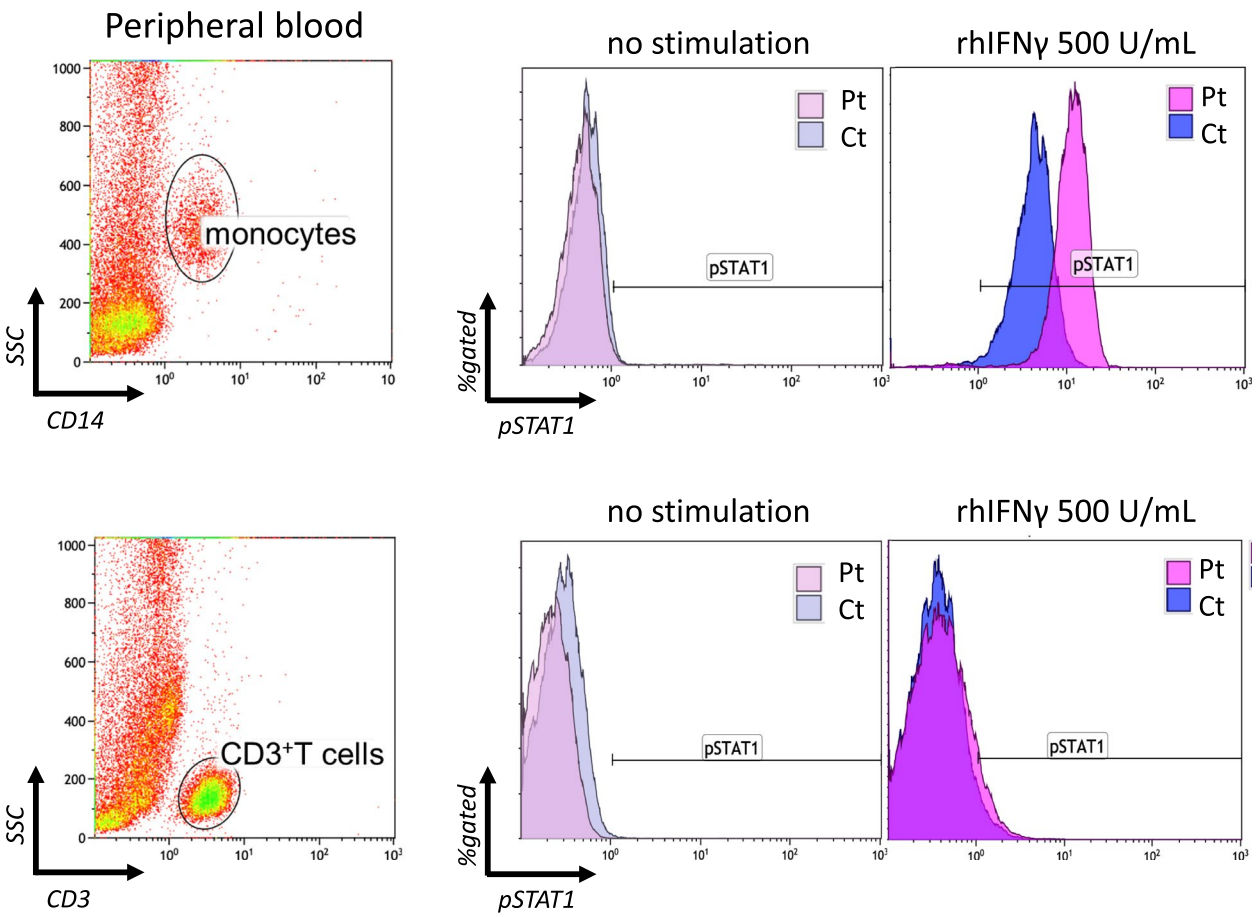

Fig. 1 The expression of phosphorylated STAT1 (pSTAT1) in monocytes (*upper*) or T cells (*lower*) after IFNγ stimulation. The representative data show pSTAT1 expression in circulating CD14-positive or CD3-positive gated cells from a peripheral blood sample of newly diagnosed patients after IFNγ stimulation. Single-parameter histograms with pSTAT1 and %gated show the differences in phosphorylation with and without 500 U/mL of rhIFNγ

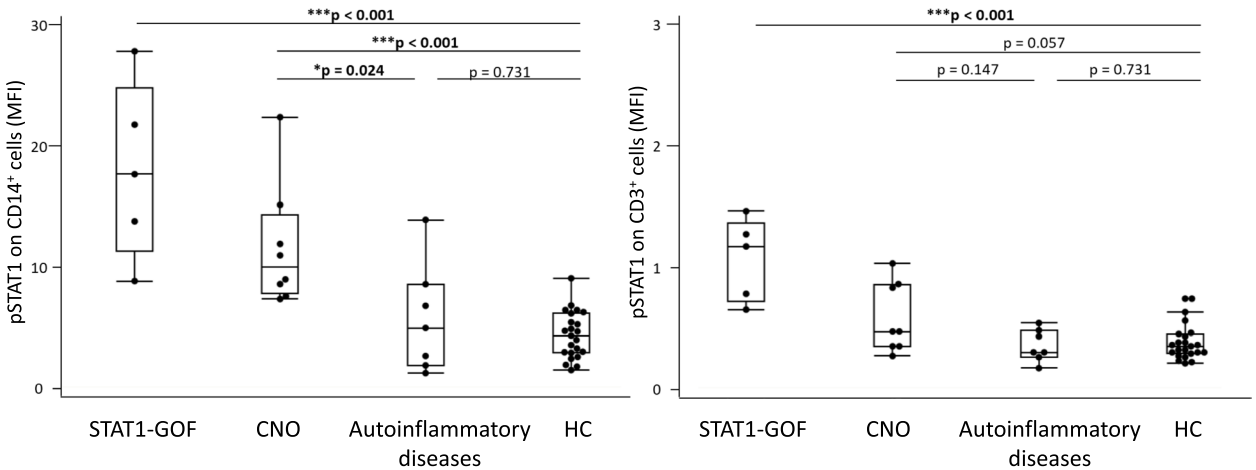

Fig. 2 pSTAT1 expression in STAT1-GOF, CNO, other autoinflammatory diseases, and healthy controls (HC). The pSTAT1 levels in monocytes (*left*) or T cells (*right*) from patients (n = 8[†]) at the time of diagnosis or active phase CNO are compared with those of patients with STAT1-GOF (n = 5, from 3 participants) and autoinflammatory diseases (n = 7) and healthy controls (HC, n = 23). The box-and-whisker plot represents the median with the lower (Q1: 25th %tile) and upper quartiles (Q3: 75th %tile) between minimum (Q1-1.5 × interquartile range [IQR]) and maximum levels (Q3 + 1.5 × IQR). Significant differences are determined by Wilcoxon's signed-rank test. * $p < 0.05$, *** $p < 0.001$, [†]We failed to evaluate P4 at the time of active disease phase or before the start of the JAK inhibitor. MFI: median fluorescence intensity

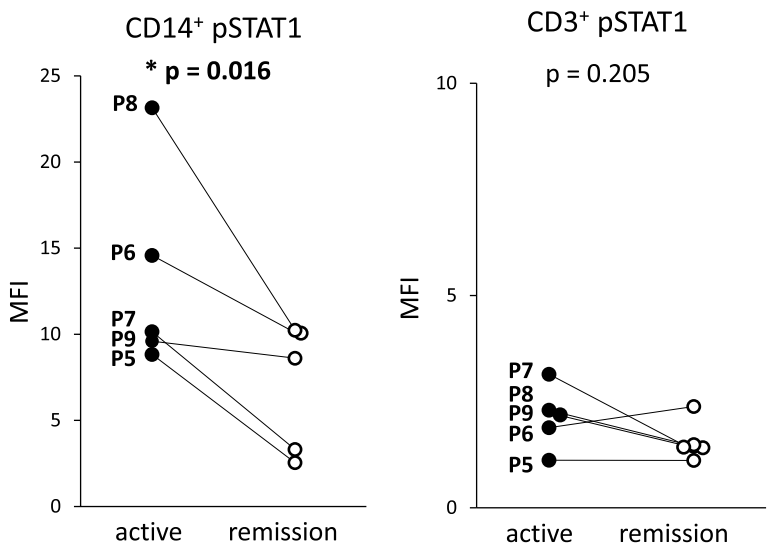

Fig. 3 The pSTAT1 levels in CD14$^+$ monocytes or CD3$^+$ T cells are in 5 patients (P5-P9) with CNO at *active* and *remission* phases that responded to NSAIDs alone with or without cDMARDs. The pSTAT1 levels in CD14$^+$ monocytes but not CD3$^+$T cells significantly decrease after the effective treatments ($p = 0.016$). Significant differences are determined by the paired-sample *t*-test. $^{**}p < 0.01$, MFI: median fluorescence intensity

ciencies. Genetic screening for osteogenesis imperfecta was not performed due to the clinical findings. He had a family history of autoimmunity including Hashimoto's disease in his mother and fibromyalgia and vasculitis syndrome in his sisters. Foot magnetic resonance imaging (MRI) and ^{18}F-fludeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) revealed multiple edematous bone marrow lesions and an accumulation in the distal tibia and tarsal bones (Fig. 4A-C). After the

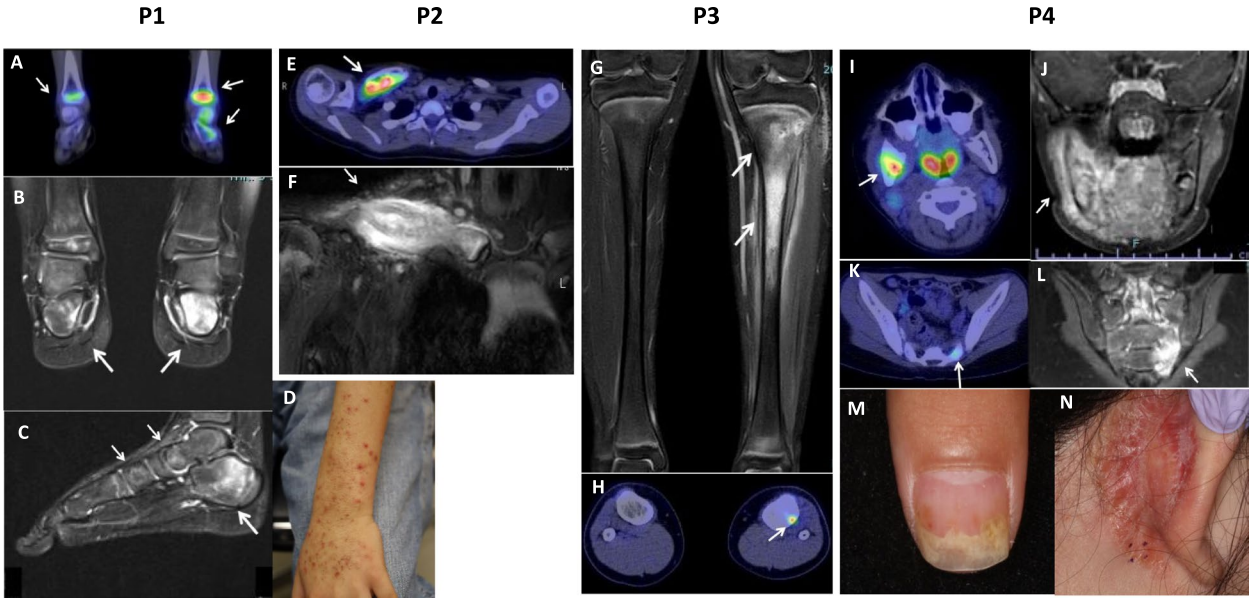

Fig. 4 Radiographic findings at diagnosis in four patients (P1-4) with CNO and extraosseous complications. **P1. A** FDG-PET/CT revealed an abnormal accumulation in the bilateral distal tibia and tarsal bones. **B** T2 prolongation and augmentation effects on the bilateral distal tibia and tarsus indicate bone marrow edema in foot MRI. **C** Sagittal MRI view in foot MRI. **D** Acne vulgaris on bilateral limbs observed during follow-up. **P2. E** FDG-PET/CT revealed irregularities and an abnormal accumulation in the right clavicle. **F** The swollen right clavicle showed T2 prolongation and enhancement of internal and surrounding soft tissues, indicating edematous changes in MRI. **P3.** The proximal left tibial lesion in MRI (**G**) and FDG-PET/CT (**H**). **P4.** FDG-PET/CT and MRI revealed bone lesions in the right mandible (**I** and **J**) and left sacrum (**K** and **L**), respectively. Edematous bone lesions with swelling of the surrounding soft tissues in MRI. P4 presented a nail deformity due to psoriasis (**M**), and pyoderma (**N**). FDG-PET/CT: fludeoxyglucose-positron emission tomography/computed tomography. White arrows indicate lesions

diagnosis of CNO following a biopsy, NSAIDs controlled foot pain at first, but came to be ineffective at 12 years old. Because of partial response to methotrexate and adalimumab (40 mg/dose), he received step-up treatment consisting of tacrolimus, liposomal dexamethasone palmitate (lipo-DEX), and an increased dose of adalimumab (80 mg/dose). Thereafter, uncontrollable leg pain resulted in difficult walking. Subsequently, symptoms such as diarrhea, weight loss, and acne vulgaris on bilateral limbs (Fig. 4D) emerged as extraosseous manifestations, presenting clinical features resembling SAPHO syndrome. Genetic testing for inborn errors of immunity (IEI), including autoinflammatory diseases, was conducted and did not identify any known pathogenic variants. Before the use of JAK inhibitors at 13 years of age, he was limping due to a painful right tibia. The CDAS score increased to 9.7, but the mean CHAQ score was 0.14 (range, 0–3). T2 signal prolongation and enhancement on foot MRI were scattered across the right distal tibial and tarsal bones with bone marrow edema in the calcaneus (Fig. 5, P1 *before*). Blood test indicated no systemic inflammation or autoimmunity. The lymphocyte subsets and cytokine profiles were unremarkable. Monocyte-pSTAT1 levels increased after IFNγ stimulation (Fig. 6, P1). With the approval of the institutional ethics committee and consent of the patient and his guardians, baricitinib (4 mg/day, once a day orally) was initiated after the discontinuation of adalimumab and tacrolimus. He experienced prompt relief of foot pain and walking difficulty, with an improved CDAS score (4.0) and foot MRI findings (Fig. 5, P1 *after*). The patient's acne vulgaris, resistant to topical steroids and antibiotics, and diarrhea also disappeared promptly after the JAK inhibitor started. He declined to undergo the endoscopic study because of improved gastrointestinal symptoms and catch-up growth (Supplementary Fig. 2) [27].

Patient 2. A 10-year-old girl complained of leg pain at 6 years old, followed by coccygeal and clavicular pain. She had an unremarkable family history. At 8 years old, MRI and FDG-PET/CT revealed irregularities and an abnormal accumulation in the right clavicle, determining a diagnosis of CNO after workup with a biopsy (Fig. 4E, F). The patient developed palmoplantar pustulosis subsequently. NSAIDs controlled clavicular pain on dose escalation, along with methotrexate and then switched to biological agents including infliximab (5 mg/kg/dose), adalimumab (40 mg/dose), and tocilizumab (8 mg/kg/dose) in order. Because of poor pain control, we decided to use additional JAK inhibitors. Upon a re-evaluation, clavicular pain persisted with a CDAS score of 14.5, and the CHAQ score increased to a mean of 1.1 (range: 0–3). MRI revealed residual irregularities in the right clavicle (Fig. 5, P2 *before*). Blood test results and lymphocyte subsets were unremarkable. The serum cytokine profile showed elevated levels of IL-6 (130 pg/mL) and IL-8 (1,467 pg/mL). IFNγ-stimulated monocytes showed increased expression of pSTAT1 (Fig. 6, P2). Baricitinib (4 mg/day) ameliorated bone pain, and lipo-DEX and tocilizumab were discontinued without recurrence. Following 11 months of baricitinib therapy, the CDAS score was 1.0, and the CHAQ score was 0.1, with improved MRI findings (Fig. 5, P2 *after*).

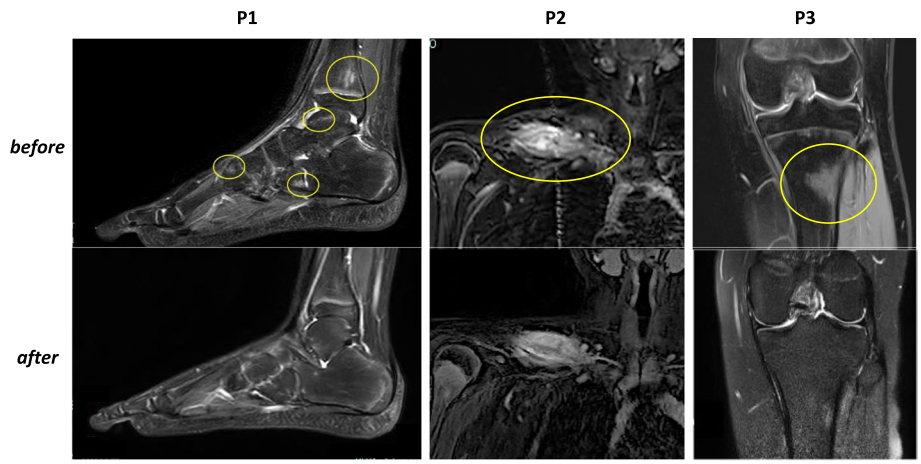

Fig. 5 MRI findings of patients with refractory CNO before and after JAK inhibitor therapy. **P1)** Multiple edematous bone marrow lesions remained on the right distal tibia and tarsus (*before*, yellow circle). The T2 prolongation and potentiation effects were improved (after). **P2)** An edematous lesion in the right clavicle with inflammation of surrounding tissue (*before*) and subsequent improvement (*after*). **P3)** Edematous changes in the left tibia from the proximal metaphyseal end to the diaphysis (*before*) and subsequent improvement at 15 months after evaluation (*after*)

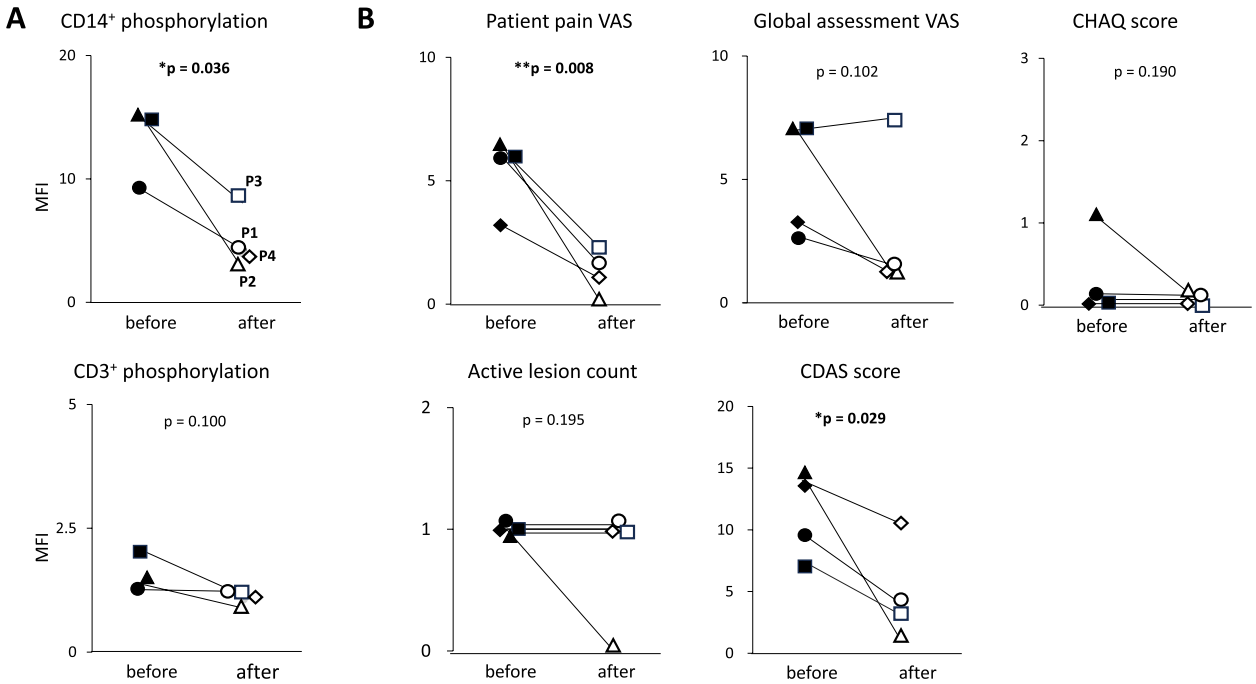

Fig. 6 Clinical and immunological parameters of patients with refractory CNO before and after JAK inhibitor therapy. **A** Increased pSTAT1 levels in $CD14^+$ cells significantly decreased about 4 weeks after the start of adjunctive JAK inhibitor (*left panel,* $p = 0.036$), but not in $CD3^+$ cells (*right panel,* $p = 0.100$). Patient 1 (P1: *blue bar*), P2 (*red bar*), P3 (*green bar*), and P4 (*purple bar*). **B** Functional measures using CDAS and CHAQ scores. CDAS is calculated as a sum score of patient pain VAS, active lesion count, and global assessment VAS. Patient pain VAS and CDAS significantly improved after JAK inhibitor therapy ($p = 0.008$ and $p = 0.029$, respectively). Significant differences are determined by the paired-sample *t*-test. * $p < 0.05$, ** $p < 0.01$, MFI: median fluorescence intensity

Patient 3. A 9-year-old girl with an unremarkable personal or family history presented with recurrent pain of the left knee. MRI and FDG-PET/CT revealed the proximal left tibial lesion (Fig. 4G, H). A biopsy from the left tibial lesion, which represented chronic inflammation without evidence of infection or malignancy, led to a diagnosis of CNO. NSAIDs failed to control relapsing leg pain, and painful lesions extended to the lumbar vertebrae. Because methotrexate, tacrolimus, and adalimumab (40 mg/dose) failed to control bone pain, the disease severity was re-evaluated before the start of JAK inhibitors. The left tibial pain persisted, with a CDAS score of 14.0 and a CHAQ score of 0. MRI showed abnormal signals of the residual lesion in the proximal left tibia (Fig. 5, P3 *before*). Blood test results and lymphocyte subsets were unremarkable. Serum IL-8 levels increased to 642 pg/mL. IFNγ-stimulated monocytes showed increased pSTAT1 levels (Fig. 6, P3). Baricitinib (4 mg/day) initially controlled bone pain (patient pain VAS score, 2 cm) with the partial improvement of CDAS score (10.5). Six months later, the JAK inhibitor was switched to upadacitinib (15 mg/day, once a day orally) because of occasional pain. Upadacitinib has successfully controlled pain, with improved MRI findings (Fig. 5, P3 *after*).

Patient 4. A 7-year-old healthy girl with an unremarkable family history had afebrile jaw pain recurrently. MRI and FDG-PET/CT revealed edematous bone lesions with swelling of the surrounding soft tissues, and an abnormal accumulation in the right mandible and left sacrum (Fig. 4I). Histopathological and microbiological studies of the bone lesion confirmed the diagnosis of CNO. She subsequently presented with palmoplantar pustulosis and inflammatory bowel disease. NSAIDs, methotrexate, and infliximab, appreciably controlled the bone pain, although psoriasis, pyoderma, and an occasional fever developed (Fig. 4M, N). Additional tacrolimus failed to control these symptoms. Upadacitinib (15 mg/day) was initiated at 14 years old for the treatment of the fever and skin and residual bone lesions (CDAS 7.5). Blood tests, lymphocyte subsets, and cytokine profiles were unremarkable, with an elevated erythrocyte sedimentation rate (ESR) of 21 mm/hr (Supplementary Table 2). She received no pSTAT1 testing before JAK inhibitor therapy. Upadacitinib ameliorated the skin lesions but not control the fever or elevated ESR.

The JAK inhibitor was then switched to tofacitinib (5 mg orally twice a day) but thereafter switched back to upadacitinib because of progressive skin lesions. After 11 months of re-administration of upadacitinib, systemic, cutaneous, and painful bone lesions improved to a patient pain VAS of 1 cm with CDAS of 3.1.

Monocyte pSTAT1 monitoring in intractable cases after JAK inhibitor therapy

We assessed the changes in clinical and immunological variables using imaging data before and after an adjunctive JAK inhibitor. IFNγ-stimulated pSTAT1 levels significantly decreased in monocytes but not in T cells after JAK inhibitor therapy ($p=0.036$), in concert with the improved patient pain VAS score and CDAS score (Fig. 6B, $p=0.008$ and $p=0.029$, respectively) but not the CHAQ score. Serum cytokine levels did not change remarkably even after the administration of JAK inhibitors (Supplementary Fig. 3).

Discussion

We demonstrated that IFNγ-stimulated pSTAT1 levels in monocytes, but not T cells, are a useful immunological marker at the presentation of active CNO. Four intractable cases showed a drastic improvement in VAS scores, CDAS, and imaging data during JAK inhibitor therapy, along with the reduced monocyte-pSTAT1 levels in all three cases with monitored pSTAT1 levels. Cytokine levels and CHAQ scores did not change after effective treatment with the JAK inhibitor. These results indicate a pathophysiological role of the STAT1 pathway in CNO and the utility of monitoring to assess the treatment effects of JAK inhibitors.

CNO or SAPHO syndrome has various clinical manifestations, including a relapsing fever, skin manifestations, and painful bone and joint lesions, leading to a wide range of treatment targets [28, 29]. However, the molecular pathophysiology of this disease spectrum remains unknown. Many studies addressing the mechanisms focusing on bone lesions in CNO have indicated immune activation and cytokine dysregulation, but there is limited information on the causative or associated genes [30, 31].

CNO and SAPHO syndrome arise from the activation of innate immunity in childhood and adaptive immune dysregulation with cutaneous expressions in adults [32]. We found split pSTAT1 levels in monocytes from T cells at presentation, indicating innate immune activation during the prodromal stage. This is the first demonstration for the diagnostic utility of monocyte-pSTAT1 for CNO with the screening advantage. Increased monocyte-pSTAT1 levels may represent the initial activation of innate immune cells that affect osseous remodeling. TNF and IL-1β promote osteoclastogenesis through the upward regulation of RANKL in osteoblasts and bone marrow macrophages [33, 34]. Inflammatory cytokines, including TNF and IL-8, have been implicated in the development of local bone lesions in adult SAPHO patients [35, 36]. Increased serum IL-6 levels are positively correlated with C-reactive protein (CRP) or ESR levels in patients [37, 38]. In the present study, two of three patients had higher levels of serum IL-6 or IL-8 at the time of the diagnosis of CNO than healthy controls. Meanwhile, serum TNF and IL-1β levels showed no obvious changes. In contrast to previous reports, the lack of detectable TNF was possibly due to the characteristics of pediatric CNO patients or the effect of prior therapies at the time of evaluation. Alternatively, the bioactivity could have been difficult to detect in serum as in previous reports, thus requiring improved analytical methods and biomarkers [39, 40]. Previous reports have shown that IFNγ, which is the focus of this evaluation of pSTAT1, is a potent inhibitor of osteoclastogenesis [3, 41]. A study of patients with Mendelian susceptibility to mycobacterial disease suggests that augmented osteoclastogenesis due to poor response to IFNγ is important for the development of multiple bone lesions [4]. IFNγ concentration-dependent inhibition of osteoclast formation was reportedly enhanced in granulocyte macrophage colonies from patients with STAT1-GOF but not in patients with IFNγR1 deficiency or STAT1-loss of function [42]. The dysregulated response to IFNγ disrupts bone homeostasis, which possibly explains the pathogenesis in pediatric patients with CNO. In this context, a monocyte-pSTAT1 analysis is a useful tool for diagnosing CNO after excluding infection, monogenic autoinflammatory diseases, or systemic bone diseases.

A major concern is the treatment of progressive osteopathy. A survey of the Coalition for Rheumatology Research Alliance reported that 95% of patients received NSAIDs as the first-choice analgesics to control bone pain [43]. NSAIDs affect inflammasome assembly and osteoclast activation [44] but do not control extensive bone or skin inflammation. Methotrexate, TNF inhibitors, and bisphosphonates are used for second-line treatment under recent consensus [7]. Bisphosphonates were not used off-label in this case series; however, recent studies have supported the safety and efficacy. Zhao et al. [22] reported improved bone mineral density without increasing adverse events. Marini et al. [45] found enhanced vertebral strength with potentially reducing compression fracture risk. These highlight clinical utility of bisphosphonates to control CNO under careful consideration. Furthermore, several reports have suggested a treatment benefit of JAK inhibitors in SAPHO

syndrome on bone or skin lesions [10–17]. This is the first report to demonstrate the effect of JAK inhibitors with monitoring by biomarkers on the control of refractory patients with CNO. Four intractable cases showed prompt and sustainable improvement of the bone lesions and other manifestations. pSTAT1 monitoring corroborated the immunological response to disease control. On the other hand, our phospho-flow data showing negative pSTAT1 results in T cells suggest that immune modulation in CNO may target different pathways. As a result, tacrolimus, a CNI that targets T cells, was not effective in treating CNO in our cohort, with three of the four patients failing to improve. Baricitinib and upadacitinib have been used safely and effectively in pediatric patients with atopic dermatitis [46]. Further studies are needed to determine the safety and tachyphylaxis associated with prolonged use of adjunctive JAK inhibitors for CNO.

There are a few studies on the role of monocytes in the pathogenesis of CNO or SAPHO syndrome. Hofmann et al. [30] reported an impaired IL-10 expression in monocytes from CNO patients in response to stimulation with toll-like receptor 4 by lipopolysaccharide. Downregulation of IL-10 production has been implicated in osteoclastogenesis by activating NLR family pyrin domain-containing 3 inflammasomes and increasing IL-1β [39]. Although we did not follow changes in serum levels of IL-10 and IL-1β in intractable cases, the cytokine-mediated JAK/STAT pathway has been implicated in the pathogenesis of autoimmune and autoinflammatory diseases [9]. IFNγ, used for stimulation in this study, activates STAT1 via JAK1 and JAK2. Many JAK inhibitors have been developed to control signal transduction in recent years [47]. Although JAK inhibitors have distinct selectivity for inhibiting the JAK-STAT pathway, their treatment effects vary in practice [48]. Our patients showed favorable treatment responses depending on the type of JAK inhibitor, sharing the clinical and immunological effects on the painful bone lesions and monocyte-pSTAT1 levels after IFNγ stimulation after the administration of an effective JAK inhibitor. In this setting, the phosphorylation analysis is a useful tool for predicting the treatment response and concurrently approaching the therapeutic target of selective JAK inhibitors. This raises the possibility that JAK inhibitors exert therapeutic effects by modulating the activation of the JAK-STAT pathway, providing a clue to the pathogenesis of CNO or SAPHO syndrome.

Several limitations associated with the present study warrant mention. The first is the small number of patients. A long-term cohort of uncommon disorders is required to validate the novel findings of this study. Second, this analysis couldn't explain the absence of baseline differences in pSTAT1, despite the phenotype being potentially driven by IFN-gamma. Further studies are necessary to clarify whether this is due to the absence of a genetic background to hyperactivation such as STAT1-GOF, transient signaling dynamics, or other compensatory mechanisms. Finally, we evaluated the phosphorylation of STAT1 after stimulation with IFNγ, but not with other stimulants, including IL-10 and type 1 IFNs. To clarify the molecular mechanism involving the STAT1 pathway in CNO or SAPHO syndrome, further molecular and pathological analyses are required, focusing on the affected bone tissues.

Conclusion

This study provides valuable insights into the potential use of JAK inhibitors as therapeutic options for refractory CNO. Using pSTAT1 as a monitoring tool can enhance our understanding of the immunological response to JAK inhibitor treatment. These findings contribute to ongoing efforts to diversify treatment strategies for CNO or SAPHO syndrome, ultimately aiming to improve the quality of life of pediatric patients.

Abbreviations

CNO	Chronic nonbacterial osteomyelitis
CRMO	Chronic recurrent multifocal osteomyelitis
JAK	Janus kinase
pSTAT1	Phosphorylation of signal transducer and activator of transcription 1
IFNγ	Interferon-gamma
GOF	Gain of function
SAPHO	Synovitis, acne, pustulosis, hyperostosis, and osteitis
NSAIDs	Nonsteroidal anti-inflammatory drugs
cDMARDs	Conventional disease-modifying antirheumatic drugs
TNF	Tumor necrosis factor
IL	Interleukin
VAS	Visual analog scale
CDAS	CNO clinical disease activity score
CHAQ	Childhood Health Assessment Questionnaire
FDG-PET	^{18}F-fludeoxyglucose-positron emission tomography
CT	Computed tomography
lipo-DEX	Liposomal dexamethasone palmitate
MRI	Magnetic resonance imaging
ESR	Erythrocyte sedimentation rate
CRP	C-reactive protein

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12969-025-01059-6.

Supplementary Material 1: Supplementary Table 1. Profiles of all participants in this study. Supplementary Table 2. Laboratory data at the diagnosis of intractable CNO [49–53].

Supplementary Material 2: Supplementary Fig. 1. Representative data of pSTAT1 expression in CD14$^+$ monocytes (*upper*) or CD3$^+$ T cells (*lower*) after IFNγ stimulation in patients 1–3. Single-parameter histograms with pSTAT1 and %gated show the differences in phosphorylation with and without 500 U/mL of rhIFNγ. Supplementary Fig. 2. The growth curve of P1 is plotted on growth standard charts for Japanese children shows a point of stunted growth and weight gain at the disease onset. The weight loss follows skin manifestations and diarrhea consistent with SAPHO syndrome (*vertical arrow*), and subsequent improvement in the growth curve and

these symptoms after starting baricitinib (*horizontal arrow*). Supplementary Fig. 3. Serum levels of inflammatory cytokines in patients with refractory CNO before and after the administration of JAK inhibitors. Patient 1 (P1: *blue*), P2 (*red*), P3 (*green*), and P4 (*purple*). Significant differences are determined by the paired-sample *t*-test.

Acknowledgements
We sincerely thank Prof. Yasuharu Nakashima (Department of Orthopedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan) and Prof. Takeshi Nakahara (Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan) for providing the diagnostic supports.

Authors' contributions
The contributions of each author are as follows: MS, KK, and SO were the principal investigators, taking primary responsibility for the paper. NH performed data curation and analysis. SP, SA, YY, KE, TF, MKN, NK, and MI performed the clinical management with helpful discussion regarding the completion of the work.

Funding
This work was supported in part by a Grant-in-Aid (20FC1053) from the Health and Labor Sciences Research grants (Research on Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan and JSPS KAKENHI Grant Number JP23K14953.

Data availability
Individual data are available upon reasonable request.

Declarations

Ethics approval and consent to participate
The research protocols were approved by Kyushu University Hospital Ethics Committee and informed written consent was obtained from a subject or a parent or guardian, participating in the study.

Consent for publication
Written informed consent was obtained from a subject or a parent or guardian for the publication.

Competing interests
The authors declare that they have no competing interests.

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Received: 6 September 2024 Accepted: 7 January 2025
Published online: 23 January 2025

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