with proliferative LN into MMF targeted to 3 g/day and monthly IVCY and evaluated treatment response at 6 months [9]. MMF was not superior to IVCY as an induction treatment, but there was a difference in clinical response among races/regions. As a maintenance therapy for LN, AZP has been mainly used so far. MAINTAIN Nephritis Trial found no difference between MMF and AZP as a maintenance therapy [10]. On the other hand, ALMS compared the efficacy of MMF and AZP as a maintenance therapy for LN [11] and found MMF to be superior.

Based on these clinical evidences, induction and maintenance therapy for proliferative LN recommended by American college of rheumatology (ACR) is GCs with either IVCY or MMF and GCs with either AZP or MMF, respectively [12]. European league against rheumatism/European renal association-European dialysis and transplant association (EULAR/ERA-EDTA) also recommends mycophenolic acid (MMF or enteric-coated mycophenolic acid sodium) or CY in combination with GCs as an initial treatment for class $III_{A\ or\ A/C}$ ($\pm$ V) or class $IV_{A\ or\ A/C}$ ($\pm$ V) nephritis and mycophenolic acid or AZP in combination with low-dose GCs as a subsequent treatment for class III–IV or class V nephritis [13]. Although CY, AZP, tacrolimus, and mizoribine have been approved for the treatment of LN in Japan, the use of MMF for LN is yet to be approved, inducing off-label use of this immunosuppressant for patient care.

In order to clarify the real-world use of MMF as a treatment for LN in Japan, Japan College of Rheumatology surveyed the use of MMF in daily clinical practice.

Patients and methods

Adult patients with LN who visited enrolled hospitals from October 2008 to September 2013 were surveyed by referring their clinical records. Adult patients refer to patients older than 15 years when they started treatment with MMF. The enrolled hospitals were Gunma University Hospital, Hokkaido University Hospital, Hospital of University of Occupational and Environmental Health, Hyogo College of Medicine, Japanese Red Cross Wakayama Medical Center, Juntendo University Hospital, Kagoshima University Hospital, Kyoto University Hospital, National Center for Child Health and Development, National Center for Global Health and Medicine, Nippon Medical School Hospital, Okayama University Hospital, Saitama Children's Medical Center, St. Marianna University School of Medicine Hospital, Tokyo Women's Medical University Hospital, and Yokohama City University Hospital (alphabetical order). All patients in this cohort fulfilled the 1997 revised ACR criteria for SLE [14]. The following information of the patients were surveyed: age; gender; height; body weight; disease duration; pathological classification of LN according to International Society of Nephrology (ISN)/Renal Pathology Society (RPS) [15]; initial, maximum, and maintenance dose of MMF; concomitant use of GCs and other immunosuppressants; and adverse events. The efficacy was evaluated by urine protein, serum protein, albumin, creatinine, complement levels, and anti-DNA antibody titers. This observational study has been approved by the ethical committee of each institution.

Statistical analyses

Variables were summarized in numbers (percentages) for categorical data and as means $\pm$ standard deviations (SDs) or median (range) for continuous variables. Differences between baseline and the last observation were compared using Wilcoxon signed-rank sum test for urine protein and anti-DNA antibody, and paired *t*-test for other continuous variables. All tests were two-sided, and $P < 0.05$ was considered significant.

Results

Clinical features of the patients

A total of 137 patients (116 females and 20 males, 1 unidentified) from 16 institutions were enrolled. Renal specimens were obtained from 127 patients. Pathological diagnosis according to the ISN/RPS classification revealed high prevalence of class-IV nephritis followed by class-III nephritis (Table 1). Class-V nephritis combined with other classes was also prevalent.

Dose and duration of MMF

The mean of initial, maximum, and maintenance doses of MMF were 0.95 ± 0.40 g/day, 1.84 ± 0.48 g/day, and 1.37 ± 0.60 g/day, respectively. The median of initial, maximum, and maintenance doses of MMF were 1.0 g/day, 2.0 g/day, and 1.0 g/day, respectively. Time from initiation of MMF to the maximum dose was 35 days in median. Duration of MMF administration/observation period was 28 months in median, ranging from < 1 to 112 months (data available from 129 patients). Doses of MMF, according to categories of age and body weight, were compared (Table 2). Categorization was done according to the median values of age (30 years old) or body weight (55 kg). There was no correlation between the initial/maximum dose of MMF and age, body weight, or estimated glomerular filtration rate (eGFR) (data not shown). MMF was discontinued in 39 patients because of adverse event(s) in 16 patients, insufficient effect in 13 patients, and economical reason in 1 patient (some patients had more than one reason for discontinuation, and for some patients the reasons for withdrawal were not indicated).

Concomitant use of other immunosuppressive drugs

Information on the dose of GCs was available for 120 patients. These patients were given GCs with a median daily dose of 30 mg. Tacrolimus was concomitantly administrated in 35.7% of the enrolled patients. Cyclosporin, CY, AZP, and other immunosuppressants were concomitantly used in 3, 2, 1, and 4 patients, respectively (Table 1). The median of initial, maximum, and maintenance doses of MMF were 1.0 g/day, 1.5 g/day, and 1.0 g/day, respectively. Average initial, maximum, and maintenance doses of MMF in patients without concomitant immunosuppressants

Table 1. Clinical features of the patients at baseline.

	All patients ($n = 137$)	Availability of the data (n)
Age (years)	30 (16–68)	134
Weight (kg)	58.1 ± 13.8	120
Disease duration (months)	72 (1–411)	134
LN classification (ISN/RPS)		127
Class I (*N*, %)	1 (0.7%)	
Class II (*N*, %)	14 (10.2%)	
Class III (*N*, %)	20 (14.6%)	
Class IV (*N*, %)	49 (35.8%)	
Class V (*N*, %)	19 (15.0%)	
Class VI (*N*, %)	0	
III + V (*N*, %)	9 (6.6%)	
IV + V (*N*, %)	15 (10.9%)	
GCs (prednisolone, mg/day)	30 (1.5–60)	120
Concomitant use of IS		
TAC	49 (35.7%)	
CsA	3 (2.1%)	
CY	2 (1.4%)	
Others	5 (3.6%)	

GCs glucocorticoids except steroid pulse therapy, *IS* immunosuppressant, *TAC* tacrolimus, *CsA* cyclosporine, *CY* cyclophosphamide.
Values are expressed as median (minimum–maximum), mean $\pm$ SD, or number (percentage) unless otherwise stated.

Table 2. Initiation, maximum, and maintenance doses of MMF according to the patient profiles.

	Initiation dose (g/day)	Maximum dose (g/day)	Maintenance dose (g/day)	Duration (months)
Overall	0.95 ± 0.40, 1.0 (0.25–2.0)	1.57 ± 0.56, 1.5 (0.25–3.0)	1.37 ± 0.60, 1.0 (0.25–3.0)	28 (< 1–112)
Age (years)				
< 30 (n = 65)	0.88 ± 0.36, 1.0 (0.5–2.0)	1.63 ± 0.54, 1.5 (1.0–3.0)	1.54 ± 0.55, 1.5 (0.5–3.0)	50 (1–112)
30 ≤ (n = 69)	1.02 ± 0.42, 1.0 (0.25–2.0)	1.52 ± 0.58, 1.5 (0.25–3.0)	1.20 ± 0.58, 1.0 (0.25–3.0)	21.5 (< 1–100)
Body weight (kg)				
< 55 (n = 57)	0.88 ± 0.37, 1.0 (0.5–2.0)	1.51 ± 0.55, 1.5 (0.25–3.0)	1.28 ± 0.53, 1.0 (0.25–2.5)	30 (< 1–102)
55 ≤ (n = 63)	1.02 ± 0.42, 1.0 (0.5–2.0)	1.63 ± 0.60, 1.5 (1.0–3.0)	1.42 ± 0.63, 1.25 (0.5–3.0)	23 (< 1–102)

Values are expressed as mean ± SD and/or median (minimum–maximum).

were 0.99 ± 0.48 g/day, 1.65 ± 0.60 g/day, and 1.45 ± 0.59 g/day, respectively. Average initial, maximum, and maintenance doses of MMF in patients with concomitant immunosuppressant were 0.98 ± 0.30 g/day, 1.50 ± 0.57 g/day, and 1.27 ± 0.61 g/day, respectively.

Efficacy of the treatment

Median urine protein decreased from 1.88 g/gCr to 0.21 g/gCr, mean C3 level elevated from 66.4 mg/dl to 80.3 mg/dl, and median anti-DNA antibody titer decreased from 40.6 IU/ml to 10.6 IU/ml (Table 3). Daily dose of GC except steroid pulse therapy was significantly reduced during the treatment including MMF.

Adverse events

Sixty-one adverse events were reported from 39 patients during the follow-up period: alopecia in 3, cytomegalovirus infections in 6 (2 colitis and 4 viremia alone), herpes zoster in 6, common cold in 5, pneumonia in 3, sinusitis in 3, acute colitis in 1, tonsillitis in 1, bacterial infection of unknown region in 1, diarrhea in 4, nausea in 2, sigmoid perforation in 1, skin abscess in 3, leukocytopenia in 8, pancytopenia in 4, thrombocytopenia in 1, anemia in 1, thrombotic microangiopathy in 1, hyperglycemia in 2, hyperuricemia in 1, palpitation in 1, myoma uteri in 1, ovarian cancer in 1, and uterus cancer in 1. Among patients who experienced one or more adverse events, MMF was discontinued in 16 patients and MMF dose was decreased in 8 patients.

Discussions

According to the ACR guideline, the recommended dose of MMF, as an induction therapy for proliferative LN, is 2–3 g/day, whereas the recommended maintenance dose is 1–2 g/day [12]. In this recommendation by ACR, the Task Force Panel voted that Asians compared with non-Asians might require lower doses of MMF for similar efficacy at level-C evidence. Therefore, physicians might recommend 2 g/day as an induction therapy for Asian patients. Recommended target dose of MMF as an induction therapy by EULAR/ERA-EDTA is 3 g/day [13]. Specific treatment dose concerning Asian ethnicity has not been mentioned in this recommendation. Our survey revealed that relatively lower doses of MMF were given to Japanese LN patients compared with those to non-Asian patients, but appropriate induction/maintenance doses should be established in Japan. Considering the rarity of diarrhea in our survey, the selected treatment doses may partly vary because of the cost of this relatively expensive drug for off-label users or concomitant use of tacrolimus in ~35% of the patients.

Table 3. Efficacy of the treatment including MMF.

	Baseline	Last observation	p values
Urine protein (g/gCr)	1.88 (0.04–29.2) (n = 76)	0.21 (0–10.2) (n = 76)	< 0.0001*
Serum total protein (g/dl)	6.15 ± 1.26 (n = 114)	6.52 ± 1.02 (n = 114)	0.0022
Serum albumin (g/dl)	3.20 ± 0.89 (n = 115)	3.84 ± 0.70 (n = 115)	< 0.0001
Serum creatinine (mg/dl)	0.77 ± 0.50 (n = 124)	0.80 ± 0.50 (n = 124)	0.5
eGFR (ml/min/1.73 m^2)	102.3 ± 49.0 (n = 114)	89.7 ± 36.1 (n = 114)	< 0.0001
C3 (mg/dl)	66.4 ± 29.0 (n = 104)	80.3 ± 20.6 (n = 104)	< 0.0001
aDNA or anti-dsDNA (IU/ml or U/ml)	40.6 (1.67–7,400) (n = 105)	10.6 (0.5–440) (n = 105)	< 0.0001*
Dose of GCs (mg/day, PSL)	30 (1.5–60) (n = 119)	8.0 (0–50) (n = 119)	< 0.0001

Values are expressed as median (minimum–maximum) or mean ± SD.
*P values were calculated using paired t-test or Wilcoxon signed rank-sum test.

MMF used together with GCs and other immunosuppressants in some cases demonstrated significant clinical improvement, but it was not possible to evaluate the exact potential of MMF in this retrospective surveillance. Reduction in the dose of GCs indicates possible steroid-sparing effect of MMF, but this reduction seems to be a part of induction regimen in many patients.

Safety profile of MMF in Japan was presumably acceptable. MMF was discontinued in ~12% of the patients because of adverse events, but decreased doses were tolerable in some patients with adverse events. Diarrhea was not as frequent as reported, probably due to the lower induction dose of MMF. Opportunistic infections especially herpes zoster, as well as hematological abnormalities such as leukocytopenia, anemia, and thrombocytopenia, require careful observation of the patient under treatment with MMF. In terms of malignancy, gynecological neoplasms developed in 2 patients.

High-dose GCs with IVCY or MMF is the mainstream of induction treatment for proliferative LN and recommended by the above guidelines. Other reports indicate the efficacy of tacrolimus as an induction therapy for patients with proliferative LN [7,16]. Recent systematic review concludes that there was no significant difference in the potential for the induction of renal remission among CY, MMF, and tacrolimus, but MMF and tacrolimus had a tendency to be superior to CY [17]. Obviously, evidences for MMF are more rigid compared with those for tacrolimus, influencing the guidelines by ACR and EULAR/ERA-EDTA.

Clearly, this study has limitations in that this is a non-randomized retrospective observation study based on a surveillance chart. In addition, the number of patients is relatively small, patients are not necessarily consecutive, and observation periods are not long enough to evaluate clinical outcome of the patients with LN. In the near future, larger post-marketing surveillance that comprises all of the LN patients treated with MMF would help in better understanding and thus better clinical use of this immunosuppressant.

In conclusion, MMF was commonly used for the treatment of adult LN patients with acceptable efficacy and safety in Japan. The doses of MMF in daily clinical practice in Japan tended to be relatively lower than those of the Western standards, and nearly half of the patients were under treatment with other

immunosuppressants represented by tacrolimus. MMF would be an essential immunosuppressive drug for the treatment of LN in Japan, as it is in other areas of the world.

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Conflict of interest

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ORIGINAL ARTICLE

A national survey on current use of mycophenolate mofetil for childhood-onset systemic lupus erythematosus in Japan

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Abstract

Purpose. To conduct a national survey of systemic lupus erythematosus (SLE) patients treated with mycophenolate mofetil (MMF). Based on current information on the use of MMF, we aimed to evaluate its efficacy and safety for childhood-onset (c-) SLE.
Target. We evaluated 115 patients by questionnaire on MMF use for c-SLE in medical facilities specializing in pediatric rheumatic and renal diseases.
Results. Average age at SLE onset was 10.6 (range, 2–15) years; average age at the time of starting MMF was 12.3 (range, 2–15) years. Average dose per body surface area was 1,059.3 mg/m^2/day. Corticosteroid dosing was 20.9 mg/day before treatment but 7.7 mg/day after treatment. Laboratory values before and after MMF treatment were as follows: C3 increased from 67.0 to 84.9 mg/dl ($p < 0.001$), C4 increased from 10.2 to 15.1 mg/dl ($p < 0.001$), and anti-DNA antibody decreased from 154.2 to 18.4 IU/ml ($p < 0.001$). 24 adverse events in 21 cases were reported, but MMF was not discontinued in any.
Conclusions. The amount of MMF for c-SLE in Japan is similar to the standard dose in other countries. Reduction of corticosteroid dose and improvement of laboratory values represent efficacy of MMF. The side effects recorded here indicated tolerability of the drug.

Keywords

Childhood-onset systemic lupus erythematosus, Effectiveness, Lupus nephritis, Mycophenolate mofetil, Tolerability

History

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Background

According to a Japanese nationwide survey from the year 2000 conducted by Yokota et al., childhood-onset systemic lupus erythematosus (c-SLE) is a rare disease with a prevalence of 4.7 per 100,000 children. It is thought that lupus nephritis (LN) lesions affect prognosis. Since a higher frequency of LN is found in children than adults, controlling the symptoms of LN is especially important in managing pediatric SLE. Traditionally, corticosteroids (CS) are used for SLE treatment, but several problems are associated with their use in children, such as growth retardation. Additional common side effects include hyperglycemia, compromised immunity, and osteoporosis seen on high-dose long-term single-drug administration of CS. Currently, combining CS treatment with immunosuppressive drugs is an evolving strategy to minimize the amount of steroid necessary for good disease control. Cyclophosphamide (CY) is also utilized in Japan because of its proven efficacy, but a major side effect of this drug is the development of gonadal disorders related to the cumulative administered dose. Thus, safer treatment with greater efficacy is urgently required.

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Mycophenolate mofetil (MMF) is an inhibitor of *de novo* purine biosynthesis and is used as an immunosuppressive drug after organ transplantation, because lymphocytes rely on such purine synthesis for their function. Recently, several reports have shown that MMF also possesses therapeutic efficacy in LN. For moderate-to-severe LN (class III and IV), the efficacy of MMF for inducing remission was reported to be similar to or greater than CY [1–8]. Infection resulting from immunosuppression is a known complication requiring special vigilance, but it has also been shown in several studies that the infectious complications resulting from MMF treatment are also comparable to or less than that with CY [2–4,7,8]. Finally, the efficacy and safety of MMF have been reported to be similar to or higher than that with azathioprine (AZA) [9]. Based on these data, it is recommended in the clinical practice guidelines for LN by the current Europe College of Rheumatology, the European League Against Rheumatism (EULAR) [10], and the American College of Rheumatology (ACR) [11] that MMF, even though it is not approved for this indication, should be a first-line treatment. Since the number of patients for whom off-label MMF has been used has increased gradually in Japan as outlined above, the time may have now come to license it for SLE. The national survey of SLE patients treated with MMF was performed mainly by the Japan College of Rheumatology (JCR). From the collected information, here we have analyzed changes in clinical laboratory values in childhood-onset cases over the disease course under

MMF therapy. In our study, we aimed to evaluate the efficacy and safety of MMF in c-SLE.

Methods

The recorded use of MMF and its efficacy and safety were analyzed retrospectively using data from pediatric rheumatic disease and/or renal disease specialist clinics in Japan for patients with LN at onset or recurrence. We evaluated cases where MMF treatment had started before the patients reached 16 years of age.

For the purpose of acquiring data to support the licensing of MMF for c-SLE in Japan, nationwide medical facilities were surveyed concerning their use of the drug in August 2014 by the "Subcommittee on Surveillance of Mycophenolate use in Lupus Nephritis" of the Japan College of Rheumatology (Chairman: Prof. Tatsuya Atsumi, Hokkaido University Graduate School of Medicine, Division of Rheumatology, Endocrinology and Nephrology). For c-SLE patients, a request for research cooperation was sent to 12 facilities specialized in pediatric rheumatic and kidney disease treatment through accreditation from the Pediatric Rheumatology Association of Japan and the Japanese Society for Pediatric Nephrology in August 2014. A total of 147 cases were reported from these 12 facilities. Of these, 115 patients under 16 years of age had developed SLE in childhood and received MMF treatment for the onset or recurrence of LN.

The questions asked in the questionnaire were as follows: date of birth, gender, date of SLE onset, date of LN onset, histopathological diagnosis of LN before MMF administration and clinical history at MMF treatment initiation (onset or recurrent), height and weight, laboratory findings at the start of MMF administration, changes in the MMF dose, concomitant medications, MMF withdrawal or laboratory findings at the last observation time (blood biochemistry, urine, and anti-DNA antibody titer), date of onset of side effect(s), and MMF dose when the side effect occurred. We evaluated the statistical significance of changes of laboratory findings by paired Student's *t*-test, and the anti-DNA antibody titer after log transformation.

The protocol of this survey and research plan was approved by the Clinical Ethics Committee of Yokohama City University Hospital (approval number: B140601012). This study was performed also with the approval of the Ethical Committees in each of the medical facilities. The questionnaire was anonymous and unlinked to any documentation collected.

Results

Of the 147 cases submitted by the cooperating facilities, 115 were SLE patients who had started MMF treatment in childhood (defined as under 16 years of age). The cohort consisted of 23 boys and 92 girls, with an average age at SLE onset of 10.6 ± 3.4 (range, 2–15) years. Age at initiation of MMF treatment was 12.3 ± 2.7 (range, 2–15) years. The mean duration of MMF administration was 58.2 (range, 3–141) months.

Histopathology of LN (ISN/RPS classification)

Of the 115 cases, histological classification of renal status had been described in 112 prior to initiating MMF treatment, as follows: 6 class I, 25 class II, 22 class III, 48 class IV, 5 class V, 1 class III + V, and 5 class IV + V. Classification of the remaining three was unknown due to lack of response in the questionnaire (Table 1).

Table 1. Histological findings of the biopsy specimens before administration of MMF.

Class (ISN/RPS)	Cases
I	6
II	25
III	22
IV	48
V	5
III + V	1
IV + V	5
Not filled	3
Total	115

Dose

The total average MMF dose for maintenance therapy was 1,355.1 ± 423.1 mg/body/day (range, 500–2500 mg). However, there was a large degree of variability in the dose per unit weight and body surface area because of the differences in age and sizes of the patients. The average dose per body surface area was 1,059.3 ± 295.1 mg/m^2/day (range, 353.1–2192.7 mg), per body weight was 33.8 ± 11.4 mg/kg/day (range, 9.4–86.2 mg). Variation in total dose was still great even when corrected for age, body weight, and surface area (Figure 1).

Renal function and MMF dose

Uehara et al. had analyzed renal function in Japanese children aged between 2 and 18 years and established a formula for estimated glomerular filtration rate (eGFR) based on body length and the measured serum creatinine (Cr) level [12]. Here, we examined correlations of eGFR and MMF dose in 80 patients where data on body length and serum Cr level before drug administration were available. We found no correlations with body surface area or body weight (Figure 2). We identified 3 cases with eGFR decreased to under 60 mL/min/1.73 m^2, the reference value for chronic kidney disease (CKD). Further 12 cases with eGFR < 90 mL/min/1.73 m^2 required management for chronic renal failure. All these 15 patients had been given similar doses of MMF as the remaining 100. Most of the latter 15 cases experienced no adverse events over the observation period, except for one 12-year-old girl with hair loss, whose eGFR was 65.3 mL/min/1.73 m^2.

Concomitant medications in addition to CS

There were 35 patients where MMF was combined with CY and 11 with other immunosuppressive drugs including 2 receiving azathioprine (AZA), 7 tacrolimus, 2 cyclosporine, and 5 mizoribine (with some patients receiving more than one of these).

Efficacy

Almost all cases had undergone MMF therapy for at least 6 months but only one case had received MMF for 3 months, so we had excluded the case and had analyzed factors associating with efficacy in 114 patients. The CS dose was 20.9 ± 11.7 mg/day (prednisolone equivalent) at the time of starting MMF, but 7.7 ± 5.8 mg/day at the last observation time. Thus, MMF treatment was associated with a tapering of the CS dose. A significant improvement in laboratory values was observed over this period: C3 increased from 67.0 ± 28.7 to 84.9 ± 17.4 mg/dl ($p < 0.001$), C4 increased from 10.2 ± 7.7 to 15.1 ± 6.3 mg/dl ($p < 0.001$), and geometric mean anti-DNA antibody titer decreased from 48.6 to 12.7 ($p < 0.001$). Serum albumin (Alb) tended to increase from 3.8 ± 0.7 g to 4.9 ± 4.9 g/dl ($p = 0.052$). Regarding urinary findings, the number of cases was insufficient for evaluation (Figure 3).

Safety

24 adverse events of MMF were reported in 21 patients. Total person-years for MMF therapy of 115 cases are 563.0, so there

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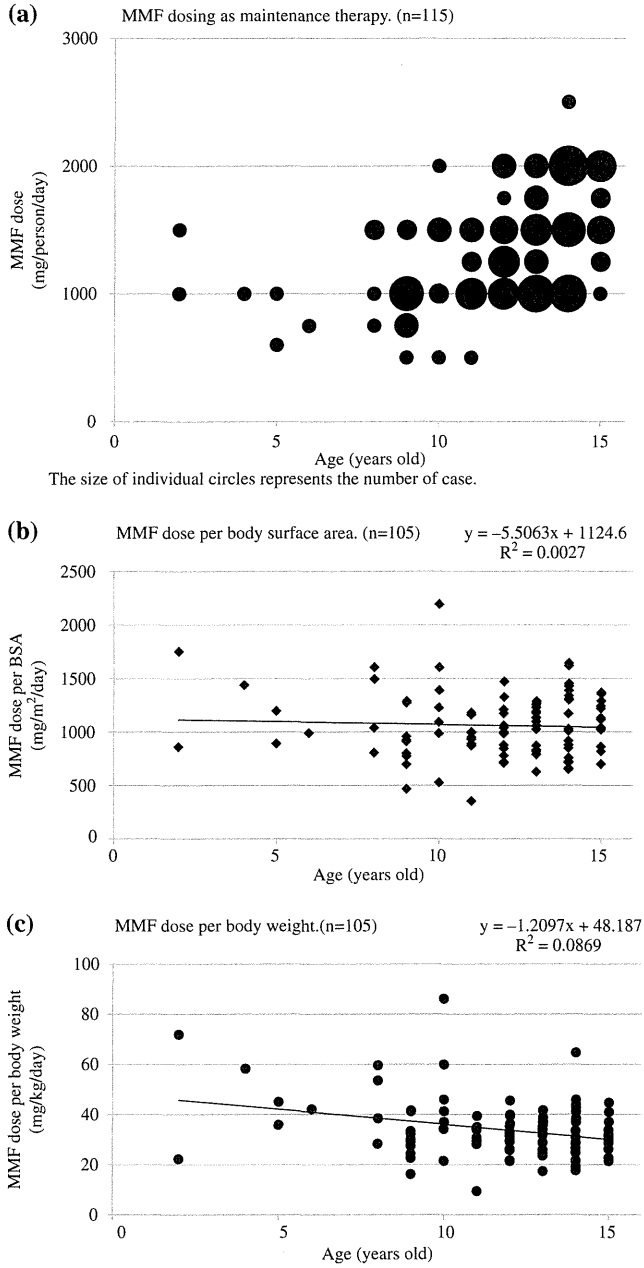

Figure 1. MMF total dose and per body indices. (a) shows total MMF dose classified by age of patients. Generally, there seemed to be some correlation between patient's age and MMF total dose. (b) presents a correlation between patient's age and MMF dose per body surface area. There were much differences of MMF dose per BSA in each age, so we did not seek any correlation between MMF dose per BSA and patient's age. (c) presents a correlation between patient's age and MMF dose per body weight. Because of much differences in each age, there also were no correlations between MMF dose per BW and patient's age. (b, c) suggest that MMF dose was determined by BSA or BW of patient, regardless of one's age.

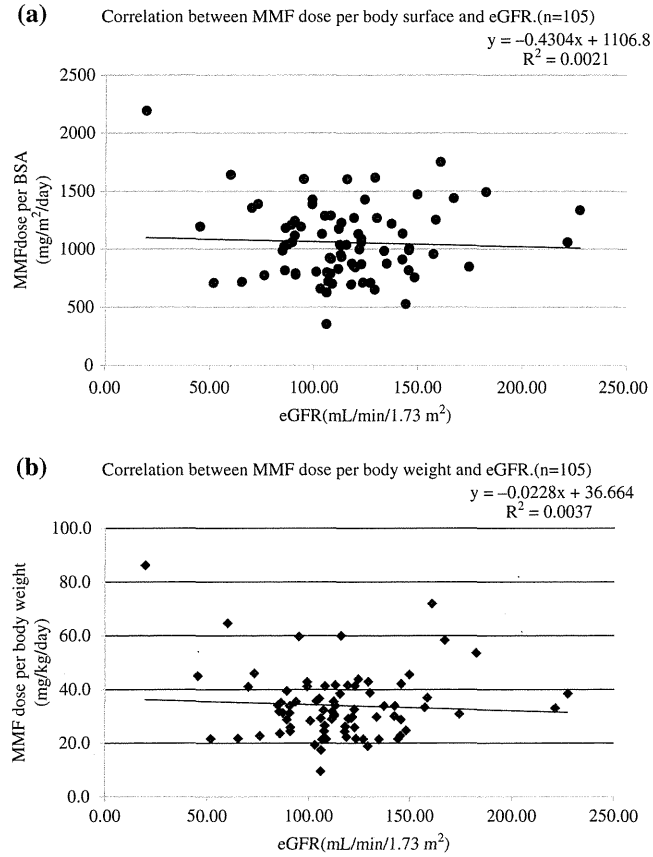

Figure 2. Correlations corrected MMF dose and renal function. (a) shows correlation between MMF dose per BSA and eGFR. There was no correlation between them. Average dose per BSA was almost equal to that for patient of renal transplantation in each age. (b) shows correlation between MMF dose per BW and eGFR. MMF dose per BW also did not correlate with eGFR. (a, b) present that physicians did not seem to count patient's renal function, but the result may rely on absence of patient with poor renal function.

were 4.3 events per 100 person-years. The most common side effect was herpes zoster, seen in 8 cases. MMF administration was restarted after a washout period in all these patients. Herpes zoster developed at different ages, and was not associated with any large differences in MMF dose relative to the average received by all the patients. The second most common side effect was the occurrence of gastrointestinal symptoms such as diarrhea, abdominal pain, and vomiting (7 cases). Gastrointestinal symptoms were treated with washout, symptomatic treatment, and herbal medicine. Finally, other side effects such as decreased levels of IgG (3 cases) and leukopenia (2 cases) were also reported. The MMF dose was decreased as a result of side effects in only 6 patients, and was not discontinued in any (Table 2).

Discussion

c-SLE is a relatively common autoimmune disease in childhood, which can be life-threatening. It is well-known that c-SLE causes systemic organ damage, and especially LN is observed more frequently (in about 70% of cases) in childhood-onset disease than in adult-onset SLE [13]. Since it is generally accepted that LN is one of the complications significantly affecting prognosis, its management is important for improving the care of SLE patients. Relative to treatment with CS alone, the prognosis of SLE, especially renal prognosis, has been greatly improved by co-administration of CY, which also allowed a reduction in the CS dose [2,14]. This is important due to the growth-stunting effect of CS in childhood-onset cases. However, side effects of CY include gonadal failure, raising later problems such as amenorrhea and fertility decline. This is therefore a challenge for the long-term management of pediatric cases. Thus, there is an urgent need for treatments with reduced side effects but with efficacy similar to or better than the combination of CS and CY.

MMF is approved in Japan for use in preventing organ transplant rejection. Due to its lymphocyte inhibitory effect, use of MMF for LN treatment has been trialed and several reports since

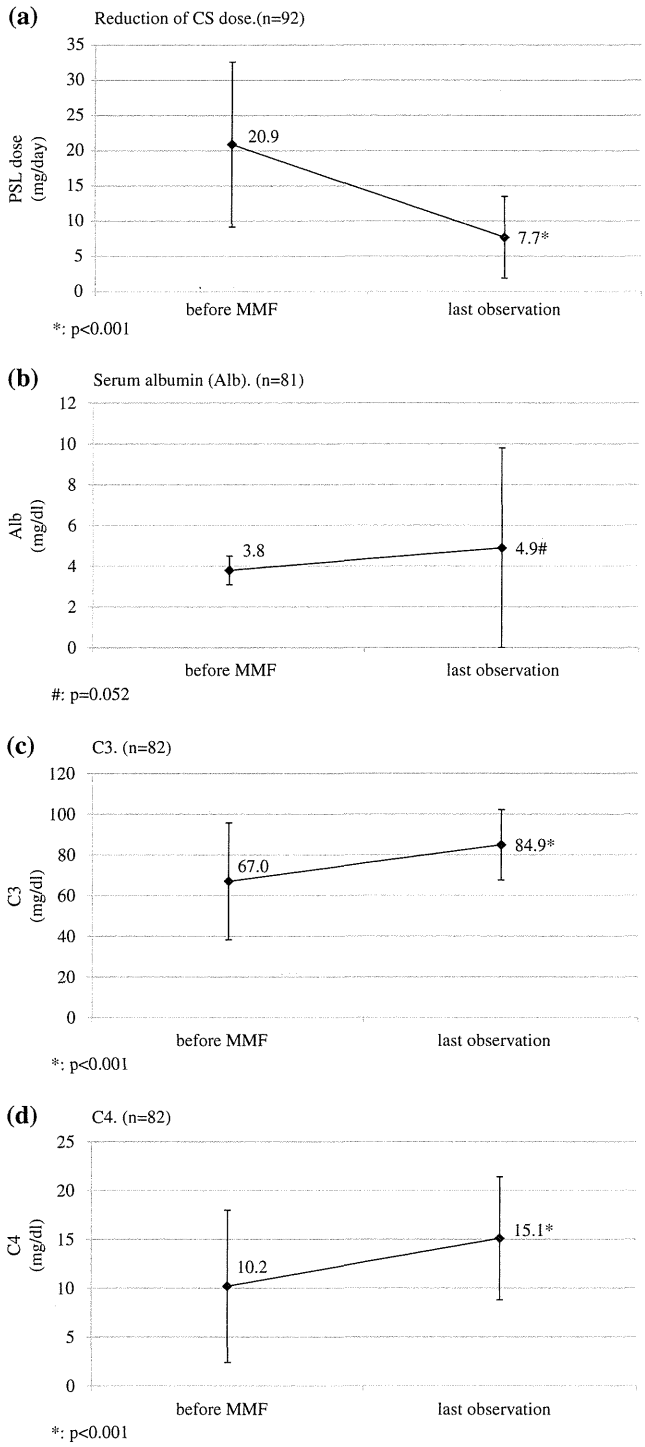

Figure 3. Changes of steroid dose and clinical values after MMF therapy. (a) presents reduction of CS dose. Significance of steroid reduction after MMF therapy shows effectiveness of MMF. (b) presents change in serum albumin. Alb concentration was not so low, but there seems to be tendency to improve after MMF therapy. (c, d) shows changes of serum complement level. Both C3 and C4 were elevated after MMF therapy, so that also suggests effectiveness of MMF therapy. (e) shows change of titer of anti-DNA antibody. Logarithm values of Ab titer were analyzed here. There also was a statistically significant improvement of anti-DNA Ab.

1998 suggest its efficacy. According to the results of studies comparing CY, AZA, and MMF, it was concluded that the efficacy of MMF was similar to that of the other drugs, but that its side effects were relatively mild [1–6,8,15,16]. Treatment with MMF was associated with lower relapse rates than AZA [9]. On the other

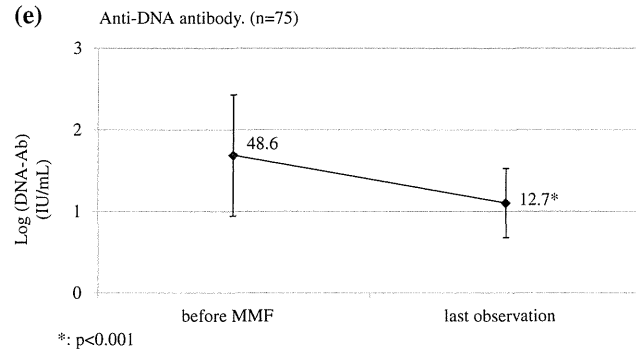

Figure 3. (*Continued*).

hand, gastrointestinal disorders and herpes zoster were observed as side effects of MMF, although the frequency of these side effects was similar to what is seen with the other drugs [2–4,7,8,15]. In addition, because CY causes gonadal failure, it was considered that the safety profile of MMF was sufficiently favorable in comparison with other immunosuppressive drugs. Based on these data, although MMF is still not approved for SLE, either MMF or intravenous CY (IVCY) was strongly recommended by EULAR/European Dialysis and Transplant Association (EDTA) in 2012 for the first-line treatment of class-III and class-IV (± V) LN and as maintenance therapy [10]. MMF administration is also positioned as a standard treatment for LN in the guidelines of the ACR [11]. According to the latter, the risk of kidney lesions in pediatric SLE patients is relatively high, but it was recognized that evidence for this is limited, and management needs to be based on an extrapolation from data obtained in adults.

Background of the survey on the current use of MMF in Japan

Due to the predicted increase of SLE/LN in Japan, a request for licensing MMF for this indication was submitted to the Ministry of Health, Labour and Welfare by the Japan College of Rheumatology (JCR) in 2011. After discussing this issue in the "Evaluation Committee on unapproved or off-label drugs with high medical needs," this request was considered to have a high degree of medical utility. Chugai Pharmaceutical Co. Ltd., accordingly received a request for drug development from the Ministry of Health, Labour and Welfare. The desire for approval was seen by the public as an industry-biased view, however. Therefore, as a result of this public knowledge-based evaluation by the Pharmaceuticals and Medical Devices Agency (PMDA), the JCR was requested to survey the current actual domestic use of MMF and to establish guidelines for optimal use and dosage. This investigation was mainly performed by the "Subcommittee on Surveillance of Mycophenolate use in Lupus Nephritis" (Chairman: Prof. Tatsuya Atsumi, Hokkaido University Graduate School of Medicine, Department of Rheumatology, Endocrinology and Nephrology) established by the JCR.

Subjects

According to the ACR guidelines and the EULAR/EDTA recommendations, patients eligible for MMF treatment were those with severe LN classified as class III/IV [10,11]. However, in the present survey, we found that in practice, relatively mild LN cases, such as class I/II, were also treated with MMF. We speculate that the reason why MMF was selected even in these mild cases was because of its ease of administration, lack of irreversible gonadal failure accompanying the alternative CY treatment, and because pediatric arthritis physicians and kidney specialists were focusing on long-term renal prognosis for the purpose of improving treatment. In the event that the efficacy and safety of MMF will be

Table 2. Reported adverse effects.

Case number	Gender	Adverse event	Age at presentation	MMF dose at presentation: Total dose (mg/d)	MMF dose at presentation: Per BSA (mg/m^2/d)	Resolution	CS dose (mg/day)	Suspect drugs
1	F	Herpes zoster	11	1250	1237	Restart with lower dose after washout (1000 mg/d)	10	MMF, PSL
2	F	Herpes zoster	10	1000	935	Restart with lower dose after washout (500 mg/d)	10	MMF, PSL
3	F	Herpes zoster	13	1000	892	Restart after washout	ND	PSL
4	F	Herpes zoster	15	1750	1115	Restart after washout	ND	CY, PSL
5	F	Herpes zoster	22	2000	1308	Restart after washout	ND	PSL
6	F	Herpes zoster	13	1750	1328	Restart after washout	ND	PSL, CY, AZA
7	F	Herpes zoster	11	1000	1091	Restart after washout	7.5	ND
8	M	Herpes zoster	14	1000	788	Restart after washout	ND	ND
9	F	Varicella	7	750	991	Restart after washout	9	MMF, PSL,CY
10	F	Diarrhea	13	1250	1096	Restart after washout	ND	ND
11	F	Diarrhea	15	2000	1451	Restart after washout	ND	ND
12	F	Diarrhea, abdominal pain	13	1000	1064	Restart after washout	7.5	MMF
13	F	Vomiting, abdominal pain	13	1250	–	Reduce dose (1000 mg/d)	ND	ND
14	F	Vomiting, abdominal pain	14	1500	1130	Chinese herb	30	MMF
		Decrease of IgG	14	1500	1130	Observation only	35	MMF, PSL, CyA
15	F	Abdominal pain	10	1000	926	Reduce dose (750 mg/d)	ND	ND
16	F	Abdominal discomfort	13	1500	1059	Add H-2 blocker	ND	MMF
17	F	Decrease of IgG	13	1250	870	Observation only	15	MMF
18	M	Decrease of IgG leukopenia	12	2000	1667	Reduce dose (1250 mg/d)	25	ND
			12	2000	1667	Reduce dose (1250 mg/d)	25	ND
19	F	Leukopenia	19	2000	1370	Reduce dose (1500 mg/d)	ND	ND
20	M	Decrease of platelet	5	500	746	Restart after washout	15	AZA
21	M	Hair loss	12	1000	718	Observation only	40	MMF
		Compromised	13	1000	718	Observation only	20	ND

MMF mycophenolate mofetil, *PSL* prednisolone, *CY* cyclophosphamide, *AZA* azathioprine, *CyA* cyclosporine A, *ND* no data.

established in future trials, it could be proposed that use of MMF is extended to other stages.

Dose

According to the ACR guidelines, 2–3 g/day in non-Asians or 2 g/day in Asians is recommended for induction MMF therapy and 1–2 g/day for maintenance therapy [11]. The EULAR consensus also approved 3 g/day as induction therapy [10]. Monitoring the plasma mycophenolic acid (MPA) concentration in renal-transplanted adult patients revealed that this was correlated with body weight rather than surface area. In that study, it was reported that the optimal dose was 24 mg/kg/day [17]. According to a report by Kazyra on c-SLE [7], the optimal dose was 20–25 mg/kg/day and the median dose at the start of MPA was 500 mg/day, and at 12 months, 1500 mg/day. On the other hand, the optimal dose in the US and EU is set to 1200 mg/m^2/day when used for suppression of rejection of renal transplants in children. A PK model has also been developed as part of the investigations into the optimal dose for c-SLE [18]. In addition, correlations between weight-adjusted dosing and MPA exposure in renal transplantation remained moderate, and it was reported that there was a large individual difference in the PK parameters [19]. Moreover, there was no difference in treatment failure after renal transplantation between a concentration-controlled group and fixed-dose group of patients [20]. As we could not locate any studies in the literature in which the relationship between dose and therapeutic effect had been determined in c-SLE, further investigation of optimal dosage is required.

Regarding the relationship between dose and side effects, published results of a meta-analysis revealed that hematologic abnormalities, infectious diseases, and gastrointestinal symptoms were the most common reasons for MMF dose reduction [21]. However, there were no differences in treatment failure or in side effects such as gastrointestinal symptoms, hematologic abnormalities, and infectious disease, between patients concentration-controlled by therapeutic drug monitoring and patients on a fixed-dose schedule. In addition, it was reported that herpes simplex infection was higher in the concentration-controlled group [22]. Our present study showed that the average dose used in Japan was 33.8 mg/kg/day and 1,061 mg per m^2/day, which is similar to that used for LN and post-renal transplantation in other countries. We also saw 4 of 26 cases with side effects during administration of 20–25 mg/kg/day for one year, as described in a report by Kazyra [7]. This is also consistent with the likelihood that our data on dose and occurrence of side effects in Japan are similar to the reported experience in other countries.

Efficacy

We compared the dose of CS patients received before starting MMF with the dose they were receiving at the end of MMF treatment, or at the last observation. Where laboratory values were available, further comparison took the disease activity index of SLE or LN into account, such as complement titers, the presence of anti-DNA antibodies, and urinary protein. The CS dose was significantly lower at the last observation than that before MMF therapy was started, and complement activity and the amount of anti-DNA antibody also improved. This suggests that MMF is able to reduce the amount of CS required at the same time in addition to suppressing SLE and LN. For the urinalysis, there was too little data on quantitative indicators, such as 24-h urine protein, urine protein:creatinine ratio, or creatinine clearance, so we could not make any comparisons. In a meta-analysis of several other reports comparing MMF with other drugs such as AZA and IVCY, it was suggested that the efficacy of MMF for remission induction and maintenance was similar to or better than the other drugs [8]. Although there are very few reports on pediatric cases, a therapeutic effect on complement activity, renal function, and urinary findings, in addition to a reduction in the amount of CS required,

was noted in 26 cases, including patients switched from AZA as well as those where MMF was used for induction and maintenance therapy [7]. Specifically in Asia, Kizawa et al. also reported such MMF efficacy in 9 cases [13].

Side effects

According to previous reports on MMF administration, the side effects of this drug are similar to or less than those of AZA and IVCY [2–4,7–9,15]. The main side effects of MMF are reported as diarrhea [3]. In the present study, herpes zoster was the most often observed disease due to side effects of MMF, followed by gastrointestinal symptoms, such as abdominal pain and diarrhea. All patients with zoster recovered after temporary cessation of MMF treatment and MMF administration could be resumed even up to the original dose in many cases. However, in two cases, the dose of MMF had to be reduced. Regarding the gastrointestinal side effects, symptomatic treatment or temporary cessation of MMF administration was also sufficient except in one patient where resumed treatment had to be at a reduced dose. Other reported side effects included decreased leukocyte counts, thrombocytopenia, and reduction of IgG levels. The types and frequencies of all these side effects were similar to those of previous reports. In the present study, no life-threatening side effects occurred and temporary MMF therapy withdrawal could always be followed by treatment resumption.

Teratogenicity of MPA

MPA teratogenicity was reported for the first time in 2008 [23]. It was then shown in a prospective study that the incidence of fetal malformation was increased by MPA [24]. In animal experiments and in humans, typical features of MPA embryopathy were cleft lip and palate, microtia, external auditory canal atresia, chorioretinal coloboma, and hypertelorism. The FDA recommends that women of childbearing age avoid pregnancy during MMF therapy and until at least 6 weeks after cessation of MMF administration. EULAR/EDTA also recommends that MMF should not be used for three months before pregnancy [10]. Although no data on pregnancy during MMF administration or pregnancy and childbirth post-withdrawal of MMF were available in the present study, advice on teratogenicity and contraception should be similar in Japan to that in other countries. Given the possible long-term effects of administration of MMF, this should also be taken into consideration, even in prepubertal girls.

Limitations

The present study has several limitations. First, it was performed using the limited information provided by a national survey, as shown in summary data tables. Therefore, clinical parameters such as SLEDAI and changes to renal pathology could not be evaluated because this information is missing in the questionnaires. Second, this study evaluated the collected cases retrospectively. A prospective controlled study adhering to a strict protocol including collection of detailed data will be necessary in order to confirm the efficacy and safety of MMF for c-SLE.

Conclusions

We evaluated data on 115 c-SLE/LN patients obtained by questionnaire during a national survey on the actual clinical use of MMF, and conclude that MMF does improve LN disease activity indices, for example, as reflected in changed complement values, and SLE disease activity indices, for example, as reflected in anti-DNA antibody titers. Additionally, the required dose of PSL could also be reduced. Furthermore, the side effects of MMF in children were similar to those reported previously in adults, and there were no serious events requiring permanent cessation of MMF treatment. Since almost all previous reports on the efficacy and safety of MMF for c-SLE were very small-scale, we believe that our present data based on a large number of cases strongly supports the notion that MMF is efficacious and safe for treating c-SLE.

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Conflict of interest

R. H., H. M., K. N., T. M., T. N., M. K., N. S., T. K., S. S., K. S., S. T., Y. K.: none.

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ORIGINAL ARTICLE

Characteristics of FDG-PET findings in the diagnosis of systemic juvenile idiopathic arthritis

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Abstract

Objective: To examine and delineate inflammatory focus in patients with juvenile idiopathic arthritis (JIA), ^{18}F-Fluoro-deoxy-glucose (FDG)-positron emission tomography (PET) (^{18}F-FDG-PET) was applied to patients with JIA, and the images of these patients were compared.

Methods: Sixty-eight children (59 with systemic JIA (s-JIA) and 9 with polyarticular JIA) were included. The diagnosis of JIA was done to meet the International League of Associations for Rheumatology (ILAR) criteria. After 6-h fasting, whole-body positron emission tomography (PET) scans were acquired 60 min after intravenous injection of 3–5 MBq/kg ^{18}F-FDG. The interpretation of ^{18}F-FDG uptake was based on visual characteristics.

Results: Two types of PET images were outstanding in s-JIA; one was ^{18}F-FDG uptake in red bone marrow, such as the spine, pelvis, and long bones as well as spleen (12 cases), and other type was the uptake in the major joints, such as hips, elbows, wrists, knees, and ankles (8 cases). The former findings were correlated with elevated levels of inflammatory markers, while the latter were with significantly increased levels of MMP-3 ($p < 0.05$).

Conclusion: There was a noticeable accumulation of ^{18}F-FDG uptake in bone marrow of s-JIA patients which may indicate the inflammatory focus of this disease and play an important role in the pathogenic basis of arthritis and systemic inflammation of s-JIA.

Keywords:

Diagnosis, F-Fluoro-deoxy-glucose-positron emission tomography, Systemic juvenile idiopathic arthritis

History

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Introduction

Systemic juvenile idiopathic arthritis (s-JIA) is a systemic chronic inflammatory disease, the main symptoms of which are remittent fever, rheumatoid rash, and arthritis [1]. During the clinical course, about 7% of patients suffer from macrophage activation syndrome (MAS), which can be life-threatening. Some patients develop MAS as the first symptom of the disease [2,3]. In s-JIA, the whole range of symptoms is rarely observed at the onset, with remitting fever being an early symptom in most cases, while arthritis tends to appear later. Therefore, early diagnosis is required but no appropriate diagnostic marker has been established to date. However, it has been determined that heme oxygenase-1 (HO-1) and interleukin-18 (IL-18) are markedly increased at the active phase of s-JIA, unlike in polyarticular and oligoarticular juvenile idiopathic arthritis, and these two factors have been reported to be useful serological diagnostic markers [4,5]. In addition, matrix metalloproteinase 3 (MMP-3), which is produced by inflamed synovial cells and fibroblasts, is a useful activity marker for cartilage destruction. It has become possible to diagnose the disease and evaluate its activity relatively easily. Together with a marked elevation in inflammatory markers, such as C-reactive protein (CRP) and blood sedimentation rate, the diagnosis of s-JIA can become more accurate.

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Although s-JIA is currently classified as one subtype of JIA, there is a school of thought considering the disease as an autoinflammatory syndrome [6]. Affected joints are markedly different from polyarticular and oligoarticular JIA, and the process of cartilage and bone destruction is also different, suggesting that s-JIA is a different disease. Marked osteoporosis and poor development of epiphyseal nuclei are observed in the systemic type, whereas narrowing of the joint space is characteristic of the articular type [7,8], suggesting different mechanisms responsible for disease onset of systemic versus articular type.

In the present study, the primary inflammatory lesion was investigated by ^{18}F-FDG-PET (FDG-PET) in >400 cases with remittent fever. The FDG-PET findings were compared in 59 cases of s-JIA diagnosed based on serological evaluation and clinical course, so that characteristic FDG-PET findings for the disease could be determined. In cases that do not receive a firm diagnosis, these characteristic FDG-PET findings may be used as potent diagnostic tools.

Patients and methods

A total of 68 cases with JIA examined by FDG-PET between January 2002 and December 2011 at our hospital were retrospectively investigated. There were 59 cases of s-JIA (31 boys and 28 girls; average age, 9.1 ± 3.9 years) and nine polyarticular JIA (p-JIA) (5 boys and 4 girls; average age, 11.6 ± 5.2 years). Joints at the bilateral shoulders, elbows, hands, hips, knees, and ankles were evaluated. Articular symptoms of tenderness and swelling of each joint were recorded. White blood cell count (WBC), CRP,

serum amyloid A (SAA), erythrocyte sedimentation rate (ESR), ferritin, and FDP-E were assessed as inflammatory markers, while MMP-3 was used as a marker of articular destruction; plasma G-CSF, IL-6, and IL-18 were measured at the same time.

For FDG-PET, ^{18}F-FDG was intravenously infused after 6 h of fasting, and images were scanned 1 h later. SUVmax $\geq$0.5 was considered positive for defining ^{18}F-FDG accumulation. Next, clinical symptoms and laboratory data at the time of FDG-PET and accumulation patterns of ^{18}F-FDG were compared among the 12 joints listed above. In addition, cases with characteristic ^{18}F-FDG accumulation findings were extracted and their images were examined.

This study was carried out as part of advanced medical research of the hospital (Registration No. 158-1) and all patients participated in the study only after they gave informed consent.

Results

^{18}F-FDG accumulation in children without inflammation

After exploration of a remittent fever, a case without inflammation is presented here, as an example (Figure 1). In children without inflammation, ^{18}F-FDG accumulates in the brain, heart, bladder, and joints during growth. This patient is an 8-year-old girl with low-grade fever of unknown origin and pain in the extremities. Her complaints were severe and her parents wanted to have her examined as extensively as possible. We undertook FDG-PET after obtaining informed consent from her and her parents. It was confirmed that she had no inflammation and she was diagnosed as suffering from fibromyalgia.

Characteristics of ^{18}F-FDG accumulation in cases with p-JIA

In 11 cases with p-JIA, ^{18}F-FDG accumulation showed a diffuse distribution pattern in inflamed joints. It was often observed that ^{18}F-FDG accumulated in almost all large joints as in (Figure 2a) and (Figure 2b) or in those joints with severe inflammation as in (Figure 2c). There was no accumulation in the bone marrow and no significant difference in accumulation in the liver or spleen.

Relationship between arthritis and ^{18}F-FDG accumulation in cases with p-JIA

Relationships between SUVmax and other laboratory data such as CRP, ESR, WBC, and MMP-3 were examined, but no significant correlations were found (Figure 2).

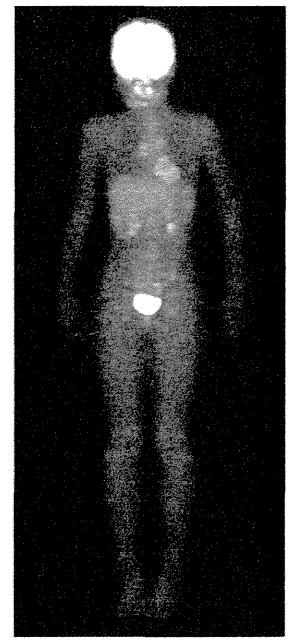

Figure 1. FDG-PET findings in a healthy child. Despite no abnormal inflammation, accumulation in the brain, heart, bladder, and joints at the growth stage is observed.

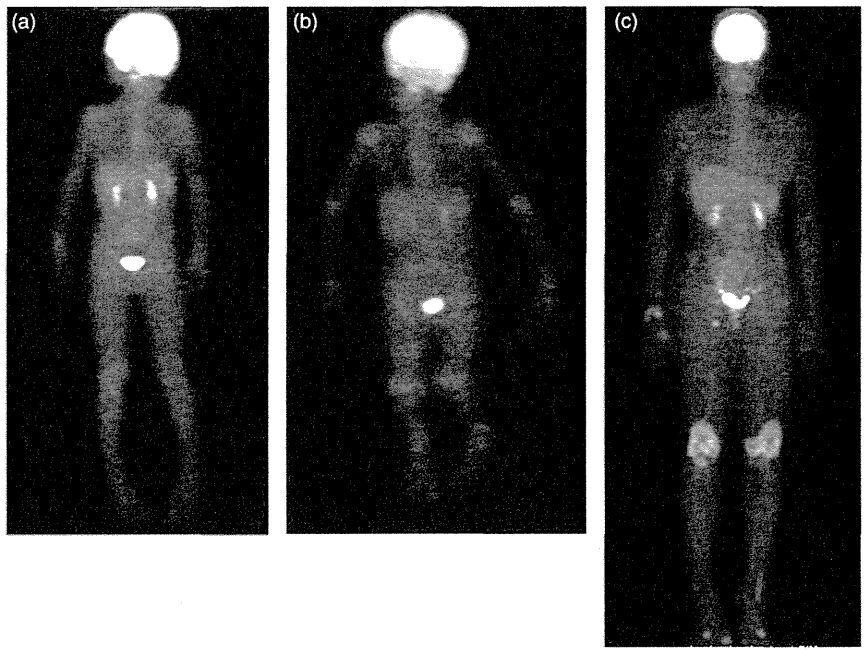

Figure 2. FDG accumulation in cases with p-JIA. (a) Accumulation is observed at the bilateral shoulders, elbows, wrists, and in the knees. In particular, marked accumulation was observed at the bilateral shoulders, the left elbow, and in the right knee. (b) Accumulation is observed at the bilateral shoulders, in the elbows, wrists, hips, knees, and ankles. (c) Marked accumulation is observed in the bilateral knees and the right wrist.

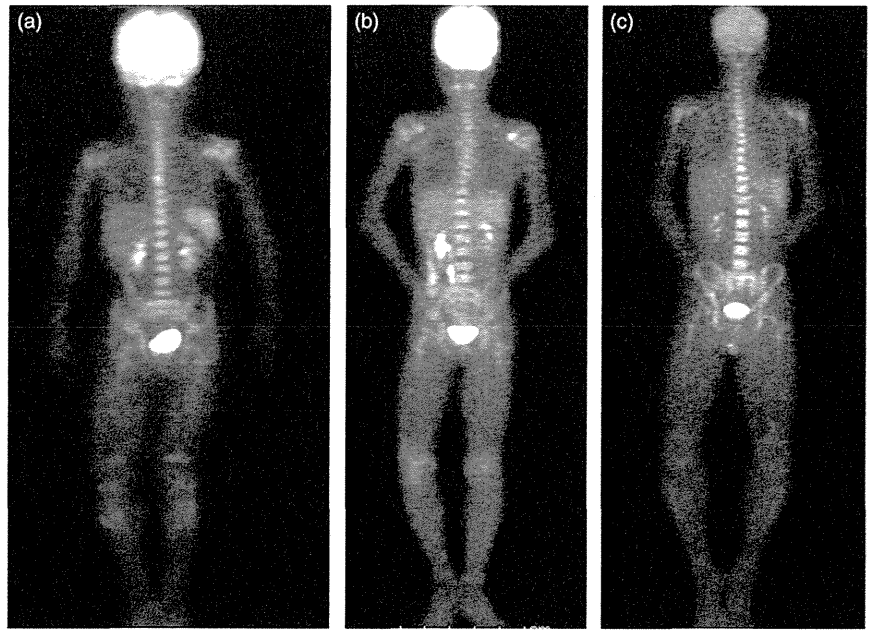

Figure 3. FDG accumulation in s-JIA (type I). (a) Accumulation is observed at the bilateral shoulders, in the vertebrae, pelvis, spleen, and bilateral knees. Accumulation is centered on the head of the humerus at the shoulder and the proximal tibial bone at the knee. (b) Accumulation is observed at the bilateral shoulders, in the vertebrae, pelvis, spleen, and bilateral knees. (c) Marked accumulation at the bilateral shoulders, in the vertebrae, pelvis, and spleen. Accumulation is also observed in the knee. At the shoulder and in the knee, accumulation is observed not in the joint but at the epiphysis (bone marrow tissue). In the pelvis, accumulation is marked at the ala of the ilium that contained red bone marrow.

Characteristics of ^{18}F-FDG accumulation in cases with s-JIA

There were two characteristic patterns in ^{18}F-FDG accumulation in the 59 cases with s-JIA. They were designated type I and type II as follows:

(1) Characteristic accumulation was found in all vertebral bodies and pelvis and around large joints, such as shoulders and knees. The accumulation was not in the joint synovia but the bone itself or at the end of the long bones. It was considered that accumulation was not in the joints but in the bone marrow. In addition, compared with the liver, greater accumulation in the spleen was characteristic (Figure 3) (type I, 12 cases).
(2) As in cases with p-JIA, diffuse accumulation in inflamed joints was recognized. There was no accumulation in the bone marrow and no significant difference between the liver and spleen (Figure 4) (type II, 8 cases).

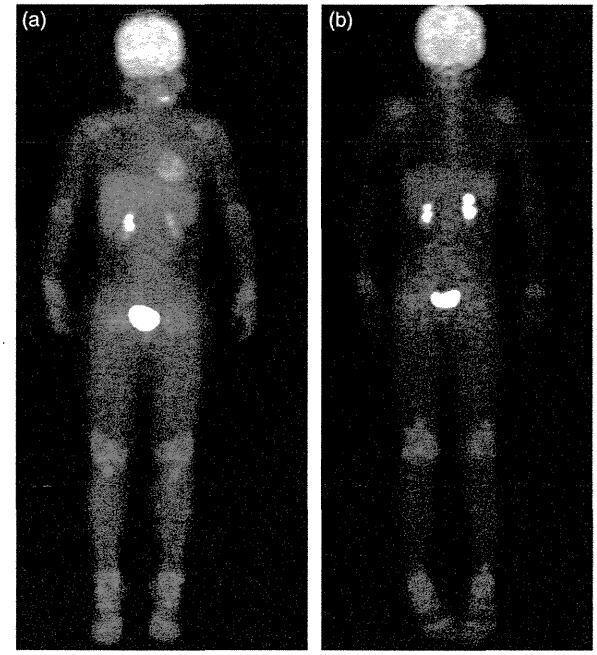

Figure 4. FDG accumulation in s-JIA (type II). (a) Accumulation is observed at the bilateral shoulders, in the elbows, hands, hips, knees, and ankles. In particular, it is marked in the hands, knees, and ankles. (b) Accumulation is observed at the bilateral shoulders, in the hands, knees, and ankles. It is marked in the knees and ankles.

Relationship between arthritis and ^{18}F-FDG accumulation in cases with s-JIA

Relationships between SUVmax and other laboratory data such as CRP, ESR, WBC, and MMP-3 were examined, but no significant correlations were found.

Comparison of different accumulation patterns in cases with s-JIA

Age, gender, duration from onset, treatment intervention, transition to MAS, reduction in the steroid dose 1 year later, cases treated with biological agents, and number of refractory cases were compared between type I and type II. With regard to laboratory data, WBC, CRP, SAA, ESR, ferritin, FDP-E, MMP-3, IL-6, IL-18, and G-CSF were compared (Tables 1 and 2, and Figure 5).

Type I accumulation in FDG-PET was observed in boys, in particular, immediately after onset and before treatment; the risk of developing MAS seemed high. There was no significant difference in treatment resistance.

The test results (Figure 5) showed that inflammation markers such as WBC, CRP, SAA, ESR, ferritin, IL-6, IL-18, and G-CSF

were significantly higher in type I, whereas the synovitis marker MMP-3 was significantly higher in type II.

Discussion

FDG-PET results for the diagnosis of remittent fever and reference findings of JIA were collected for retrospective comparison over a 10-year period at our department. Of these, 68 cases with JIA were analyzed. In cases with p-JIA, ^{18}F-FDG accumulated in the joints with synovitis, as is seen in adult rheumatoid arthritis. These findings were almost identical to the physical, ultrasonographic, and magnetic resonance imaging (MRI) findings for arthritis. Although it was reported that SUVmax correlates with the severity of arthritis, there was no such significant correlation in the present study. The degree of accumulation was influenced by the interval from radioisotope injection to scanning, a meal and exercise taken before the test, and age and body build of the subjects, and there was a large interindividual variation in measurements.

Table 1. Comparison of type-I and type-II s-JIA (at scanning).

Background of s-JIA subtypes	s-JIA type I	SJIA type II
Number of cases	12	8
Age (years)	8.8 ± 2.8	7.9 ± 4.1
(range)	(3~14)	(3~18)
Boy:girl ratio	8:4	3:5
Duration from	1.3 ± 1.7	23.4 ± 43
onset (months)	(0~10)	(0~66)
No treatment	7 cases	1 case
when PET taken	(58%)	(13%)

Table 2. Comparison of type-I and type-II s-JIA (all clinical courses).

Course of s-JIA subtypes	s-JIA type I	s-JIA type II
Number of cases	12	8
Macrophage activation syndrome	3 cases (25%)	0 cases
Reduction rate of PSL dosage (after 1 year)	−63.1%	−54.9%
Treatment with biologics	11 cases (91.7%)	6 cases (85.7%)
Refractory cases	3 cases (1 articular 2 systemic course)	5 cases (all cases had articular courses)

Characteristic images of ^{18}F-FDG accumulation in the red bone marrow tissue of the whole body were obtained in 12 cases, as shown in Figure 3. Among cases diagnosed with s-JIA, findings similar to those of p-JIA were sometimes obtained. Cases with accumulation in the bone marrow tissue had a shorter period from onset, had received less intensive treatment, and showed an increase in serological markers for systemic inflammatory status (WBC, CRP, SAA, ESR, ferritin, FDP-E, IL-6, IL-18, and G-CSF). Diagnosis of s-JIA at an early stage after onset is a critical issue for treatment selection; these findings will be useful for diagnosis in this regard.

For reference, in this study, FDG-PET images from 23 juvenile systemic lupus erythematosus patients, 20 juvenile dermatomyositis, 10 mixed connective tissue disease, 8 systemic sclerosis, and 10 Kawasaki disease patients were examined and no characteristic findings in these diseases were observed.

It has been demonstrated that imaging modalities are useful for the diagnosis and evaluation of arthritis in JIA, and recent advances in joint ultrasonography, in particular, for p-JIA, have been remarkable [9]. It is highly significant that this modality enables real-time evaluation of inflammation based on the presence of synovitis, retention of synovial fluid, stratification of synovial membrane, and increased blood flow by power Doppler imaging. Additionally, joint ultrasonography is useful for the evaluation of arthritis in cases with s-JIA, but differential diagnosis is very difficult when the patient presents with a remittent fever with unclear arthritis. However, an elevation in HO-1 [4] and IL-18 [5] is disease-specific and these can be useful serological markers for diagnosis. Therefore, it is expected that they will be widely used as serological markers.

FDG-PET showed characteristic findings in this study. In type I s-JIA, ^{18}F-FDG accumulation was observed in the bone marrow, in particular the red bone marrow, reflecting systemic inflammation; accumulation was also more marked in the spleen than in the liver. These findings are otherwise seen only in some diseases

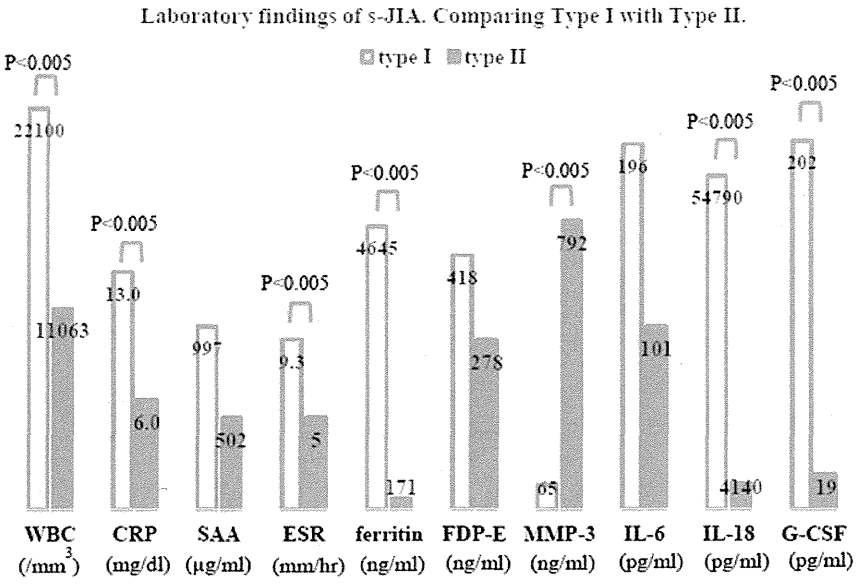

Figure 5. Laboratory findings of s-JIA: comparing type I with type II. Inflammation markers, such as WBC, CRP, SAA, ESR, ferritin, IL-6, IL-18, and G-CSF, were significantly higher in type I, whereas the synovitis marker MMP-3 was significantly higher in type II.

such as sepsis and are thus considered relatively specific for the diagnosis of s-JIA in combination with serological tests. FDG also accumulates in the bone marrow of patients with leukemia. Nevertheless, FDG-PET in s-JIA is different in that there is relatively homogeneous accumulation in the bone marrow, whereas in leukemia it has a speckled distribution.

It should be noted that adult-onset Still's disease, a disease similar to s-JIA, also exhibits characteristic accumulation of ^{18}F-FDG in the bone marrow, spleen, and lymph nodes, and FDG-PET is considered an effective modality for its diagnosis at the stage of exploration of remittent fever in adults [10]. In addition, FDG accumulation was observed in inflamed joints as a characteristic finding in type-II s-JIA, suggesting synovitis. Furthermore, there was no significant difference in the amount of FDG accumulation in the liver relative to the spleen, and there was no accumulation in the bone marrow. In type-II s-JIA, some cases manifest arthritis as the main symptom after a long clinical course, while others have a tendency to improve after anti-inflammatory treatment such as with steroids. The clinical course of type-I and type-II s-JIA is different, as reflected in differences at the sites of ^{18}F-FDG accumulation. In p-JIA, ^{18}F-FDG accumulated only at the joints with inflammation and there was no significant difference in the accumulation in the bone marrow and spleen. These findings are consistent with the FDG-PET findings observed in adult rheumatoid arthritis [11] and are interpreted as ^{18}F-FDG accumulation at the joint synovial membrane and synovial fluid in the joint capsule.s-JIA rarely develops a remittent fever, rash, and arthritis at the same time during disease progression. It starts with a remittent fever and rash, following which arthritis develops, and in the long-term eventually causes problems in daily life activities due to polyarthritis [2]. It is an inflammatory disease in which systemic inflammation precedes the appearance of arthritis that eventually becomes the main symptom.

Exploration by FDG-PET in this study showed two patterns of type-I and type-II s-JIA ^{18}F-FDG accumulation, with type-I revealing a pattern at the early stage after onset based on clinical findings and laboratory data, and showing inflammation localized to the bone marrow and spleen. In contrast, type II is an advanced inflammatory disease and progression to arthritis is expressed similarly to p-JIA and rheumatoid arthritis.

According to the national survey on exploration of ''remittent fever,'' s-JIA is the most frequent outcome with a definite diagnosis [12]. In general, it takes a long time before a definite diagnosis can be made. In addition, 6.8 to 13% of cases develop MAS and their prognosis is often poor [13]. Therefore, early diagnosis is desirable for s-JIA to initiate appropriate mitigating treatment. Our results that FDG-PET showed a characteristic accumulation pattern for early systemic inflammation indicate that this imaging modality is useful for early diagnosis of s-JIA.

Accumulation of ^{18}F-FDG in the bone marrow suggests that these cells are proliferating, and differentiating [14]. G-CSF and GM-CSF administered to counter the adverse effects of chemotherapy is associated with accumulation of ^{18}F-FDG in the bone marrow and spleen [15]. In that study, plasma G-CSF levels were markedly increased at the time of FDG-PET in cases with s-JIA [15], most of whom also showed an increase in peripheral blood granulocytes. Therefore, it was suggested that excessive G-CSF was involved in the accumulation of ^{18}F-FDG in systemic inflammation at the early stage of s-JIA, and thus not only IL-6 but also G-CSF was potentially involved in disease pathogenesis [16].

Because HO-1 and IL-18 are increased in the serum [4,5] and amyloidosis is an important factor influencing the development of joint destruction, marked osteoporosis [6], and the prognosis of s-JIA, it is clear that this disease exhibits different characteristics relative to p-JIA. The systemic type has the characteristics of an ''autoinflammatory syndrome'' as a systemic inflammatory disease lacking associations with external factors, and its tentative assignment into this disease category is currently under consideration [17,18].

Characteristics of FDG-PET findings were elucidated in the present study. After infectious disease and malignancy were ruled out based on a blood culture test and bone marrow testing, the findings of FDG accumulation in the bone marrow (red bone marrow) and spleen are consistent with the proposal that s-JIA should be classified as an ''autoinflammatory disease''. Investigations of larger numbers of similar cases to support the utility of FDG-PEG for disease diagnosis is now required.

Conflict of interest

Masaaki Mori has received lecture fees from MSD, Sumitomo Dainippon Pharma, and Phizer Japan Inc, and has served as a consultant adviser to Bristol-Myers Squbb and Astellas Pharm. Shumpei Yokota hold a patent for tocilizumab and receives royalties for Actemra. All other authors have declared no conflicts of interest.

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Increased Serum B Cell Activating Factor and a Proliferation-inducing Ligand Are Associated with Interstitial Lung Disease in Patients with Juvenile Dermatomyositis

Norimoto Kobayashi, Ichiro Kobayashi, Masaaki Mori, Shinji Sato, Naomi Iwata, Tomonari Shigemura, Kazunaga Agematsu, Shumpei Yokota, and Kenichi Koike

ABSTRACT. ***Objective.*** Rapidly progressive interstitial lung disease (RP-ILD) is an intractable and fatal complication of juvenile dermatomyositis (JDM). This study evaluated serum levels of B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) in JDM patients with complicating ILD, and their association with ILD phenotypes, clinical variables, and anti-melanoma differentiation-associated gene 5 (MDA5).

Methods. We measured the levels of BAFF, APRIL, and anti-MDA5 in the sera of 23 JDM patients with ILD [8 in the RP-ILD group and 15 in the chronic ILD (C-ILD) group], 17 JDM patients without ILD (non-ILD group), and 10 age-matched controls, using the ELISA method. ILD was identified by high-resolution computed tomography.

Results. Serum BAFF titers were significantly higher in the JDM patients with RP-ILD versus those with C-ILD ($p = 0.011$) and in healthy controls ($p = 0.0004$). The C-ILD group had significantly higher levels of BAFF versus controls ($p \leq 0.0001$). Serum APRIL was markedly elevated in the RP-ILD group as compared with the C-ILD group ($p = 0.003$) and controls ($p = 0.006$). In patients with ILD, both BAFF and APRIL levels were correlated with serum Krebs von den Lungen-6 and interleukin 18. Subjects with high titer anti-MDA5 (> 200 U) had higher levels of BAFF and APRIL than those with low titer anti-MDA5 (< 100 U; $p = 0.019$ and $p = 0.0029$, respectively), which may have been due to a relationship between RP-ILD and high anti-MDA5 titer.

Conclusion. Our findings of markedly elevated levels of BAFF and APRIL in patients with RP-ILD JDM suggest the potential importance of these cytokines in the diagnosis and treatment of RP-ILD accompanying JDM. (First Release October 15 2015; J Rheumatol 2015;42:2412–18; doi:10.3899/jrheum.140977)

Key Indexing Terms:
B CELL ACTIVATING FACTOR PROLIFERATION-INDUCING LIGAND ANTI-MDA5
JUVENILE DERMATOMYOSITIS INTERSTITIAL LUNG DISEASE

Juvenile dermatomyositis (JDM) is a childhood-onset autoimmune inflammatory myopathy characterized by skin rashes and muscle weakness. JDM prognosis was poor before the introduction of corticosteroid therapy, with death occurring in one-third of patients[1]. Although the mortality rate has since decreased to 0.7–3.1% with combination therapy of corticosteroids and immunosuppressants[2,3,4], our recent nationwide survey revealed that mortality in JDM remained higher than in other juvenile rheumatic diseases in Japan and that the major cause of death was rapidly progressive interstitial lung disease (RP-ILD)[5].

It was reported that differences in clinical DM phenotypes among several myositis-specific autoantibodies (MSA) indicated a role of autoantibodies in DM pathogenesis[6].

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Autoantibodies to melanoma differentiation-associated gene 5 (MDA5), which is found in 10-20% of Asian patients with adult-onset DM, have been correlated with both clinically amyopathic disease and a high incidence of ILD, particularly RP-ILD[7,8,9,10,11]. Similarly to adult cases, we have uncovered a relationship between anti-MDA5 titer and ILD severity in patients with JDM[5,12], suggesting a critical role of anti-MDA5 in the development of JDM-associated ILD.

B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are important physiological mediators of B cell homeostasis and play important roles in the production of antibodies. Both cytokines are expressed by myeloid lineage cells such as macrophages, monocytes, and dendritic cells, and at lower levels by T cells[13]. Serum levels of BAFF and APRIL are reportedly increased in several autoimmune diseases such as systemic lupus erythematosus (SLE), Sjögren syndrome (SS), multiple sclerosis, and systemic sclerosis (SSc), and elevated BAFF alone was reported in ANCA-associated renal vasculitis, Grave disease, autoimmune pancreatitis, and myasthenia gravis[14]. In particular, elevated BAFF is associated with the presence of disease-specific autoantibodies such as anti-SSA in SS, anti-dsDNA in SLE, and anti-histone in SSc[15,16,17,18]. Moreover, a prominent role of BAFF in the development of autoimmune diseases has been supported by the therapeutic effects invoked by BAFF antibodies in SLE and RA[19,20].

Elevated levels of BAFF in the serum and in muscle tissue have also been reported in patients with idiopathic inflammatory myopathy (IIM)[6,21,22], and BAFF titer was associated with the presence of autoantibodies and disease activity scores of myositis[6,21]. However, because the roles of BAFF and APRIL in patients with ILD accompanied by JDM remain unclear, our present study measured the serum levels of these mediators in patients with JDM-associated ILD and examined for associations with clinical course, disease variables, anti-MDA5 titer, and treatment.

MATERIALS AND METHODS

Patients and controls. Our present study was approved by the ethics committee of Shinshu University, according to the Declaration of Helsinki. We enrolled 24 JDM patients with ILD (ILD group), including 9 patients with RP-ILD (RP-ILD group) and 15 patients with chronic ILD (C-ILD group), 17 JDM patients without ILD (non-ILD group), and 10 age-matched healthy controls. The clinical records, routine laboratory data, and serum samples of the ILD group were collected from 12 centers across Japan, while those of the non-ILD group were obtained at Shinshu University Hospital and Aichi Children's Health and Medical Center. One patient in the RP-ILD group was excluded because he was found to have *Pneumocystis pneumonia*. That left 8 patients in RP-ILD group. All patients were diagnosed as having JDM prior to 16 years of age. JDM diagnosis was made according to the criteria of Bohan and Peter[23,24] for dermatomyositis or that of Gerami, *et al* for clinically amyopathic dermatomyositis[25] with some modification, whereby a disease duration for 6 months or longer was excluded because most of the subjects with ILD required prompt systemic corticosteroid and immunosuppressive drug treatment within 6 months of disease onset. ILD was diagnosed using high-resolution computed tomography (HRCT) of the lungs. RP-ILD was defined as ILD rapidly worsening within 3 months of symptom onset or at the time of ILD diagnosis. C-ILD patients included those with apparent ILD without any respiratory symptoms who responded to immunosuppressive therapy or those with slowly progressive ILD over a 3-month period.

The clinical characteristics of patients at the time of ILD diagnosis or at JDM diagnosis for patients without ILD are shown in Table 1. A total of 5 patients succumbed to ILD-related respiratory distress.

Evaluation of clinical laboratory variables and serum BAFF, APRIL, and anti-MDA5 levels. Serum samples were obtained at the time of ILD diagnosis or at JDM diagnosis for the non-ILD group, divided into aliquots, and stored between –20°C and –40°C until analysis. Routine laboratory tests included measurement of serum levels of creatine phosphokinase (CK), aspartate transaminase (AST), alanine transaminase, aldolase, ferritin, Krebs von den Lungen–6 (KL-6), antinuclear antibodies, and anti-Jo1. Two of 8 patients in the RP-ILD group and 5 of 15 patients in the C-ILD group had already been treated for JDM with methylprednisolone pulse therapy (25–30 mg/kg/day for 3 days) followed by high-dose corticosteroids (maximum dose: 1–2 mg/kg/day) at the time of ILD diagnosis. The sera of 8 patients (5 with RP-ILD and 3 with C-ILD) were also available after completion of treatment (Supplementary Table, available at jrheum.org). Serum levels of BAFF (R&D Systems), APRIL (eBioscience), and IL-18 (MBL) were measured by ELISA according to the manufacturers' protocols. Anti-MDA5 was also measured using ELISA as described[10,26], whereby the number of antibody units was calculated from optical density results at the 450 nm (normal range: < 8 U) using a recombinant MDA5 antigen.

Statistical analysis. We performed all statistical analyses using JMP 9 software (SAS Institute). Unpaired data were analyzed with the nonparametric Wilcoxon test. Paired data were examined by the Wilcoxon signed-rank test. Correlations were assessed using Spearman's rank-order test (r_s). A p value < 0.05 indicated statistical significance.

RESULTS

Serum levels of BAFF and APRIL at the time of diagnosis of ILD. Figure 1 presents the BAFF levels in the 2 ILD subgroups (RP-ILD and C-ILD) and the non-ILD group. Both the RP-ILD [median 4734 pg/ml, interquartile range (IQR) 2198–9219 pg/ml] and C-ILD (median 1721 pg/ml, IQR 1122–2315 pg/ml; p = 0.011) groups had higher levels of BAFF in comparison with the control group (median 534 pg/ml, IQR 361–582 pg/ml; p = 0.0004 and p ≤ 0.0001, respectively). BAFF levels also tended to be higher in the non-ILD group (median 2310 pg/ml, IQR 1036–3662 pg/ml) than in healthy controls (p = 0.0017). Although patients with RP-ILD had significantly higher circulating BAFF levels than did C-ILD patients (p = 0.011), there were no remarkable differences between the RP-ILD group and the non-ILD group (p = 0.076) or the C-ILD group and the non-ILD group (p = 0.35).

Serum levels of APRIL were significantly elevated in the RP-ILD group (median 13,350 pg/ml, IQR 4975–26,850 pg/ml) as compared with the C-ILD group (median 2400 pg/ml, IQR 1600–6700 pg/ml; p = 0.003), non-ILD group (median: 2500 pg/ml, IQR 1300–3475 pg/ml; p = 0.003), and healthy controls (median: 2000 pg/ml, IQR 475–4200 pg/ml; p = 0.006). There were no marked differences among the remaining groups.

Correlation of serum BAFF and APRIL levels with laboratory variables. Next, we determined the correlation

Table 1. Clinical and laboratory data of patients with and without ILD. Values represent the median (interquartile range) or number (percentage) unless otherwise indicated.

Variables	ILD, n = 23	RP-ILD, n = 8	C-ILD, n = 15	Non-ILD, n = 17
Age, yrs	9.4 (5.1–12.0)	6.3 (4.5–8.7)	11.4 (8.3–12.2)	6.0 (3.5–9.5)
Sex, male:female	11:12	3:5	8:7	6:11
JDM:JCADM	20:3	6:2	14:1	16:1
Fatal outcome, n (%)	5 (21.7)	5 (62.5)	0 (0)	0 (0)
CK, IU/l	104 (45–231)	78 (28–163)	121 (45–237)	333 (112–1977)
AST, IU/l	83 (21–178)	193 (84–297)	61 (18.4–83)	80 (23–152)
Aldolase, U/l	12.6 (6.6–20.4)	17.6 (12.5–20.5)	8.9 (5.4–15.0)	23.3 (5.6–30.5)
Ferritin, ng/ml	243 (185–526)	325 (213–511)	216 (142–307)	86 (36–221)
KL-6, U/ml (normal: < 500)	836 (306–1575)	1976 (1109–2670)	466 (193–940)	250 (216–312)
Anti-Jo1, n (%)[a]	2 (8.7)	1 (12.5)	1 (6.7)	0 (0)
IL-18, pg/ml	543 (379–1018)	1189 (643–2150)	426 (252–640)	348 (245–1136)
Anti-MDA5, units (normal: < 8)	51.4 (9.5–357.6)	495 (342–830)	10.8 (6.7–51.4)	2.2 (1.6–3.0)
n (%)[a]	18 (78.3)	8 (100.0)	10 (66.6)	0 (0)
ANA[b], n (%)[a]	5[c] (22.7)	4 (50)	1[d] (7.1)	7[e] (43.8)
No. of pretreatment at blood sampling	7 (30.0)	2 (25.0)	5 (33.3)	0 (0)

[a] Values represent the number of antibody-positive patients. [b] ANA nuclear or/and cytoplasmic HEp2 cell immunofluorescence, titer ≥ 1:80; [c] n = 22; [d] n = 14; [e] n = 16. ILD: interstitial lung disease; RP-ILD: rapidly progressive ILD; C-ILD: chronic ILD; JDM: juvenile dermatomyositis; JCADM: juvenile clinically amyopathic dermatomyositis; CK: creatine phosphokinase; AST: aspartate transaminase; KL-6: Krebs von den Lungen–6; IL: interleukin; anti-MDA5: anti-melanoma differentiation-associated gene 5; ANA: antinuclear antibodies.

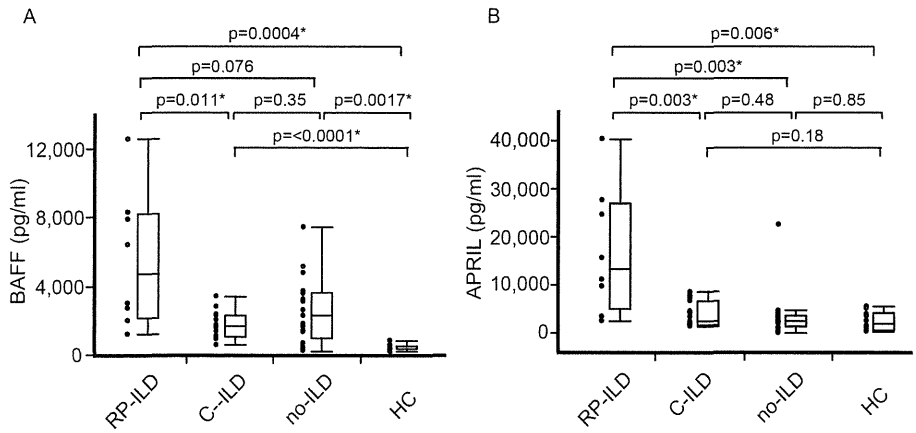

Figure 1. Serum BAFF and APRIL levels in patients with juvenile dermatomyositis (JDM) with interstitial lung disease (ILD). Those with ILD are divided into rapidly progressive ILD (RP-ILD) and chronic ILD (C-ILD). Other categories are patients without ILD (no-ILD), and healthy controls (HC). The horizontal line within boxes represents the median, the upper and lower boundaries of the boxes represent the 75th and 25th percentiles, respectively, and the top and bottom whiskers represent the 95th and 5th percentiles, respectively. Dots represent individual patient cytokine values. Significant differences are indicated by asterisks. BAFF: B cell activating factor; APRIL: a proliferation-inducing ligand.

coefficients of BAFF and APRIL with laboratory variables in the ILD group (Table 2). Serum BAFF and APRIL were positively correlated with AST and aldolase ($p < 0.05$), but not with CK ($p = 0.51$ and $p = 0.88$, respectively). Serum BAFF and APRIL were significantly associated with KL-6 ($r_s = 0.60$, $p = 0.003$ and $r_s = 0.62$, $p = 0.0019$, respectively) and IL-18 ($r_s = 0.46$, $p = 0.0026$ and $r_s = 0.57$, $p = 0.0045$, respectively). Ferritin had a positive correlation with APRIL ($r_s = 0.49$, $p = 0.026$), but not with BAFF ($p = 0.23$).

Correlation of serum BAFF and APRIL levels with anti-MDA5. Among MSA, anti-aminoacyl-tRNA synthetases (anti-ARS; anti-Jo1) and anti-MDA5 have been associated with ILD in patients with dermatomyositis[27]. We previously reported that serum anti-MDA5 was frequently observed in patients with ILD and that extremely high serum levels of anti-MDA5 (> 200 U) were found in all patients with RP-ILD as compared with C-ILD patients, whose anti-MDA5 was comparably lower (< 100 U)[5]. Here, all RP-ILD subjects had

Table 2. Correlations of serum BAFF and APRIL levels with clinical and laboratory variables in ILD patients with JDM.

Variables	ILD Group, n = 23	
	r_s	p value
BAFF vs CK	0.14	0.51
BAFF vs AST	0.67	0.0004
BAFF vs aldolase	0.46	0.031
BAFF vs KL-6	0.60	0.003
BAFF vs IL-18	0.46	0.0026
BAFF vs ferritin	0.28	0.23
APRIL vs CK	0.03	0.88
APRIL vs AST	0.61	0.0020
APRIL vs aldolase	0.61	0.0025
APRIL vs KL-6	0.62	0.0019
APRIL vs IL-18	0.57	0.0045
APRIL vs ferritin	0.49	0.026

r_s: Spearman's correlation coefficient; BAFF: B cell activating factor; APRIL: a proliferation-inducing ligand; ILD: interstitial lung disease; JDM: juvenile dermatomyositis; CK: creatine phosphokinase; AST: aspartate transaminase; KL-6: Krebs von den Lungen–6; IL: interleukin.

high titers of anti-MDA5, while 10 of 15 C-ILD subjects had lower values (Table 1). To evaluate the relative influence of BAFF and APRIL on anti-MDA5, the C-ILD group was subdivided into patients with and without anti-MDA5. The RP-ILD group having high anti-MDA5 exhibited significantly increased levels of BAFF and APRIL than did the C-ILD group with low anti-MDA5 ($p = 0.019$ and $p = 0.0029$, respectively; Supplementary Figure, available at jrheum.org).

Associations of BAFF and APRIL with clinical course in JDM patients with ILD. To determine the effect of immunosuppressive therapy on serum levels of BAFF and APRIL in JDM patients with ILD, we analyzed these cytokines in 8 JDM-associated ILD patients before and after treatment. Three patients with RP-ILD did not respond to therapy and succumbed to respiratory distress. Additionally, nonresponders exhibited pulmonary involvement as detected by HRCT (data not shown) and persistently high levels of serum KL-6 (nonresponders: mean 2375 U/ml, range 1767–6190 U/ml vs responders: mean 208 U/ml, range 242-750 U/ml), suggesting that disease activity was present after completion of treatment. In contrast, ILD symptoms and chest CT findings of the other 2 patients with RP-ILD and the 3 with C-ILD were both improved.

BAFF levels were significantly decreased after treatment compared with those at the diagnosis of ILD ($p = 0.0078$; Figure 2). Interestingly, the posttreatment BAFF levels in the patients who subsequently died remained higher than those of healthy controls. APRIL levels were reduced by treatment in 6 of 7 patients (Figure 2). However, 1 patient who succumbed to RP-ILD had markedly high values of APRIL in comparison with healthy controls, and the APRIL levels of the remaining 2 dead and 1 recovered patient with RP-ILD did not return to the levels of healthy controls.

DISCUSSION

In our present study, we saw that serum values of BAFF and APRIL were significantly higher in the RP-ILD group than in the C-ILD group, which suggested that these cytokines

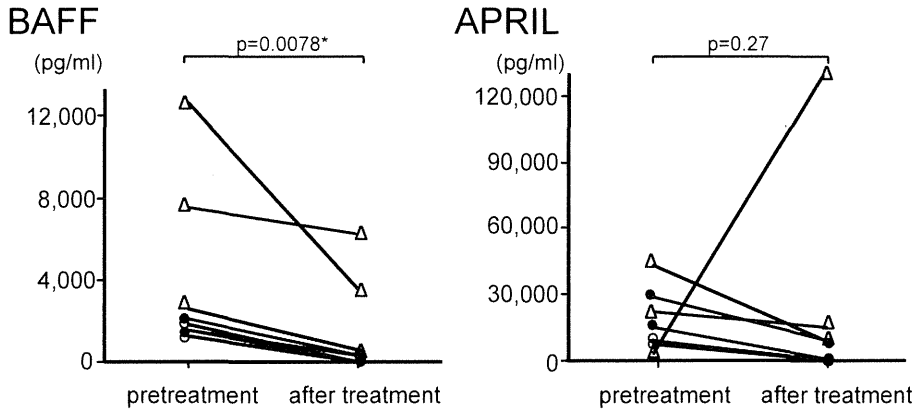

Figure 2. Serum BAFF (left panel) and APRIL (right panel) levels in dead patients with JDM and rapidly progressive-ILD (RP-ILD; open triangles), recovered patients with RP-ILD (closed circles), and patients with C-ILD (open circles) at the diagnosis of ILD (pretreatment) and after completion of the treatment protocol shown in Table 2. Eight data points are shown for BAFF and 7 are displayed for APRIL. One C-ILD patient could not be examined for APRIL because of insufficient serum volume. The therapies used until the second sampling point are described in the supplementary table and are summarized as follows: all 5 patients with RP-ILD and 2 of 3 patients with C-ILD received methylprednisolone pulse therapy (25-30 mg/kg/day for 3 days) followed by high-dose corticosteroids (1-2 mg/kg/day) and cyclosporine. One patient with C-ILD was treated with corticosteroids and tacrolimus. Cyclophosphamide (500 mg/m^2/day for 2-4 weeks) was added to the regimens of 3 patients with RP-ILD. Intravenous immunoglobulins were administered to 1 patient who died, 1 RP-ILD patient who recovered, and 1 patient with C-ILD. Significant difference is indicated by an asterisk. BAFF: B cell activating factor; APRIL: a proliferation-inducing ligand; JDM: juvenile dermatomyositis; C-ILD: chronic interstitial lung disease.