be considered to be a process of the disease rather than a disease itself due to hypercytokinemia [29]. It can be difficult to diagnose patients as having MAS at a given time because MAS is a disease in which a series of events such as thrombocytopenia, endothelial cell damage, coagulation abnormalities, mitochondrial permeability transition and multiple organ failure occurs, fades away, and worsens in a couple of days.

Macrophage activation syndrome is clinically characterized by the rapid development of fever, hepatosplenomegaly, lymphadenopathy, purpura and mucosal bleeding. In our experience, an exact diagnosis will be made when precise laboratory examinations are performed during the course of the process. Laboratory studies primarily indicate the presence of hematocytopenia, and then, combinations of serum β2-microglobulin and ferritin, elevated tissue-derived enzymes such as mitochondrial aspartate aminotransferase (mAST), lactate dehydrogenase (LDH) and creatine phosphokinase (CK), hypoalbuminemia, increased levels of fibrin degradation products (FDP-E, D-dimer) and elevated triglycerides. A bone marrow examination, if performed with proper timing, may show active phagocytosis by macrophages and histiocytes [30]. Accompanying the progression of the process, finally, increases in creatinine, alanine aminotransferase (ALT) and amylase levels are present, indicating multiple organ failure.

The pathogenesis of MAS remains to be established. The first report described the pathogenic role of TNF-α in MAS [2]. The increased levels of IFN-α, TNF-α and other proinflammatory cytokines correlate with the rapid development of clinical symptoms and the progression of abnormal laboratory parameters [31]. In addition, since systemic JIA patients display decreased levels of perforin in NK cells and diminished NK cell function, the recent investigation suggested that perforin gene (*PRF1*) mutations also play a role in the development of MAS in systemic arthritis patients [5]. Thus, MAS would be the transition form of the disease process from IL-6 cytokinemia in systemic JIA to multiple proinflammatory cytokinemia for the background of *PRF1* gene mutation and diminished NK cell function.

Biologic function of IL-6 & tocilizumab

IL-6 is one of the most pleiotropic cytokines known that is involved in regulating a wide variety of inflammatory and immune functions, B-cell differentiation, T-cell growth, acute-phase reactions and hematopoiesis [32,33].

The first step in the induction of the transduction signals by IL-6 is the binding to its IL-6R, which is either localized at the cell surface or present in a soluble form in serum. The association of the IL-6/IL-6R complex with another receptor, gp130, forms a high-affinity complex that triggers specific transduction signals. Three members of the janus kinase family, JAK1, JAK2 and TYK2, are closely related to gp130 and are rapidly activated in the presence of IL-6 [34]. These kinases phosphorylate the tyrosine residues of the gp130 cytoplasmic domain, which allows the recruitment and phosphorylation of transcriptional factors of the signal transducers and activators of transcription family (STAT1 and STAT3) [35]. Once activated, the STAT proteins may activate different genes. Thus, the blockade of IL-6R by tocilizumab can result in invalidity of the formation of phosphorylated STAT proteins, which inhibits inflammatory responses [36].

Pathogenesis of systemic JIA & MAS

The pathogenic role of proinflammatory cytokines in systemic JIA has long been investigated. IL-6 is reported to be markedly elevated in blood and synovial fluid [37]. The IL-6 level increases before each fever spike and correlates with the systemic activity of the disease, arthritis and an increase in acute-phase reactions [38]. Abnormalities in the regulation of IL-6 are also responsible for the thrombocytosis and anemia seen in this disease [7]. *In vitro* studies have documented increased production of IL-6 by peripheral blood mononuclear cells from patients with systemic JIA [39]. An imbalance in IL-6 homeostasis is suggested by the observations that sIL-6R concentrations are significantly increased in children with systemic JIA. Growth retardation was found in IL-6 transgenic mice overexpressing human IL-6, similar to that in children with systemic JIA [8]. In contrast to IL-6, TNF-α levels are not increased in systemic JIA. Taken together, IL-6 and IL-6R might play a central role in the induction and progression of systemic disease and its complications. However, direct evidence in humans is not yet available.

During the course of recurrent inflammatory episodes of systemic JIA, MAS often follows a viral infection, such as Epstein–Barr virus or influenza virus [40]. Changes in medications, as well as the introduction of nonsteroidal anti-inflammatory drugs, gold compounds or methotrexate, were reported to trigger the syndrome [41]. However, it seems likely that changes in medications were coincidental, that is, occurring in a child who was susceptible to MAS and who required additional therapy for uncontrolled systemic JIA. The histopathologic features of skin biopsy specimens are the presence of microthrombi and endothelial cell proliferation [42], indicating that due to overwhelming proliferation of various proinflammatory cytokines such as IFN-γ and TNF-α, continuing damage to endothelial cells and the resultant vasculitis induce disseminated intravascular coagulation (DIC) and, subsequently, multiple organ failure. This is the whole spectrum of clinical MAS.

Clinically, MAS starts with thrombocytopenia and leukocytopenia, and then abrupt improvements in erythrocyte sedimentation rate (ESR) and CRP levels can be seen. Fibrin degradation products (FDP-E, D-dimer) and hypofibrinogenemia are present, indicating DIC due to activated and destroyed endothelial lining of the vasculature by combinatorial effects of proinflammatory cytokines [43]. Markedly increased levels of cytokine-induced proteins, serum ferritin by TNF-α [44] and β2-microglobulin by IFN-γ [45] can be observed during this stage. Subsequently, rising levels of serum mAST, LDH and CK indicate apoptosis due to mitochondrial permeability transition by TNF-α [46], which can solely be protected by cyclosporine [47]. In the late phase of MAS, increased levels of triglycerides and decreased levels of total cholesterol are present due to inhibited lipoprotein lipase activity by TNF-α [48]. Finally, multiple organ failure along with DIC will progress.

Clinical signs & symptoms seen in patients with systemic JIA & proinflammatory cytokines

Fever & sickness behavior in systemic inflammation

During the clinical trials, the most prominent features of the effects of tocilizumab were the abrupt normalization of fever and disappearance of fatigue, lethargy or anorexia in children with long-lasting inflammation with regard to systemic JIA [19,20].

Systemic inflammation is accompanied by changes in body temperature and behavior. The proinflammatory cytokines, IL-1β, IL-6 and TNF-α, synthesized by activated macrophages in response to experimental administration of the bacterial pyrogen lipopolysaccharide (LPS), are considered important mediators of fever and sickness behavior [6].

Recent investigations revealed that experimental fevers are generally polyphasic, and that different mechanisms underlie different febrile phases [49]. Signaling mechanisms of the most common pyrogen used, LPS, have been found to involve Toll-like receptor 4 [50]. The roles of endogeneous cytokines, particularly IL-6 and the cytokine-like hormone leptin, but not IL-1β or TNF-α, have been confirmed by using cytokine-specific antisera to be the key mediators of fever and sickness behavior assessed by voluntary exercise and food intake induced by LPS in rats [51].

Proinflammatory cytokines are then switched to a downstream mediator, prostaglandin (PG)E2 [52]. An indispensable role of PGE2 in the febrile response to LPS has been demonstrated in studies with targeted disruption of genes encoding either PGE2-synthesizing enzymes or PGE2 receptors. EP3 (a G-protein-coupled receptor) is likely to be the primary fever receptor, and the effector pathways of fever start from EP3-bearing neurons [38]. The neurons project to the raphe pallidus in the hypothalamus. Inflammatory signaling and thermoeffector pathways involved in fever and sickness behavior are further modulated by neuropeptides and peptide hormones such as leptin [51].

Tocilizumab is an IL-6R-specific monoclonal antibody, and clinical studies involving children with systemic JIA have demonstrated the targeted blockage of the IL-6 signaling pathway in humans. Consequently, tocilizumab treatment attenuates the body temperature rise and sickness behavior, indicating that the major manifestations of this systemic inflammatory disease are apparently IL-6 related. Skin rash, which appears to accompany a rise in body temperature, could be supposed to be an IL-6-related skin manifestation of systemic JIA, but this topic needs further investigation.

C-reactive protein

C-reactive protein is an acute-phase protein and a sensitive marker and mediator of inflammation in the clinical setting. CRP was used as one of the surrogate markers for assessing the flares of the disease in tocilizumab trials. The administration of tocilizumab to children with systemic JIA rapidly attenuated the increased levels of CRP in serum within a few days.

The synergistic induction mechanism of *CRP* gene expression by IL-1β and IL-6 in the human hematoma cell line, Hep3B cells, was recently investigated [14,53]. In the early induction phase, IL-1β and IL-6 activate NF-κB p65 and the janus kinase family, respectively. The activation of janus kinases by IL-6 allows the recruitment and phosphorylation of the transcriptional factor, STAT3. NF-κB p65 forms a complex with STAT3, which inhibits expression of the *CRP* gene. In the late induction phase, synergistic stimulation by IL-1β and IL-6 causes the formation of a heterodimeric complex with c-Fos, STAT3 and hepatocyte nuclear factor (HNF)-1-α, which in turn induces synergistic expression of the *CRP* gene. Thus, transcriptional complex formation of c-Fos/STAT3/HNF-1α plays an essential role in cytokine-driven *CRP* gene expression. Tocilizumab treatment, therefore, ameliorates the CRP rise by inhibiting the formation of the transcriptional complex.

Chronic anemia

Anemia occurs in patients with chronic inflammatory disorders such as infection, autoimmune disease or chronic kidney disease [11]. Anemia of chronic disease is characterized by normocytic or microcytic iron-deficiency anemia and preserved marrow iron. Proinflammatory cytokines, particularly IL-6, are believed to have an important role in this syndrome.

Early clues regarding the role of IL-6 in the pathogenesis of chronic inflammatory anemia were discovered in the cancer setting [54], where IL-6 has been evaluated as an antitumor immunotherapy. In patients with advanced ovarian cancer, anemia was so severe that the IL-6 level was the only factor other than disease stage to independently predict hemoglobin levels in a multivariate analysis. In addition, administration of human recombinant IL-6 was found to induce a rapid-onset, dose-dependent, progressive form of anemia that was quickly reversible after cessation of therapy [55].

Recent studies revealed the role of IL-6 in chronic anemia [56]. During acute-phase reactions, proinflammatory cytokines impair iron metabolism, particularly plasma iron turnover and ferritin synthesis, with the result that patients with acute or chronic infections have lower serum iron, lower tranferrin saturation and higher ferritin concentrations than do persons without apparent inflammation.

As a key regulator of transmembrane iron transport, hepcidin controls the absorption of iron in the intestine, the mobilization of iron from hepatic stores and iron recycling by macrophages [56]. At the first stage, inflammation leads to macrophage activation to produce IL-6, which acts on hepatocytes to induce hepcidin production. Under the influence of elevated hepcidin concentrations, hepcidin inhibits macrophage iron release and intestinal iron absorption, leading to hypoferremia, which limits the availability of iron for erythropoiesis, thereby contributing to the anemia associated with inflammation.

During inflammation, IL-6, but not IL-1β or TNF-α, rapidly induces hepcidin synthesis in human hepatocytes and corresponding hypoferremia. Anti-IL-6 antibodies block the induction of hepcidin mRNA in primary human hepatocytes treated with the bacterial endotoxin, LPS [57]. IL-6-knockout mice failed to produce hepcidin in response to inflammatory challenges [58]. In our studies, chronic anemia was gradually improved in association with the blockade of the IL-6 signaling pathway by tocilizumab. Thus, IL-6 is presumably the most potent cytokine of chronic inflammatory anemia in children with systemic JIA.

Development of tocilizumab as a blocking agent of the IL-6 signaling pathway

Tocilizumab is a genetically engineered monoclonal antibody of the IgG1 subclass that was humanized by the technique of complementary-determining region grafting from mouse anti-human IL-6R monoclonal antibody [18,59]. Tocilizumab binds to both membrane-bound and sIL-6R and inhibits the formation of the IL-6/IL-6R complex that results in a decrease in signal transduction via gp130. The IL-6R molecule is theoretically the exclusive target of tocilizumab, and thus, the roles of the IL-6 signaling pathway in inflammatory immune diseases can be clearly elucidated when tocilizumab is administered in humans as a therapeutic agent. Thus, tocilizumab treatment can be termed a 'molecular intervention.'

The use of intravenous tocilizumab in patients with rheumatoid arthritis was investigated and found to be more effective than placebo in reducing disease activity and to have a safety profile consistent with that of other biological and immunosuppressive therapies [60]. Notably, tocilizumab appears to provide an additional option for those patients who do not respond sufficiently to methotrexate and other biological response modifiers. In turn, tocilizumab administration proved that IL-6 is the key pathogenic cytokine in the induction and progression of rheumatoid arthritis. Together with evidence that targeting and inhibiting TNF-α with infliximab or etanercept can result in significant improvement in signs and symptoms of rheumatoid arthritis, the combined role of IL-6, TNF-α and probably IL-1β rather than the single independent action of each cytokine in inflammatory responses might be important. In other words, a vicious cycle is formed by the combination of these proinflammatory cytokines from a pathologic viewpoint, causing progression and continuation of inflammatory responses in rheumatoid arthritis. Thus, in many patients but not all, any monoclonal antibodies or specific receptors can block the overwhelming joint inflammation.

Efficacy of tocilizumab for children with systemic JIA

Phase II trial

The Phase II clinical study of tocilizumab was conducted in children with severe and active systemic JIA refractory to high-dose, long-term corticosteroids to investigate the safety, tolerability, antigenicity, pharmacokinetics and efficacy of the drug [19]. Eight boys and three girls were enrolled. At enrollment, these 11 children were between 3 and 18 years of age. The median duration of the systemic disease was 3.8 years. The mean number of active joints was 4.5. Six of them exhibited severe growth retardation and osteoporosis with complicating compression vertebrae fractures. CRP and ESR values were high and the white blood cell count was over 15,000/ml. The profile of proinflammatory cytokines and soluble receptors in serum was investigated, and IL-6 and sIL-6R, but not IL-1β and TNF-α, were persistently detected at high levels, indicating the failure of IL-6 signaling pathway homeostasis.

The study, designed as a dose-escalating trial, began with three infusions of tocilizumab 2 mg/kg at 2-week intervals. When the CRP value was demonstrated to be positive at least 5 days after the initial and second administrations of tocilizumab, the dose was increased to 4 mg/kg and was administered three times every 2 weeks. If CRP levels did not improve, then three infusions of 8 mg/kg were administered at 2-week intervals. Assessment of disease response was made according to American College of Rheumatology (ACR) Pedi responses to the following six items [61], although it is not yet validated for systemic JIA: physician's and patients'/parents' general assessment on a 10 cm visual scale, functional ability, number of active joints, number of joints with restriction of motion, and CRP/ESR values (the original ACR Pedi used the ESR as a laboratory parameter instead of CRP).

After the first administration of tocilizumab, high-grade or quotidian fever abruptly subsided and vague complaints such as fatigue, lethargy and anorexia disappeared. Severe arthritis improved in all 11 children within a few weeks. Laboratory examinations revealed that CRP and ESR levels had returned to the normal range. Ten out of the 11 children improved at 2 weeks after the first administration of tocilizumab as assessed by ACR Pedi 30/50% responses. Before the second administration, eight children had increases in CRP and 4 mg/kg of tocilizumab was infused. Three of these eight children had subsequent elevations in CRP and, consequently, three infusions of 8 mg/kg tocilizumab were administered, with no further increases in the CRP value. Overall improvement in arthritis and systemic features assessed by an ACR Pedi 30, 50 and 70% improvement was 90.9, 90.9 and 63.6%, respectively. In general, tocilizumab was well tolerated. No patient withdrew during the study period. The adverse events were upper respiratory tract infection, pustules on extremities and eczema. All laboratory abnormalities were mild and no serious events requiring urgent treatment were noted.

Results of the Phase II trial suggested that although 2–4 mg/kg of tocilizumab could suppress disease activity, 8 mg/kg is probably required to control disease activity in children with systemic JIA. Clinical manifestations such as quotidian fever, fatigue, lethargy and anorexia, and laboratory abnormalities such as increased levels of CRP and ESR seen in active disease disappeared with tocilizumab treatment by blocking IL-6R alone, indicating that the IL-6 signaling pathway is directly implicated in the pathogenesis of this disease.

Phase III trial

The Phase III trial was conducted to investigate the safety and efficacy of tocilizumab for children with systemic JIA who were refractory to conventional treatment [20]. The study consisted of three phases: an open-label lead-in phase of 6 weeks, a double-blind, randomized, placebo-controlled phase of 12 weeks, and an open-label extension phase of at least 48 weeks. Tocilizumab was administered intravenously at 8 mg/kg, every 2 weeks. The primary end points in the open-label phase were the proportion of children achieving an ACR Pedi 30% improvement and the proportion of those with a reduction of CRP concentration to less than 5 mg/l. Children who achieved ACR Pedi 30% responses and low CRP concentrations were randomly assigned

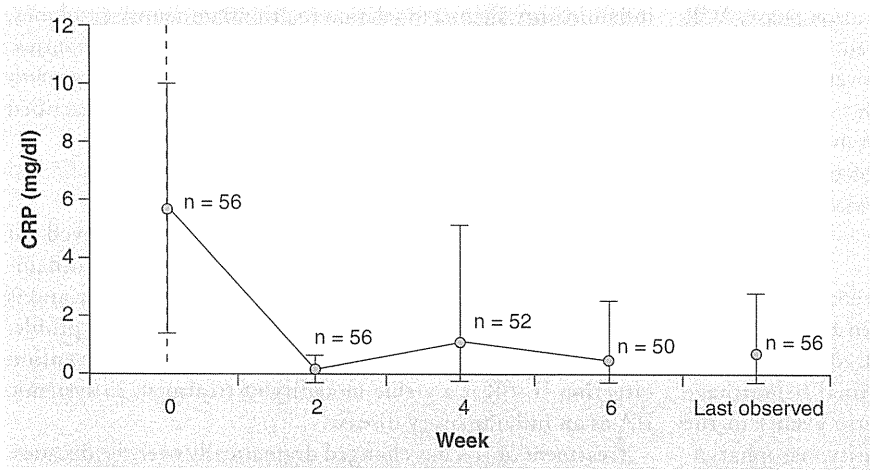

Figure 1. Effects of tocilizumab on C-reactive protein value. Increased level of CRP was decreased rapidly to the normal range after the first administration of tocilizumab. Vertical lines represent the standard error bars.
CRP: C-reactive protein.
Data from Phase III trial of tocilizumab for patients with systemic juvenile idiopathic arthritis [20].

to receive an infusion of tocilizumab 8 mg/kg or placebo every 2 weeks in a double-blind manner. Children who did not maintain an ACR Pedi 30% response or those whose CRP concentrations increased to at least 15 mg/l were withdrawn for rescue medication.

Initially, 56 children with severe systemic JIA were enrolled in the open-label study and six patients were withdrawn; three had anti-tocilizumab IgE antibody, two had adverse events (anaphylactoid reaction and gastrointestinal hemorrhage) and one was withdrawn because of absence of efficacy. Six patients did not meet the response criteria for randomization for the double-blind phase. In the double-blind phase, one patient was excluded because of being disqualified. The study mask for this patient was broken by mistake and pharmacokinetic data were unexpectedly unmasked. Therefore, 43 patients were included in the efficacy analysis. Overall, 23 children were placed in the placebo group and 20 children in the tocilizumab group in the 12-week double-blind phase. One patient was withdrawn from each treatment group in the double-blind phase because of adverse events (leaving a total of 41 patients). In the extension phase, nine patients previously withdrawn from the open-label and the double-blind phases were re-enrolled and a total of 48 children completed the 48-week open-label extension study.

Again, high-grade or quotidian fever abruptly subsided, and vague complaints disappeared after the administration of tocilizumab (Figure 1). At the end of the open-label phase, ACR Pedi 30, 50 and 70% responses were achieved by 91, 86 and 68% of the enrolled children, respectively. In the double-blind, placebo-controlled phase, 17% of children in the placebo group maintained an ACR Pedi 30% response and CRP concentrations of less than 15 mg/l compared with 80% of children in the tocilizumab group, indicating the remarkable efficacy of

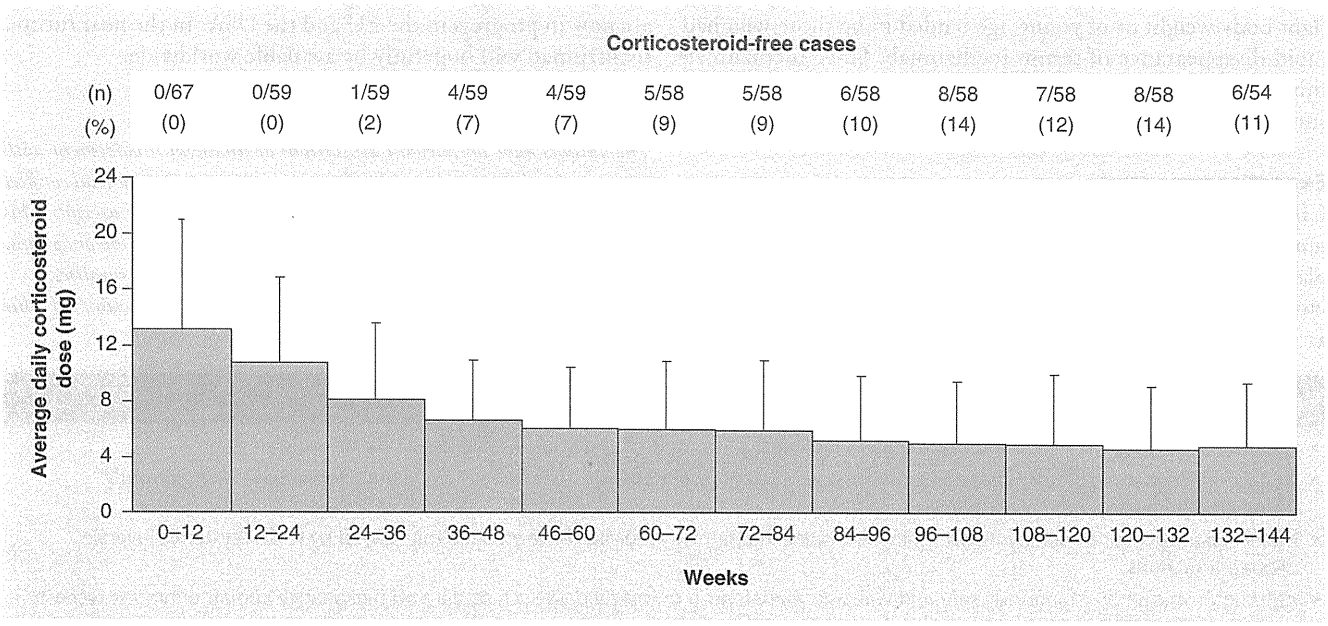

Figure 2. Sparing effects of tocilizumab on corticosteroid therapy. Corticosteroid doses were gradually decreased with repeated tocilizumab administration. The number of corticosteroid-free patients was increased during tocilizumab treatment. Vertical lines represent the standard error bars.

tocilizumab. By week 48 of the open-label extension phase, ACR Pedi 30, 50 and 70% responses were achieved by 98, 94 and 90% of the 48 children, respectively, and improvements in osteoporosis and catch-up growth in children with retarded growth were notably observed. Among the 48 children in the open-label extension study, 69 and 46% were able to reduce corticosteroid doses by at least 30 and 50%, respectively (Figure 2).

Safety & tolerability

No deaths or MAS cases occurred during the entire span of the study and no cases of TB were reported. Two serious adverse events were recorded during the open-label lead-in study: one anaphylactoid reaction and one gastrointestinal hemorrhage from chronic ulceration. Most of the adverse events in the Phase III study were mild or moderate in severity; nasopharyngitis, respiratory tract infection and gastroenteritis were frequently observed, suggesting that owing to inhibition of the IL-6 signaling pathway by tocilizumab, there might have been a potential absence of acute-phase reactions in response to infectious agents. Increases of at least grade 2 ALT and AST were recorded in some patients. Transferases tended to increase early during tocilizumab administration and then subside during continuation of treatment. Mild increases in total cholesterol, mostly within the normal range, were noted. Thus, tocilizumab was safe and well tolerated.

Pharmacodynamics & pharmacokinetics

The Phase II trial revealed that the sufficient dose of tocilizumab was 8 mg/kg at 2-week intervals. Serum concentrations of tocilizumab in the Phase III trial were achieved at steady state during 8–14 weeks after the initial administration and the trough level was 57.4 µg/ml. Children with low body height, light body weight or of young age tended to be those who had rapid disappearance of serum tocilizumab. Since tocilizumab inhibited the IL-6 signaling pathway, CRP could be used as a surrogate marker of inflammation seen in systemic JIA.

Expert commentary

Clinical and laboratory improvement in children with systemic JIA treated with tocilizumab indicates the possible roles played by IL-6 in this inflammatory disease. As described previously, the precise mechanisms of symptoms and signs such as fever, sickness behavior, *CRP* gene expression and chronic inflammatory anemia in relation to proinflammatory cytokines, particularly IL-6, have been revealed, although other manifestations and laboratory changes such as osteoporosis, growth retardation and polyarthritis are likely to be clearly described in the future.

Five-year view

Tocilizumab is the first IL-6-targeted therapy approved for children with systemic JIA in Japan. It modulates the inflammatory process by blocking the IL-6 signaling pathway and is associated with a favorable clinical outcome and safety profile. This provides proof of concept that molecular intervention targeting IL-6R is a viable modality of treatment in systemic JIA as an inflammatory disease.

Treatment of JIA has changed dramatically over the decades. Introduction of weekly methotrexate administered orally has provided remarkable clinical improvement of oligoarthritis and polyarthritis of JIA. For intractable cases, anti-TNF biologic response modifiers, etanercept and adalimumab, have recently emerged as therapeutic options. However, systemic JIA has been left alone behind these therapeutic progresses. The overall efficacy of anti-TNF therapy (etanercept, infliximab and adalimumab) and anti-IL-1 therapy (anakinra) for patients with systemic JIA was reported to be approximately 10% or less [61] and less than half of the patients, respectively [24]. Children with this disease are still under the long-term use of systemic corticosteroids, which inevitably leads to various disorders including iatrogenic Cushing's disease, growth retardation, bone fracture or cataracts. Tocilizumab is effective in children with systemic JIA and is generally safe and well tolerated. It might therefore be a suitable treatment in the control of this disorder, which has so far been difficult to manage. Phase III trials of tocilizumab are now in progress in the EU and the USA. In the near future, tocilizumab will hopefully be available worldwide.

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Key issues

- Tocilizumab has shown clinical improvements in signs and symptoms of systemic juvenile idiopathic arthritis.
- Disease-associated laboratory changes, acute-phase reactant levels, chronic anemia and hypoalbuminemia have been abruptly normalized with tocilizumab treatment.
- Molecular intervention therapy targeting the IL-6 signaling pathway indicated the possible role played by IL-6 in systemic juvenile idiopathic arthritis.
- Although tocilizumab is generally safe and well tolerated, long-term safety data such as data on malignancy and autoimmune diseases are unavailable at this time.

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ORIGINAL ARTICLE

PET assessment of disease activity in children with juvenile idiopathic arthritis

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Abstract
Background The degree of 18-fluorodeoxyglucose (FDG) uptake is previously reported to correlate with physical examination and laboratory tests for evaluating disease activity in patients with rheumatoid arthritis. The clinical validity of ^{18}F-FDG positron emission tomography (PET) has not been evaluated in juvenile idiopathic arthritis (JIA).
Objective To assess the relationship between ^{18}F-FDG PET uptake and disease activity in children with JIA.
Materials and methods A total of 560 joints in 28 children (mean age, 5.4 years; range, 1–16 years) with JIA who had undergone whole-body ^{18}F-FDG PET before treatment were retrospectively assessed clinically, biochemically and radiographically. PET images were assessed independently by two readers. We investigated the relationships between the degree of synovial ^{18}F-FDG uptake and radiographic and clinical symptoms and laboratory findings.
Results Joint tenderness and swelling had a positive association with abnormal ^{18}F-FDG uptake in the joint [odds ratio (OR) 5.37, 7.12, respectively]. The standardized uptake value (SUV) max correlated with the neutrophil count, plasma C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and matrix metalloproteinase (MMP) 3. Joint erosion (OR, 6.17), soft-tissue swelling (OR, 3.77), major joints involvement (OR, 3.50), tenderness (OR, 5.22), and CRP concentration in plasma (OR, 1.81) were positively associated with SUVmax.
Conclusion The degree of ^{18}F-FDG uptake may be associated with the severity of synovitis in children with JIA.

Keywords Juvenile idiopathic arthritis · Disease activity · PET · Child

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Introduction

Juvenile idiopathic arthritis (JIA) is a childhood rheumatic disease defined as clinically heterogeneous arthritides in one or more joints with swelling, pain, or limited range of movement for at least 6 weeks, with age at onset younger than 16 years [1, 2]. Detection of structural changes over time provides valuable prognostic information and helps guide therapy in active JIA. Active arthritis often prompts consideration of commonly referred to disease-modifying antirheumatic drugs (DMARDs), tumour necrosis factor (TNF) inhibitor, interleukin (IL)-1 receptor antagonist, or IL-6 receptor antagonists [3–10].

Although the activity of joint inflammation can be assessed on radiographs, the early radiographic changes are nonspecific and late changes are often irreversible. Despite a close correlation between radiographic score and inflammatory activity, several studies have revealed that MRI is superior to radiography for detecting early changes [11–15]. More importantly, by focusing exclusively on bone erosion and cartilage damage, MRI seems promising in terms of reliability and validity. On the other hand, previous studies have ignored low capability in assessment of weight-bearing major joints because acquisition of small structures in children is limited [13, 14].

Radiographs are insensitive to acute erosive changes in the cartilage. ^{18}F –fluorodeoxyglucose (FDG) is taken up by macrophages and immature granulation tissue [16]. TNF-α, which plays a major role in synovitis in patients with rheumatoid arthritis, regulates glucose transport and metabolism [17]. Becker et al. [18] reported that the degree of ^{18}F-FDG uptake correlated with physical examination and laboratory tests for evaluating disease activity in patients with rheumatoid arthritis. The clinical validity of ^{18}F-FDG PET has not been evaluated in JIA. We hypothesized that ^{18}F-FDG PET helps identify active synovitis and that ^{18}F-FDG uptake correlates with serum markers of inflammation in patients with JIA.

Materials and methods

Patients

Twenty-eight consecutive children with proven JIA who were evaluated for disease activity using PET between 2003 and 2007 at Yokohama City University Hospital, Yokohama were retrospectively enrolled. Diagnostic criteria were based on the International League of Association for Rheumatology (ILAR) classification for JIA [2]. Medical records were reviewed for patient age, gender, treatment history, physical examinations, laboratory tests and imaging studies including radiographs and PET. Evaluation of PET was performed independently by operators who had no knowledge of the results of the other studies. The clinical status and treatment of these children were unaltered during the evaluation period. This study was approved by the Institutional Review Board at our institution and all children provided their informed consent.

Radiographic study

Plain radiographs of 12 major joints: both shoulders, elbows, wrists, hips, knees, and ankles; and minor joints: both temporo-mandibular and sacroiliac joints, both hands and feet were obtained prior to treatment in all children. These had been taken in the posterioanterior projection using a computed radiography system (DRX-866HD or DRX-3535HD; Toshiba Medical Systems, Tokyo, Japan) with general plates and the following setting: 200 mA, 45–80 kVp, and 250 ms. Two radiologists (K.S. and N.K., 12 and 3 years of experience respectively) experienced in musculoskeletal radiography assessed the plain radiographs without knowledge of any information regarding clinical status. They evaluated all joints for five radiological features based on modified criteria [12]: periarticular osteopenia, erosion, joint deformities, soft-tissue swelling, and narrowed joint spaces. Erosion was defined as a cortical defect with an irregular floor. Soft-tissue swelling was defined as an eccentric or irregular fusiform swelling around a joint.

PET study

PET scans were performed before or after radiography with interval mean, 2.6 weeks; range, 0–5 weeks. Children fasted for 6 h before the PET scan. Before injecting ^{18}F-FDG, the blood glucose level was measured in all children (mean, 88 mg/dl). ^{18}F-FDG (3–5 MBq/kg) was injected in the antecubital or a more peripheral vein through an indwelling catheter. The mean injected dose was 120.5 MBq (range: 34.0–228.0 MBq). Whole-body emission and transmission scans were performed in all children. After a 60 min equilibration period during which the child rested, both emission and transmission scans were obtained to generate attenuation corrected images of all table positions. A dedicated PET scanner (SET 2400, Shimadzu, Kyoto, Japan) was used for acquisition with the following parameters: field of view (FOV), 595 mm; single position body-axis length, 20 cm; slice thickness, 3.125 mm; central resolution, 4.2 mm; and half width 5.0 mm. Reconstructed data were rendered in three-dimensional (3-D) imaging using a Butterworth filter with the following parameters: ordered subsets expectation maximization, conversion condition 1.0; subset count 20; cut-off value 10; order 2; and iterations 2. The 3-D image sets were available for review at slice thickness of 12 mm. The maximum standardized uptake value (SUVmax) was analyzed according to the following equation: SUV max = maximal count × calibration factor (kBq/ml)/injected activity (MBq)/body weight (kg).

Image interpretation

PET images were reviewed on a commercially available image viewer (Synapse; Fujifilm Medical, Yokohama, Kanagawa, Japan). Images were analyzed visually and quantitatively by two nuclear medicine physicians (U.T. and T.O., 15 and 3 years of experience) who were unaware of clinical and radiographic findings. They recorded their findings after reaching a consensus. For the visual analysis, abnormal ^{18}F-FDG uptake was defined as substantially greater activity than in the aortic blood on attenuation-corrected images. A region of interest (ROI) was outlined within areas of joints with increased ^{18}F-FDG uptake and measured on each slice. ROIs for determination of regional uptake were hand drawn around the areas of uptake. When the lesion was extensively heterogeneous, the ROI was set so as to cover all of the components of the lesion. When no significant uptake was identified, ROIs were arbitrarily drawn on an axial image in an area thought to represent

synovium. The maximum pixel value in each ROI was recorded for determination of SUVmax. Radiologist (N.K., 3 years of experience) reviewed the medical records. We defined the standard of reference for diagnosis as follow-up imaging findings of conventional radiography based on visual analysis and clinical status.

Clinical evaluation

Two experienced paediatric rheumatologists (>20 years of experience) performed the clinical evaluation. Symptoms at presentation were evaluated based on the International League of Associations of Rheumatology (ILAR) criteria [2]. Recorded details for all children included age at onset, major or minor joint involvement, numbers of involved joints, quotidian fever, tenderness, swelling, limited range of motion, non-fixed erythematous rash, generalized lymphadenopathy, hepatosplenomegaly, pericarditis and macrophage activation syndrome. Routine physical examinations were assessed on the same morning by independent paediatricians 1 day before or after the PET study. Blood samples were taken for biochemical investigations in all children 1 day before or after the PET study. Results of these results were masked to the investigators.

Treatment and follow-up

Treatment consisted of intra-articular corticosteroids (n=24), methylpresonisolone pulse (n=24), immunosuppressants (n=20), DMARDs (n=26), and tocilizumab (n=4). Clinical manifestations were carefully monitored with physical examinations, urinalysis, blood examinations, and screening for active infections in all children during the course of the disease. The follow-up period was dated from the time of diagnosis and the mean follow-up period was 25 months (range 6–60 months). Therapeutic response was evaluated using the American College of Rheumatology Pediatric (ACR Pedi) 30 response and systemic features including quotidian fever, non-fixed erythematous rash, generalized lymphadenopathy, hepatosplenomegaly and pericarditis [19].

Statistical analysis

Comparison of categorical variables between groups was performed using chi-square test and Fisher exact test. Fisher exact test was used when the number of cells was <5. Continuous values were compared with Student's t-test. Simple linear regression was used to compare continuous variables. Univariate regression analyses were performed to assess the variables affecting metabolic activity by comparing categorical distributions with calculation of odds ratio (OR). Multivariate analyses were applied with regression modelling for parameters affecting metabolic activity with marginally significant ($P \leqq 0.1$) variables from univariate analyses offered using a manual step-wise procedure to test the independence of factors for metabolic activity. A priori variables based on previous studies were tested. A P value <0.05 (two-tailed) was considered to

Table 1 Characteristics at presentation in 28 children with JIA

Age, years	5.4±4.0
Boys:girls	12:16
Number of involved joints	
Major joint [a]	6.3±3.6
Minor joint [a]	1.1±0.8
General symptoms	
Quotidian fever	24/28 (86%)
Non-fixed erythematous rash	20/28 (71%)
Macrophage activation syndrome	5/28 (18%)
Pericarditis	4/28 (14%)
Generalized lymphadenopathy	3/28 (11%)
Hepatosplenomegaly	3/28 (11%)

[a] Data are presented as mean ± standard deviation

Table 2 Laboratory results at presentation in 28 children subsequently diagnosed with JIA

	Value	Normal range
Red blood cell, /mm^3	453.8±47.4	Boys:420.0–550.0
		Girls:380.0–480.0
Haemoglobin, g/dl	11.8±1.8	Boys:13.5–17.0
		Girls:11.5–14.5
Haematocrit, %	35.6±5.0	Boys:40.0–52.0
		Girls:34.0–44.0
White blood cells, /mm^3	15,100±9,316	3,500–9,000
Neutrophils, %	75.9±14.2	40.0–70.0
Lymphocytes, %	17.9±10.9	20.0–45.0
Platelets, /mm^3	46.4±16.7	16.0–36.0
Aspartic aminotransferase, U/l	26.4±14.5	10.0–35.0
Creatinine phosphokinase, U/l	30.9±37.9	Boys:55.0–290.0
		Girls:40.0–290.0
Lactate dehydrogenase, U/l	281.3±123.0	120.0–240.0
CRP, mg/dl	5.3±4.9	0.0–0.2
Serum amyroid A, μg/ml	513.3±720.5	0.0–10.0
Ferritin, ng/ml	568.9±138.6	Boys:37.0–281.0
		Girls:5.0–71.0
ESR, mm/hr	43.6±31.9	Boys:2.0–10.0
		Girls:3.0–15.0
MMP 3, ng/ml	239.0±190.5	17.3–59.7
Rheumatoid factor, IU/ml	113.0±383.3	0.0–16.0
Antinuclear antibody, titre	69.6±68.5	<40.0

Data are presented as mean ± standard deviation

indicate a statistically significant difference. Statistical analysis was performed with the PASW Statistics 18 software program (IBM SPSS Inc., Chicago, IL, USA).

Results

Twenty-two out of 28 children (79%) had systemic-onset JIA and 6 (21%) polyarticular JIA (more than four joints affected). The patient characteristics are shown in Table 1. The mean age was 5.4 years, range 1–16 years. The numbers of involved major joints were 6.6±3.3 [mean±standard deviation (SD)] in systemic-onset JIA and 5.2±4.9 in polyarticular JIA. The number of involved minor joints was 1.0±0.9 in systemic-onset JIA and 1.3±0.5 in polyarticular JIA. Extra-articular ^{18}F-FDG accumulations were found in 21/28 children (75%). Frequent locations were spleen in 9/28 (32%) children and bone marrow in 6/28 (21%) children. The results of laboratory tests are summarized in Table 2.

On ^{18}F-FDG PET, 123 of 560 joints (22%) had abnormal uptake (Figs. 1 and 2). The most frequent major joints were shoulder (34/123, 28%), knee (23/123, 19%), hip (21/123, 17%) and wrist (17/123, 14%). Of these, frequencies of joint involvement in each location were similar between children with systemic-onset JIA and children with polyarticular JIA, whereas involvement of joints including ankle, elbow, metacarpophalageal (MCP), metatarsophalangeal (MTP), sacroiliac, and temporo-mandibular (TM) was found only in children with systemic-onset JIA (Table 3). The mean SUVmax of all joints of all cases was 1.0±0.3 (Fig. 3). The SUVmax in clinically involved joints was significantly higher than in unaffected joints (2.0±0.6 versus 0.7±0.3, $P<0.0001$).

Frequent radiographic findings were soft-tissue swelling (104/560, 19%) followed by narrowed joint spaces (46/560, 8%) and joint deformity (40/560, 7%). Significant differences between systemic-onset JIA and polyarticular JIA were found in frequencies of radiographic narrowed joint spaces ($P<0.0001$), joint deformity ($P=0.001$), erosion ($P=0.004$) and soft-tissue swelling ($P=0.008$). However, there was no difference in observed osteopenia ($P=0.198$).

In univariate analyses, non-fixed erythematous rash ($P=0.04$), generalized lymphadenopathy ($P<0.01$), hepatosplenomegaly ($P<0.01$) and macrophage activation syndrome ($P<0.01$) were significantly associated with quantitative ^{18}F-FDG uptake. No association was found with other variables, including age at onset, quotidian fever, pericarditis and extra-articular accumulations. Multiple major or minor joints involvement was associated with quantitative ^{18}F-FDG uptake. The joint-specific symptoms (tenderness and swelling) were also positively correlated with SUVmax, whereas limited range of motion was not (Table 4). Radiographic findings including soft-tissue swelling, nar-

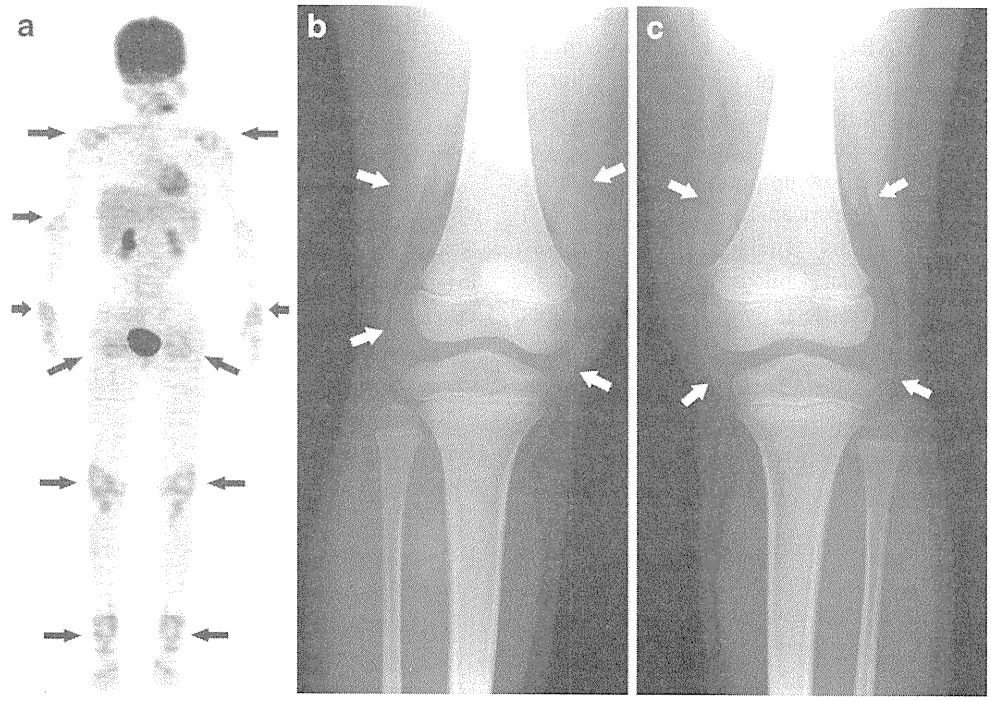

Fig. 1 **a** Whole-body ^{18}F-FDG PET performed at presentation in a 5-year-old girl with systemic-onset JIA shows abnormal uptake of 12 major joints as well as bilateral MCP and MTP joints (*arrows*). The SUVmax of the involved joints ranged from 1.0 (left elbow) to 2.8 (left knee). Physical examination revealed pain and swelling in the wrist, knee, ankle and MCP joints bilaterally. **b**, **c** Radiographs of the knee joints (highest SUVmax) revealed soft-tissue swelling only (*arrows*)

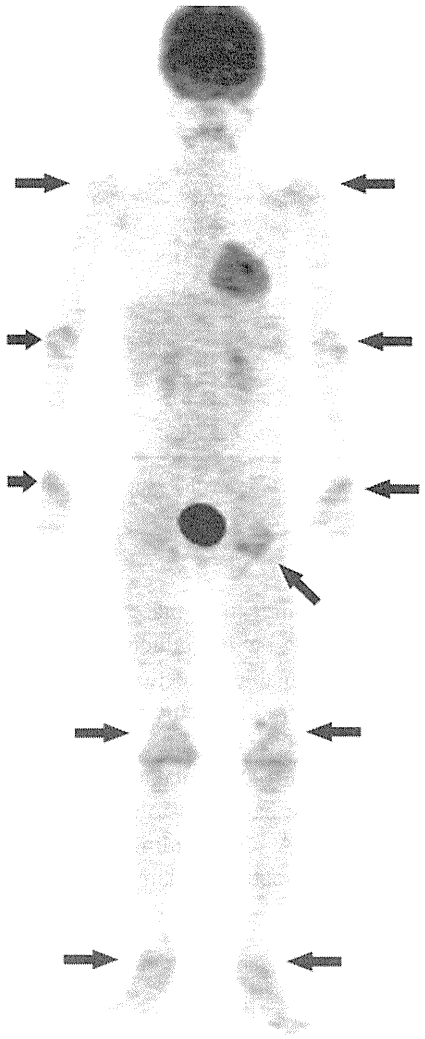

Fig. 2 9-year-old girl with polyarticular JIA. Whole-body ^{18}F-FDG PET performed at presentation shows abnormal uptake of six major joints (*arrows*). The SUVmax of the involved joints ranged from 0.9 (left elbow) to 2.3 (left hip and right ankle). Physical examination revealed tenderness and swelling in elbows, wrists, knees, and ankles as well as limited range of motion in elbows

rowed joint spaces, joint deformity, erosion and osteopenia were associated with higher ^{18}F-FDG uptake. There was a correlation between degree of ^{18}F-FDG uptake and several pathological parameters, including, CRP (r=0.610, P=0.001), neutrophil count (r=0.385, P=0.043), ESR (r=0.376, P=0.049) and MMP 3 (r=0.388, P=0.049) correlated significantly with increased SUVmax (Fig. 4). However, there was no relationship seen between increased SUVmax and other laboratory findings.

Results from the multivariate regression modelling are shown in Fig. 5. Factors associated with increased SUVmax of the joint included erosion [OR, 6.17; 95% confidence interval (CI), 2.60–14.66; P<0.0001], tenderness (OR, 5.22; 95%CI, 2.85–9.57; P<0.0001), soft-tissue swelling (OR, 3.77; 95%CI, 2.22–6.41; P<0.0001), the presence of

Table 3 Clinically involved joints at presentation in 28 children with JIA

Location	Systemic-onset JIA	Polyarticular JIA
Shoulder	26/44 (59%)	7/12 (58%)
Knee	19/44 (43%)	4/12 (33%)
Hip	18/44 (42%)	3/12 (25%)
Wrist	13/44 (30%)	4/12 (33%)
Ankle	10/44 (23%)	0
Elbow	7/44 (16%)	0
MCP	3/44 (7%)	0
MTP	2/44 (5%)	0
Sacroiliac	2/44 (5%)	0
TM	1/44 (2%)	0

multiple major joints involvement (OR, 3.50; 95%CI, 2.10–5.83; P<0.0001) and CRP (OR, 1.81; 95%CI, 1.09–3.02; P=0.022). However, swelling of the joint, the presence of multiple minor joints involvement, osteopenia, joint deformity, narrowed joint spaces, neutrophil count, ESR and MMP 3 were not associated with uptake. During follow-up, 23 children (85%) achieved ACR Pedi 30 response or improvement of systemic features. The remaining 4 children (15%) received subsequent treatment.

Discussion

We found a significant association between the degree of ^{18}F-FDG uptake and typical clinical, radiographic and biochemical findings in JIA. These factors were erosion, soft-tissue swelling, multiple major joint involvement,

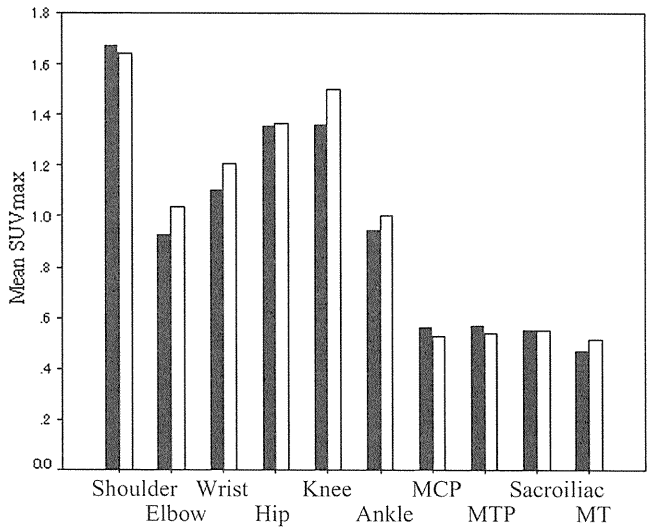

Fig. 3 Graph shows mean SUVmax in 28 children with untreated JIA at ^{18}F-FDG PET. Bar represents right (*black*) and left (*white*) joints. TM, temporo-mandibular

Table 4 Radiographic and clinical findings in major and minor joints at presentation in 28 children with JIA

Variables	*n*	OR	95%CI	*P* value
Radiographic findings				
Soft-tissue swelling	103 (18%)	3.38	2.17–3.27	<0.0001*
Narrowed joint spaces	46 (8%)	4.14	2.32–7.40	<0.0001*
Joint deformity	40 (7%)	3.23	1.72–6.07	<0.0001*
Erosion	29 (5%)	5.68	2.83–11.38	<0.0001*
Osteopenia	6 (1%)	18.47	3.86–88.43	0.002**
Symptoms				
Swelling	63 (11%)	5.37	3.24–8.92	<0.0001*
Tenderness	60 (11%)	7.12	4.27–11.87	<0.0001*
Limited range of motion	32 (6%)	1.67	0.77–3.60	0.191*

Comparisons were performed using chi-square test* or Fisher exact test**. The OR was calculated from the ratio of positive to negative radiographic or clinical findings

tenderness and elevated CRP. In a previous study, the feasibility of glucose metabolism was assessed among patients with rheumatoid arthritis [20]. Quantification of ^{18}F-FDG uptake within joints correlated well with standard clinical measures, suggesting that the degree of ^{18}F-FDG uptake can reflect anatomic and physiologic changes of synovitis and may be used to distinguish active synovitis from chronic change. The intra-individual variability in degree of ^{18}F-FDG uptake was small among patients with rheumatoid arthritis, indicating that quantification of glucose metabolism may be reproducible so that ^{18}F-FDG uptake may be a reasonable reflection of active synovitis.

We also found that radiographic findings of osteopenia, joint deformity and narrowed joint spaces did not correlate with the degree of ^{18}F-FDG uptake, suggesting that PET findings represent joint pathophysiology in the acute phase of inflammation in JIA. Our findings are consistent with previous reports that active inflammation relates to in-

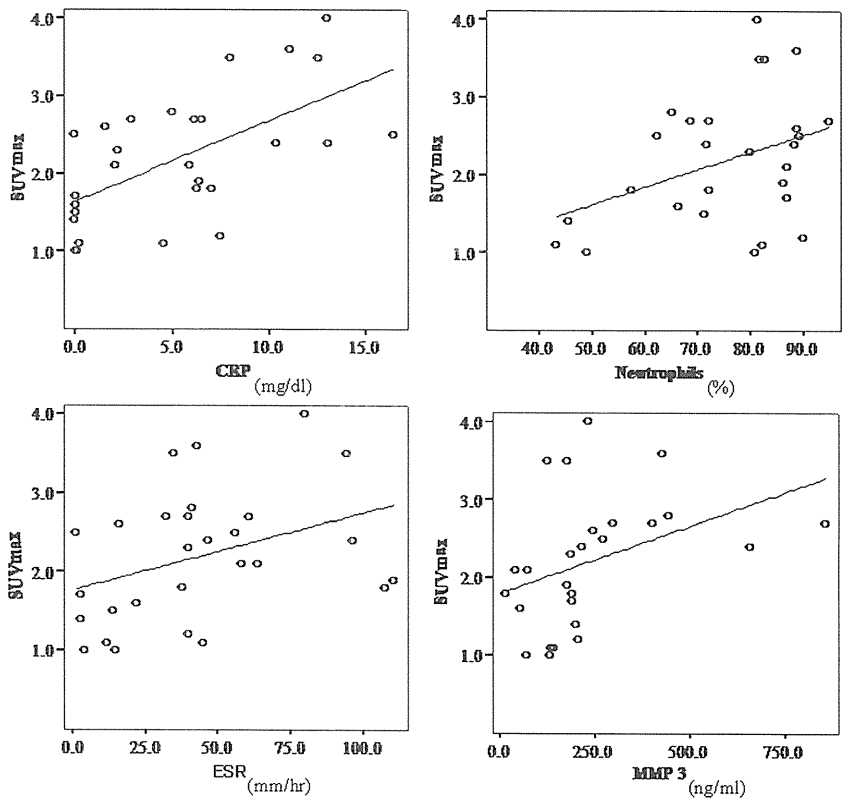

Fig. 4 Scatter plots of SUVmax and CRP, SUVmax and neutrophil, SUVmax and ESR, and SUVmax and MMP 3 with fitted regression lines are shown, whereas no relationship was observed between SUVmax and other laboratory findings. Correlations of SUVmax and CRP [(mg/dl); r=0.610; P=0.001], neutrophil [(%); r=0.385; P=0.043], ESR [(mm/hr); r=0.376; P=0.049] and matrix metalloproteinase MMP 3 [(ng/ml); r=0.388; P=0.049]

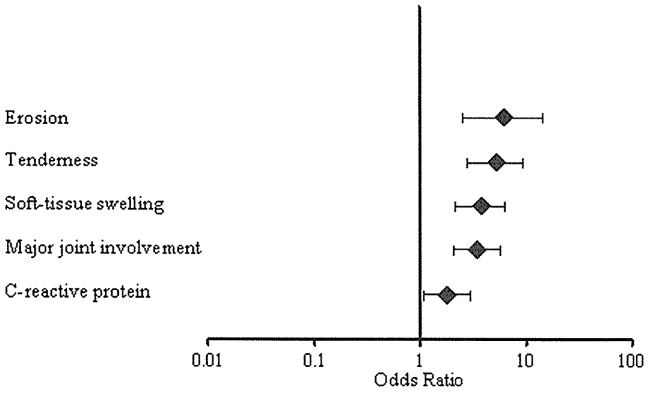

Fig. 5 Graph shows factors associated with SUVmax at ^{18}F-FDG PET in 28 children with JIA. These radiographic findings and symptoms were compared with SUVmax in the same joint. OR was calculated based on positive ratio of radiographic findings and symptoms compared with negative

creased glucose metabolism in patients with rheumatoid arthritis [20, 21]. This suggests that ^{18}F-FDG PET may be helpful in monitoring therapeutic response in JIA.

The association of the degree of ^{18}F-FDG uptake with radiographic erosion was interesting. Erosion may be caused by destruction of bone and cartilage. Elzinga et al. [22] reported increased glucose metabolism due to an inflammatory process involved in the mechanism of cartilage destruction in patients with osteoarthritis. Secondary synovitis in osteoarthritic joints may cause increased metabolic activity. As a result of active inflammation, secondary synovitis may change into fibrotic synovial tissue. Another contributing factor in the relationship between an increased glucose metabolism and erosion may be activated leukocytic infiltration within the pannus admixed with lymphocytes and fibroblasts [20].

Bone erosion is seen in the end stages of the disease process in JIA. Therefore, ^{18}F-FDG PET may underestimate erosive changes because chronic burnt-out erosions may be "cold". The capability of differentiation between acute and chronic erosive changes on ^{18}F-FDG PET is unclear. Irreversible chronic erosive changes could not be precisely evaluated on ^{18}F-FDG PET in our study. Further comparative studies of US, MRI and ^{18}F-FDG PET are required to clarify the relationship between imaging findings and acute or chronic erosive changes.

It has been established that increased glucose metabolism is found in active synovitis in patients with rheumatoid arthritis [20, 21]. The correlations between neutrophil count, CRP, ESR, MMP 3 and increased glucose metabolism in our study confirms results from a previous study on active rheumatic synovitis in which SUV increased in parallel with serum CRP and MMP 3 stimulated by TNF-α [23]. This is further suggestion that ^{18}F-FDG uptake may have a role to guide therapy, and in particular to predict response to anti-TNF-α therapy.

A few reports have investigated therapeutic monitoring by ^{18}F-FDG PET in patients with rheumatoid arthritis. Pollsson et al. [20] reported two patients with rheumatoid arthritis undergoing low-dose prednisone and methotrexate therapy, and showed that ^{18}F-FDG PET correlated well with standard activity scores. Goerres et al. [21] described the feasibility of ^{18}F-FDG PET in the assessment of Infliximab therapy in patients with rheumatoid arthritis. This suggests that a decline in ^{18}F-FDG uptake within a joint on subsequent PET scans may reflect therapeutic response over the course of systemic therapy in patients with JIA. Although our data suggest that changes in the degree of ^{18}F-FDG uptake may be a feasible marker for disease activity, it is necessary to validate these findings through clinical trials with adequate sample size, a wide spectrum of disease severity and duration and with control groups.

Despite consensus that active synovitis within a joint shows a significant degree of ^{18}F-FDG uptake in patients with rheumatoid arthritis, based largely on observational data, it is not clear what threshold values are predictive for active synovitis [24, 25]. Elzinga et al. [22] reported that 76% of joints with synovitis showed increased ^{18}F-FDG uptake, whereas many joints without clinical signs of active disease showed normal uptake in patients with rheumatoid arthritis. In contrast, false-negative and false-positive rates of ^{18}F-FDG PET to detect active synovitis in patients with rheumatoid arthritis were 13% and 11%, respectively [22]. This diagnostic accuracy may be affected by the patient populations, the onset of disease, therapeutic regimens and the presence of subclinical synovitis.

Other limitations of our study were its retrospective nature, the small population enrolled and a low prevalence of chronic erosions. It would have been preferable to compare results with other imaging modalities that measure active synovitis within joint, such as MRI or US. In addition, our interpretation has only surrogate observations for the degree of synovitis without directly measurable gold standard, e.g., biopsy. However, these tests were not feasible. A strength of our study was the availability of a detailed, comprehensive work-up of characteristics in all cases.

Conclusion

We report a possible link between the degree of synovial inflammation and ^{18}F-FDG uptake in children with JIA. We suggest that ^{18}F-FDG PET may have a role in therapeutic management of JIA, pending further validation.

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PEDIATRIC RHEUMATOLOGY

CASE REPORT Open Access

Efficacy of thalidomide in a girl with inflammatory calcinosis, a severe complication of juvenile dermatomyositis

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Abstract

We report a 14-year-old girl with juvenile dermatomyositis (JDM) complicated by severe inflammatory calcinosis successfully treated with thalidomide. She was diagnosed as JDM when she was 4 years old after a few months of increasing lethargy, muscle pain, muscle weakness, and rash. During three months, clinical manifestations and abnormal laboratory findings were effectively treated with oral prednisolone. However, calcinosis was recognized 18 months after disease onset. Generalized calcinosis rapidly progressed with high fever, multiple skin/subcutaneous inflammatory lesions, and increased level of CRP. Fifty mg/day (1.3 mg/kg day) of oral thalidomide was given for the first four weeks, and then the dose was increased to 75 mg/day. Clinical manifestations subsided, and inflammatory markers had clearly improved. Frequent high fever and local severe pain with calcinosis were suppressed. The levels of FDP-E, IgG, and tryglyceride, which were all elevated before the thalidomide treatment, were gradually returned to the normal range. Over the 18 months of observation up to the present, she has had no inflammatory calcinosis, or needed any hospitalization, although established calcium deposits still remain. Her condition became painless, less extensive and less inflammatory with the CRP level below 3.08 mg/dL. Recent examination by whole-body 18F-FDG-PET-CT over the 15 months of thalidomide treatment demonstrated fewer hot spots around the subcutaneous calcified lesions.

Background

Juvenile dermatomyositis (JDM) is a systemic connective tissue disease characterized by typical skin rash and chronic muscle inflammation of uncertain etiology [1]. Classic JDM presents with an insidious progression of malaise, easy fatigue, muscle weakness, fever, and rash that may predate diagnosis by three to six months. Calcinosis is one of the severe complications of JDM, and despite recent progress in the treatment of this disorder, it still occurs in up to 40% of patients [2,3]. The onset of calcinosis usually occurs one to three years after that of the illness.

Our understanding of the pathogenesis of calcinosis is still very limited. However, it has begun to come into focus through the following recent findings. The calcinosis itself is associated with inflammation. It has been reported that macrophages and proinflammatory cytokines, such as IL-6, IL-1, and TNF-alpha, were present in the white, calcium-rich fluid (calcium milk) collected from a patient [4]. Moreover, calcinosis has been more frequently associated with TNF-alpha-308A promoter polymorphism, which is associated with increased TNF- alpha production by peripheral blood mononuclear cells [5].

The deposition of calcium, mostly in the skin, around the joints, and in the intermuscular fascial planes, may cause more long-term disability than the myositis itself. The relatively low incidence of calcinosis observed in recently reported case series suggests that earlier diagnosis and more aggressive treatment such as corticosteroid and immunosuppressant therapy are required [6]. However, once established, the disease is still difficult to treat.

Recently, the effectiveness of the monoclonal antibody to TNF-alpha, infliximab, in the treatment of refractory juvenile dermatomyositis with calcinosis has been reported [7]. In that study, calcinosis was still present in all five patients who received infliximab, but, notably, it

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became softer, painless and, in four cases, less widespread.

Thalidomide has been accepted as an immunomodulatory drug in refractory pediatric autoimmune diseases such as Behcet's disease and systemic onset juvenile idiopathic arthritis [8,9]. It has been demonstrated that thalidomide selectively inhibits the TNF-alpha and IL-6 mRNA expression in human peripheral blood mononuclear cells [10]. The inhibitory effect of thalidomide on monocyte TNF-alpha is thought to be the main mechanism of its action as an anti-inflammatory agent [11].

We report a case of severe calcinosis in a 14-year-old girl who was treated with thalidomide, a treatment encouraged by the partial effectiveness of etanercept, a soluble TNF receptor fusion protein.

Case presentation

The patient was a girl of 14 years of age, in whom JDM had been diagnosed in January 1998, when, at 4 years of age, she had been experiencing increasing lethargy, muscle pain, muscle weakness, and rash for a few months. Calcinosis had been recognized 18 months after the diagnosis was made (Fig. 1). For 3 months, clinical manifestations and abnormal laboratory findings were effectively treated with oral prednisolone. Her muscle weakness and elevated muscle-derived enzymes were normalized with this treatment, and the myositis was stable after that. However, generalized calcinosis progressed rapidly with high fever, multiple skin and subcutaneous inflammatory lesions, and an increased level of CRP. Examination of the subcutaneous calcium milk revealed markedly elevated levels of IL-6, TNF-alpha, and IL-1beta by ELISA (Fig 2). Methylprednisolone pulses, cyclophosphamide, cyclosporine, azathioprine, probenecid and magnesium hydroxide and aluminum hydroxide were administered, but these treatments failed, resulting in repeated rupture, drainage and resection at calcinosis sites (Fig 3). "Inflammatory calcinosis" events, defined as subcutaneous inflammation caused by calcification with one or more of the following, (1) pain (VAS>50 mm), (2) fever (>38.0°C, and (3) an elevated level of CRP (>5 mg/dL), were not suppressed by the conventional treatments at all. As shown in Fig. 4, fusion imaging systems combining 18F-FDG PET (Fluorodeoxyglucose-Positron Emission Tomography) and CT visualized anatomical location of the hot spot lesions of inflammatory calcinosis. Pathological evaluation of this inflammatory calcinosis revealed calcium plaques with fibrinoid vasculitis, inflammatory cell infiltration, hemorrhage and degeneration of adipose cells. (Fig. 5)

Infliximab, the monoclonal antibody to TNF-alpha, was administered in 2003, when the patient was 9 years

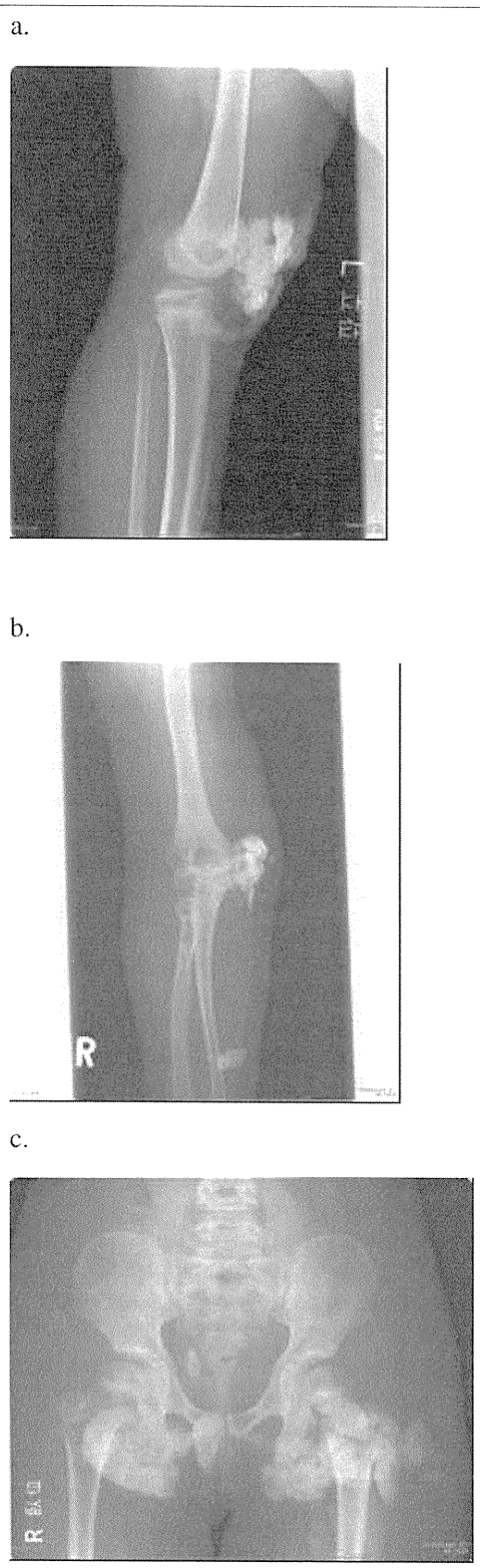
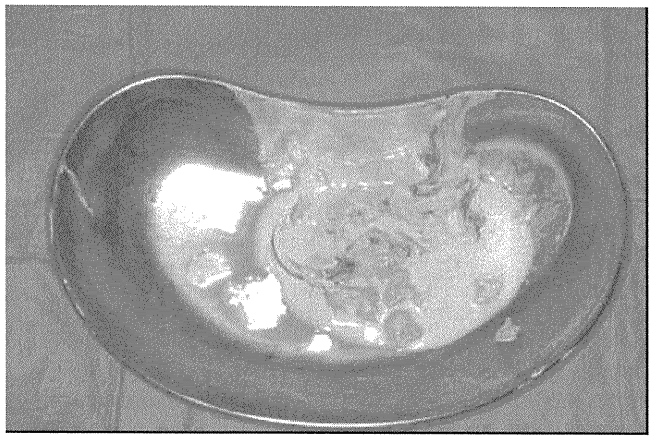


Figure 1 X-ray findings of extremities and pelvis. a. Calcium deposit around right knee in April 2001, 3 years and 3 months after JDM onset. b. Subcutaneous calcium nodule over right elbow in January 2003, 5 years after JDM onset. c. Tumoral calcinosis in the buttocks in September 2003, 5 years and 8 months after JDM onset.



	IL-12 p70	TNF-α	IL-10	IL-6	IL-1β	IL-8
Calcium fluid (pg/mL)	**6.6**	**63,148**	**460.9**	**1,090,669**	**4.325**	**67,144**
Serum (pg/mL)	**17.1**	**41.2**	**10.7**	**63.5**	**5**	**323.2**

Figure 2 Calcium milk drained with small amounts of calcium deposits from buttock (top). Characteristics of proinflammatory cytokines in serum and calcium milk. (bottom). Examination of subcutaneous calcium milk revealed highly elevated levels of IL-6, TNF-α, and IL-1β.

old, but it was discontinued due to adverse effects and replaced with etanercept. Being encouraged by the partial effectiveness of subcutaneous injection of 25 mg (0.8 mg/kg) of etanercept twice a week, her high fevers became less frequent. However, surgical approaches to the removal of the calcium deposits were still required because of inflammatory calcinosis with severe pain, rupture, or both at the calcinosis sites.

Etanercept treatment was replaced with thalidomide treatment in 2006 at the age of 12 after receiving approval from the ethical committee and informed consent from the patient and her parents. Fifty mg/day (1.3 mg/kg day) of oral thalidomide (Sauramide®, Penn Pharmaceuticals, Tredegar, UK) was given for the first four weeks, and then the dose was increased to 75 mg/day. Clinical manifestations subsided, and inflammatory markers had clearly improved (Table 1). Frequent high fever and local severe pain with calcinosis were suppressed. The levels of FDP-E, IgG, and tryglyceride, which were all elevated before the thalidomide treatment, were gradually returned to the normal range. Over the 18 months of observation up to the present, she has had no inflammatory calcinosis, or needed any hospitalization, although established calcium deposits still remain. Her condition became painless, less extensive and less inflammatory with the CRP level below

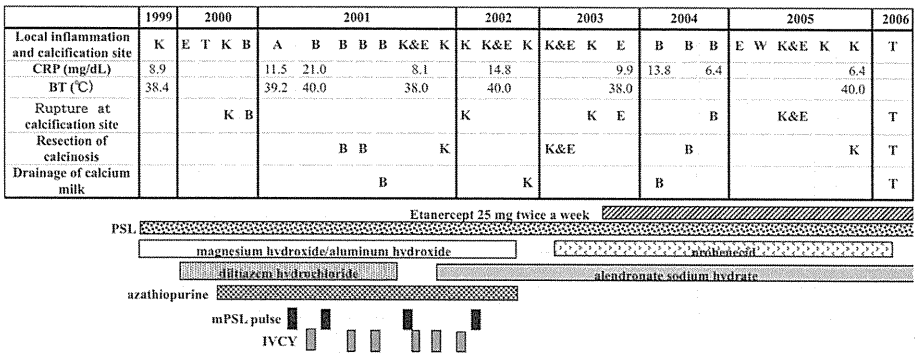


Figure 3 Clinical course of the patient and "inflammatory calcinosis" defined as subcutaneous inflammation caused by calcification with one or more of: (1) pain (VAS>50 mm), (2) fever (>38.0°), and (3) elevated level of CRP (>5 mg/dL). K: knee, E: elbow, T: thigh, B: buttock, W: wrist, mPSL: methyl prednisolone; IVCY:intravenous cyclophosphamide pulse.

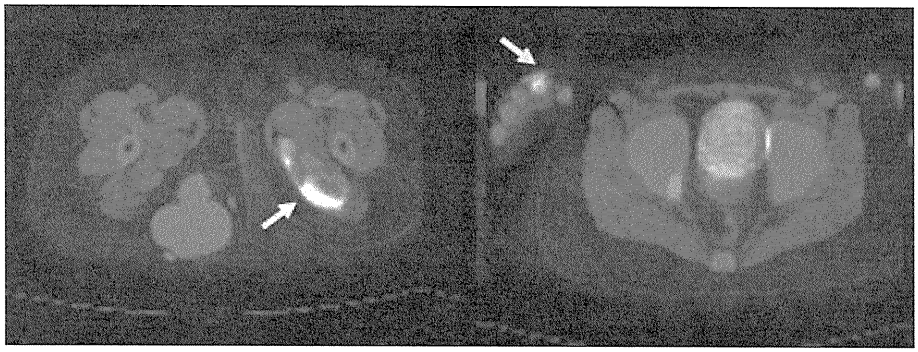


Figure 4 Findings of 18 FDG-PET/CT showing "inflammatory calcinosis"in August 2006. Subcutaneous and intramuscular calcification were recognized as in conventional plain CT. Hot spots (white arrow) along with intramuscular calcification indicate inflammation. Even the small lesion of inflammation around a right digit (yellow arrow) can be seen clearly.

3.08 mg/dL. Recent examination by whole-body 18F-FDG-PET-CT over the 15 months of thalidomide treatment demonstrated fewer hot spots around the subcutaneous calcified lesions.

Discussion

Treatment with thalidomide produced a sustained major clinical improvement in a case of JDM with inflammatory calcinosis refractory to the conventional corticosteroids and immunosuppressants and even to etanercept.

The mechanism of the inflammatory calcinosis of JDM is still unclear. Pachman *et al.* speculated that calcification occurring at sites where it is undesirable, as opposed to physiologic calcification, is usually designated as "pathologic" or, when associated with cell death, as "dystrophic" in nature [12]. Scientifically, at least, as Table 1 shows, it has been shown that inflammation accompanied by calcinosis is characterized by multiple proinflammatory cytokinemia including TNF-alpha, IL-6, and IL-1beta. The level of IL-10 in the calcium milk was also elevated. IL-10 is a cytokine with potent anti-inflammatory properties, which represses the expression of inflammatory cytokines such as TNF-alpha, IL-6 and IL-1 by activated macrophages. This finding may indicate that in parallel with the

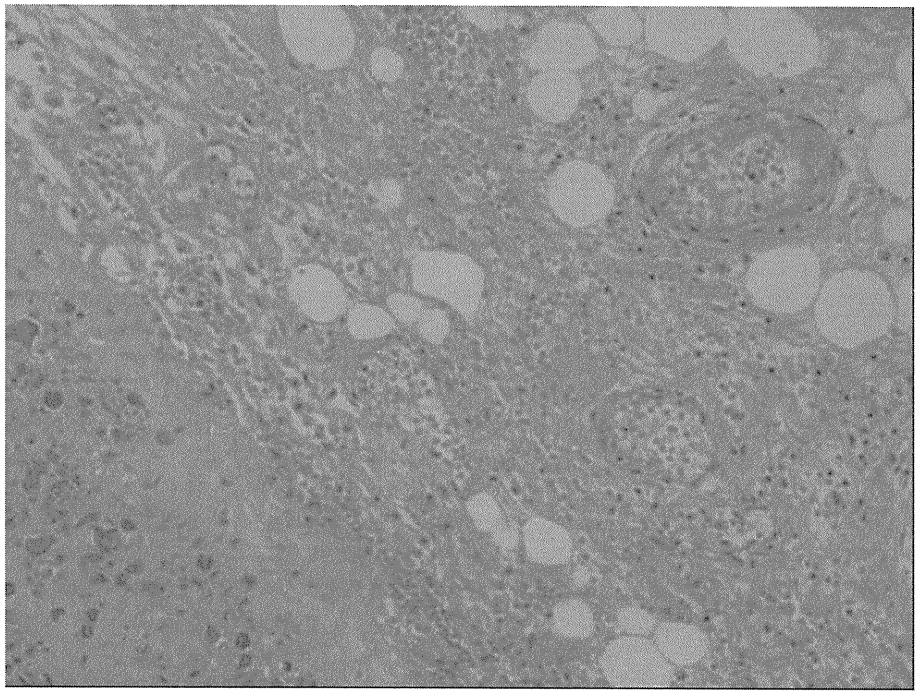


Figure 5 Light microscopy of calcium deposits with soft tissue obtained in the left thigh during surgery. Bordered with calcium plaques (arrow), fibrinoid vasculitis was observed with hemorrhage and degeneration of adipose cells. (H&E stain, 20×).

Table 1 Laboratory findings of the patient.

	2001 During inflammatory calcinosis	2006 Before thalidomide (inacitive phase)	3 months	12 months
WBC (μL)	27,300	7,100	6,400	5,200
Hb (g/dL)	11.6	11.3	9.5	12.7
Platelets ($\times 10^4$/μL)	24.7	24.6	35.7	35.7
ESR (mm/h)	27	17	3	4
FDP-E (ng/mL)	225	237	95	85
AST (U/L)	20			
ALT (U/L)	7			
LDH (U/L)	271			
CRP (mg/dL)	21.0	0.2	0.1	0.4
Triglycerides (mg/dL)	nd	251	74	93
Total cholesterol (mg/dL)	nd	121	145	156
IgG (mg/dL)	nd	2,056	813	916

The levels of FDP-E, IgG, and triglycerides were all elevated before thalidomide gradually reduced them to the normal range.

predicted activation of the immune response and tissue injury pathways caused by TNF-alpha, IL-6 and IL-1, there is simultaneous activation of pathways for the counter-regulatory and the protective mechanisms that would balance and limit the ongoing inflammatory/immune responses.

The effect of infliximab in refractory calcinosis with juvenile dermatomyositis was reported previously [7]. The anti-TNF-alpha biologics such as infliximab and etanercept are designed specifically to target TNF-alpha. Unlike the mechanism of action of the biologics, thalidomide has complex immunomodulatory and anti-inflammatory properties. It has been shown to downregulate the production of TNF-alpha and other proinflammatory cytokines, to inhibit the transcription factor nuclear factor kappa B (NF kappaB), and to downregulate cyclooxygenase 2 [13,14]. This might explain why thalidomide was more effective in the present case than etanercept. The calcified deposits contained the bone proteins osteopontin, osteonectin, and sialoprotein. Hydroxyapatite was the only mineral detected, but the tissue was distinct from bone, with an extremely high mineral content and an irregular distribution of minerals [12]. The same authors also reported that calcifications from JDM patients contained more osteonectin than is usually found in human bone. In vitro studies indicate that osteonectin can bind collagen and regulate angiogenesis, metalloproteinase expression, cell proliferation, and cell-matrix interactions [15].

Thalidomide is not only an immunomodulatory drug but also has anti-angiogenic effects [16]. It has been reported, for example, in myeloma, to suppress angiogenic factors such as vascular endothelial growth factor (VEGF), and inflammatory genes such as TNF-alpha and IL-6 [17,18]. The anti-angiogenic effect may be a part of the thalidomide mechanism that reverses inflammatory calcinosis.

The pathological specimen showed fibrinoid vasculitis surrounding calcinosis. It is hard to explain whether the inflammatory calcinosis is due to muscle vasculitis because there was no muscle vasculitis where no calcinosis was present. Muscle vasculitis arising from JDM should be symmetrical. We prefer to surmise that fibrinoid vasculitis was followed by the calcinosis universalis itself. Besides, no report of a direct relationship between vasculitis and calcinosis universalis has appeared in the literature.

The findings of 18F-FDG-PET-CT after thalidomide treatment included fewer hot spots showing local inflammation around subcutaneous calcified lesions. This suggested that the subcutaneous pooling of calcium milk may be the cause of proinflammatory cytokinemia and subsequent "inflammatory calcinosis" in JDM, and the suppression of NF kappaB activation by thalidomide is likely to be beneficial for inhibiting the systemic spread of inflammation.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor in Chief of this journal.