RHEUMATOLOGY

Rheumatology 2021;60:1387–1391
doi:10.1093/rheumatology/keaa392
Advance Access publication 25 September 2020

Concise report

Mycophenolate mofetil for methotrexate-resistant juvenile localized scleroderma

Giorgia Martini [1], Laura Saggioro[1], Roberta Culpo[1], Fabio Vittadello[1], Alessandra Meneghel[1] and Francesco Zulian [1]

Abstract

Objectives. To investigate safety and efficacy of MMF in patients with severe or MTX-refractory juvenile localized scleroderma.

Methods. Consecutive juvenile localized scleroderma patients undergoing systemic treatment were included in a retrospective longitudinal study. Patients treated with MMF because they were refractory or intolerant to MTX (MMF-group) were compared with responders to MTX (MTX-group). Disease activity was assessed by Localized Scleroderma Cutaneous Assessment Tool and thermography. Disease course was established on the number of relapses and treatment changes. Relapse-free survival was examined by Kaplan–Meier analysis.

Results. MMF and MTX groups included 22 and 47 patients, respectively. No significant difference in demographics, follow-up duration and treatment before diagnosis was observed between groups. The most represented clinical subtypes in the MMF-group were pansclerotic morphea and mixed subtype ($P = 0.008$ and $P = 0.029$, respectively), and linear scleroderma of the face in the MTX-group ($P = 0.048$). MMF was started because of MTX resistance (18 patients), relapse during MTX tapering/withdrawal (3 patients) and anaphylaxis to MTX (1 patient). After mean 9.4 years of follow-up, 90.9% of patients on MMF and 100% of those on MTX had inactive disease. No significant difference in relapse-free survival between the groups was found ($P = 0.066$, log-rank test), although MMF likely induced more persistent remission. MMF was well tolerated and combination of MMF and MTX did not increase its efficacy.

Conclusion. The present study adds strong evidence on the efficacy and tolerance of MMF in severe and/or MTX-refractory juvenile localized scleroderma. Further controlled studies are needed to prove its efficacy as first line treatment.

Key words: Mychophenolate mofetil, methotrexate, juvenile, localized scleroderma, treatment

CLINICAL SCIENCE

Rheumatology key messages

- Most patients with MTX-refractory juvenile localized scleroderma achieve sustained remission with MMF.
- MMF is well tolerated and equally effective in monotherapy or combined with MTX.

Introduction

Juvenile localized scleroderma (JLS) is a spectrum of disorders characterized by inflammation followed by thickening and sclerosis of skin and underlying tissues such as subcutaneous fat, muscle, fascia and bone, potentially leading to functional disability and cosmetic problems [1, 2]. While it is believed that most patients will enter spontaneous remission after 3–5 years of disease activity, those with more extensive deep tissue involvement, such as linear subtype and pansclerotic morphea, present considerable risk of severe course with persistent activity into adulthood and development of physical disability [1, 2].

Since the early 2000s a combination of MTX and CS has been reported as successful and safe treatment for JLS and chosen as first-choice therapy for patients with active disease [3–5]. Nevertheless, about 30% of patients do not respond to this therapy or do not tolerate it [3–5].

As alternatives to MTX, various other drugs have been used, and even biological agents such as abatacept and tocilizumab [6–8]. MMF has been suggested as potential

[1]Paediatric Rheumatology Unit, Department of Woman and Child Health, University of Padova, Padova, Italy

Submitted 30 March 2020; accepted 8 June 2020

Correspondence to: Giorgia Martini, Paediatric Rheumatology Unit, Department of Woman and Child Health, University of Padova, Via Giustiniani 2, 35128 Padova, Italy.
E-mail: giorgia.martini@aopd.veneto.it

treatment of JLS but up to now data are partial and incomplete, as only two case collections have been published so far [9, 10].

We conducted a retrospective, longitudinal study aimed to provide more evidence on MMF efficacy in reducing disease activity in MTX-resistant JLS, as well as to evaluate its safety profile.

Methods

Consecutive patients attending the Paediatric Rheumatology Unit of the Department of Woman and Child Health of Padova University, fulfilling diagnostic criteria for JLS and undergoing systemic treatment from 2000 to 2018, were included in the study and their charts were retrospectively reviewed [11]. The MMF-group included patients treated with MMF for at least 6 months, alone or in association with MTX and/or steroids, while the MTX-group included patients treated with MTX and steroids only. Institutional review board approval was not needed as the study included retrospective analysis of data. Written informed consent was obtained from parents of all subjects taking part in the study.

Data collection included: demographics (age, gender, personal or family history of autoimmune diseases), clinical data (age at disease onset, age at diagnosis, follow-up duration, presence of extracutaneous manifestations, ANA considered positive if $\geq$1:160, treatment history (start and duration of treatment with MTX and/or MMF eventually associated with oral or i.v. CS, current treatment at last evaluation) and disease course (disease relapses defined as a disease reactivation during treatment and/or tapering and/or after withdrawal).

All patients underwent periodic clinical evaluation including physical examination, laboratory work-up including complete blood cell counts, ESR, CRP and liver function tests; in patients taking MMF, levels of IgG, IgM and IgA were also routinely tested.

Disease activity was monitored by clinical evaluation combined with infrared thermography (IRT). IRT examination was performed with an infrared camera (ThermaCAM PM695, FLIR Systems AB, Stockholm, Sweden) at a room temperature, after 20 min of acclimatization, wearing underwear. Lesions were considered positive to IRT when $>0.5^{\circ}$C warmer than surrounding area or the contralateral side [12].

Long-term outcome was analysed by evaluation of disease activity at last visit including only patients with at least 6 months' treatment duration. Disease activity was assessed by using the modified Localized Scleroderma Skin Severity Index (mLoSSI) combined with IRT [12, 13]. mLoSSI is part of Localized Scleroderma Cutaneous Assessment Tool and evaluates disease activity by grading of three domains: new lesion/lesion extension, erythema and skin thickness. Each lesion was examined and, in presence of multiple lesions, the most severe was considered.

Combining mLoSSI and IRT, disease course was classified as follows:

- active disease: presence of new lesions and/or enlargement of an existing lesion within the past month, presence of erythema and/or skin thickening (equating to mLoSSI $\geq$1) and/or significant hyperthermia ($>0.5^{\circ}$C) on thermography;
- clinical remission on medication (CRM): absence of new lesions and/or enlargement of existing lesions and signs of activity (equating to mLoSSI = 0, significant hyperthermia on thermography) during treatment;
- clinical remission off treatment: absence of new lesions and/or enlargement of existing lesions and signs of activity (as for CRM) for $<$2 years from treatment discontinuation; and
- complete clinical remission: absence of new lesions and enlargement of existing lesions and signs of activity (as for CRM) after $\geq$2 years from treatment discontinuation.

Statistical analysis

Descriptive statistics for each variable included in data collection was applied to analyse patient characteristics. For quantitative variables, the main indicators of centrality and variability were calculated. Association between categorical variables was investigated by Pearson's χ^2-test and Fisher's exact test, and also in the extended Fisher–Freeman–Halton test version. Mann–Whitney test was used to evaluate differences between two groups, after verification of the non-normality distribution of variables considered in the analysis.

Kaplan–Meier analysis with log-rank test was performed for relapse-free survival comparing the two groups of patients (MMF *vs* MTX) and, within the MMF-group, comparing patients treated with MMF in monotherapy and those in combination with MTX. A *P*-value of $<$0.05 (two-tailed test) was considered statistically significant. All analyses were performed using the IBM (New York, USA) SPSS statistical software (Version 18.0).

Results

Patients

Twenty-two patients were included in MMF-group (9 males, 13 females) and 47 in MTX-group (20 males and 27 females). Clinical features of patients are summarized in Table 1. No significant difference was observed in the groups with regard to gender, age at diagnosis, diagnostic delay, family and/or personal history of autoimmune diseases, ANA and ENA positivity, follow-up duration and treatment received before diagnosis. In the MMF-group, pansclerotic morphea and mixed subtype were more frequent ($P = 0.008$ and $P = 0.029$, respectively) than in the MTX-group, where linear scleroderma of the face was slightly more represented ($P = 0.048$).

No significant difference was found for extracutaneous manifestations (ECM): in MMF- and MTX-groups CNS

Table 1 Clinical characteristics of patients

Characteristics	MMF group (22 pts)	MTX group (47 pts)	*P*-value
F	13 (59.1)	27 (57.4)	n.s
M	9 (40.9)	20 (42.6)	n.s.
F:M ratio	1.4:1	1.35:1	n.s.
Age at diagnosis, years[a]	8.3 (5.6)	7.1 (3.6)	n.s.
Diagnostic delay, months[a]	10 (8)	16 (17)	n.s.
Duration of follow-up, years[a]	9.4 (4.5)	9 (3.9)	n.s.
Disease subtype			
LiS of the trunk/limbs	4 (18.2)	13 (27.7)	n.s.
LiS of face	4 (18.2)	20 (42.6)	0.048
MS	7 (31.8)	4 (8.5)	0.029
GM	2 (9.1)	6 (12.8)	n.s.
PM	4 (18.2)	0 (0.0)	0.008
CM	1 (4.5)	4 (8.5)	n.s.

Data presented as *n* (%) unless stated. [a]Mean (S.D.). F: female; M: male; LiS: linear scleroderma; MS: mixed subtype; PM: pansclerotic morphea; GM: generalized morphea; CM: circumscribed morphea; n.s.: not significant.

abnormalities on MRI (not clinically significant) and mild dental abnormalities were present in one and two patients and in two and five patients, respectively.

Prior to MMF, 14 patients (63.6%) received a combination of MTX and oral prednisone (oPDN), 7 (31.8%) MTX with three consecutive i.v. pulses of methylprednisolone followed by oPDN, while 1 patient was started on MMF because of anaphylactic reaction to MTX. MTX full dosage was 15–17 mg/m^2/week. In the MTX-group 31 patients (66%) received MTX–oPDN and 16 (34%) MTX–methylprednisolone–oPDN. No significant differences in CS and MTX dosages and treatment duration were observed between the two groups.

MMF was started because of persistent activity on full dose of MTX in 18/21 patients (85.7%), and relapse during MTX tapering/withdrawal in 3/21 (14.3%). Mean treatment duration in the MMF- and the MTX-group was similar (mean 39.5 and 39.4 months, respectively).

MMF dosage was 700–1000 mg/m^2/day and was used in combination with MTX in 12 (54.5%) patients. Minor side effects were recorded in 12 patients of the MMF-group: headache (22.7%), mild transaminases increase (18.2%), nausea/vomiting (9.1%) and fatigue (9.1%), with no treatment discontinuation because of side effects.

Disease course and outcome

During the follow-up period 10/22 (45.4%) patients in the MMF-group and 11/47 (23.4%) in the MTX-group presented at least one disease reactivation (P = not significant). Average time from treatment start to relapse was 31 months (median 37.3, 21 S.D.) in the MMF-group and 26.2 months (median 13, 32.4 S.D.) in the MTX-group (P = not significant).

Moreover, the relapse-free survival analysis comparing the two groups of patients revealed no significant difference, as shown by Kaplan–Meier survival estimates of time to disease relapse ($P = 0.066$, log-rank test, Fig. 1A), although MMF likely induced a more persistent remission. In fact, from this analysis 50% of patients reactivated after 68 months in the MMF-group and after 22 months in the MTX-group.

Of interest, association of MMF with MTX did not increase the efficacy of therapy, as relapses occurred in 5/10 patients treated with MMF monotherapy and in 5/12 with both agents (P = not significant).

Average time from start of treatment to relapse was 30.9 months (median 40.9, 17.7 S.D.) with MMF monotherapy and 31.0 months (median 34.3, 26.0 S.D.) with MMF + MTX combination (P = not significant), respectively. Indeed, these results were confirmed by relapse-free survival analysis (Fig. 1B).

As for clinical subtype, 60% of all disease relapses in the MMF-group occurred in patients with mixed subtype and with pansclerotic morphea.

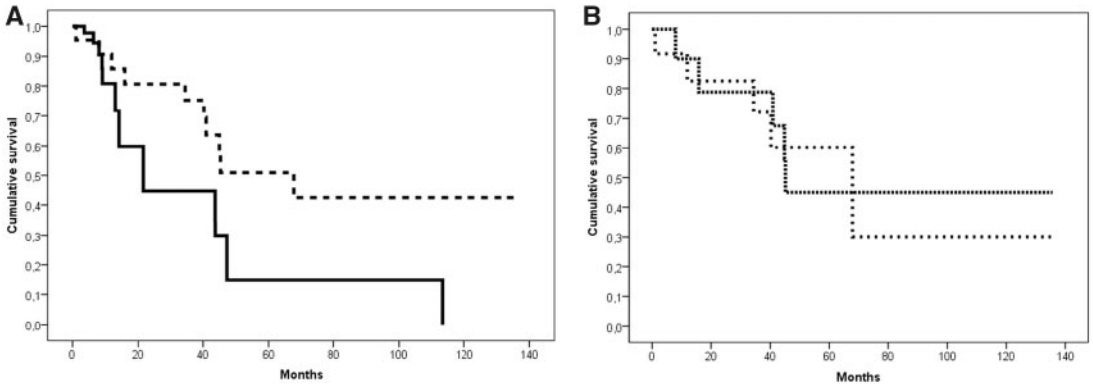
As for the long-term outcome, no significant difference in clinical course was observed in the two groups, as 90.9 and 100% of patients in the MMF- and MTX-group, respectively, had inactive disease at last evaluation. In particular, in the MMF-group 10 patients (50.0%) were in CRM, 3 (15%) in clinical remission off treatment and 7 (35%) in complete clinical remission. Furthermore, no significant difference in disease activity was found between patients treated with MMF alone and those with MMF + MTX.

Discussion

Localized scleroderma is characterized by early clinical inflammatory manifestations that are associated with extension of fibrotic lesions [14, 15]. Therefore, the aim of therapy is to cool down the inflammatory process in order to prevent progression to the fibrotic stage, thus reducing tissue damage [3].

A combination of MTX and CS is the recommended first-choice systemic treatment for JLS, but other agents

FIG. 1 Relapse-free survival analysis by Kaplan–Meier survival estimates of time to disease relapse



In this analysis, patients in the MMF-group (dashed line) were compared with those in the MTX-group (continuous line) ($P = 0.066$, log-rank test) (**A**), and patients treated with MMF alone (close dots) with those treated with MMF in combination with MTX (spaced dots, $P =$ not significant) (**B**).

such as abatacept and tocilizumab have been tried in severe cases [4–8].

In a previous report, we showed that MMF resulted in significant clinical improvement that allowed decrease and discontinuation of CS and MTX in 10 children with JLS refractory to MTX [9]. More recently, another study confirmed good response and tolerability with MMF in seven patients with difficult-to-treat localized scleroderma [10].

Although having the limitation of being retrospective, the present study is the largest to date on the use of MMF in JLS, and strengthens the evidence of its safety and efficacy in suppressing the inflammatory–fibrotic process. The comparison of the two patient cohorts, followed for 9 years on average, showed comparable efficacy between MMF and MTX. At the latest assessment, all patients in the MTX-group and 90.9% of those in the MMF-group had inactive disease. This comparable efficacy is particularly impressive in view of the composition of the MMF-group being patients that had been previously refractory or intolerant to MTX. Therefore, the result obtained further underlines the remarkable efficacy of MMF in seriously ill patients.

MMF exerts immunosuppressive effects by inhibiting lymphocytes activation and proliferation. Therefore, it has been extensively used in prevention of kidney and lung transplantation rejection and for disease-modifying treatment in adults and children with SLE [16].

The rationale for using MMF in sclerodermatous conditions, such as JLS and SSc, came from observation of its *in vitro* inhibitory effect on smooth muscle cells and fibroblasts differentiation, thus decreasing the production and exaggerated accumulation of collagen and other extracellular matrix proteins [17]. More recently, in patients with SSc treated with MMF a reduction of *monocyte chemoattractant protein-2* (*CCL2*) mRNA expression has been observed in skin [18]. CCL2 is a myeloid cell chemoattractant with an important role in SSc disease initiation, thus its inhibition by MMF was speculated to be the key responsible of skin induration improvement during treatment.

A randomized controlled trial in adult SSc showed that MMF significantly improved skin thickening and involvement as measured by modified Rodnan Skin Score, and this effect was maintained throughout the 12-month follow-up [19]. More recently, a *post hoc* analysis has been performed from two randomized placebo-controlled trials in patients with diffuse SSc in which modified Rodnan Skin Score was regularly measured by expert physicians, and this study confirmed that MMF was as effective as CYC in leading to significant improvement of modified Rodnan Skin Score but was better tolerated [20].

In our previous report 6/10 patients received both MMF and MTX, therefore we were not able to demonstrate that MMF was the only agent responsible for clinical improvement. Conversely, in the present study MMF was equally effective when used as monotherapy or combined with MTX. This observation, although based on small numbers, is important for clinicians in order to reduce the risk of a severe immunosuppressive effect stemming from the contemporary use of two agents.

MMF has a favourable safety profile and our study confirmed it: in fact, no discontinuation due to side effects was observed.

In conclusion, the results presented here strengthen the evidence of the efficacy of MMF in achieving disease inactivity in children with severe MTX-refractory JLS. These results also suggest that a substantial proportion of patients treated with MMF maintain persistent control of disease activity, as shown by the longer time from treatment start to relapse.

Further studies in larger cohorts of patients are needed to confirm these preliminary results and may clarify whether use of MMF as first-line agent, rather than only in MTX-refractory patients, likely provides better outcomes in patients with severe and extensive JLS forms.

Funding: No specific funding was received from any funding bodies in the public, commercial or not-for-

profit sectors to carry out the work described in this manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Li SC. Scleroderma in children and adolescents: localized scleroderma and systemic sclerosis. Pediatr Clin North Am 2018;65:757–81.

2 Saxton-Daniels S, Jacobe HT. An evaluation of long-term outcomes in adults with pediatric-onset morphea. Arch Dermatol 2010;146:1044–5.

3 Martini G, Fadanelli G, Agazzi A *et al.* Disease course and long-term outcome of juvenile localized scleroderma: experience from a single pediatric rheumatology centre and literature review. Autoimmun Rev 2018;17: 727–34.

4 Zulian F, Martini G, Vallongo C *et al.* Methotrexate treatment in juvenile localized scleroderma: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2011;63:1998–2006.

5 Torok KS, Arkachaisri T. Methotrexate and corticosteroids in the treatment of localized scleroderma: a standardized prospective longitudinal single center study. Rheumatology (Oxford) 2012;39:286–94.

6 Adeeb F, Anjum S, Hodnett P *et al.* Early- and late-stage morphea subtypes with deep tissue involvement is treatable with abatacept. Semin Arthritis Rheum 2017;46: 775–81.

7 Martini G, Campus S, Raffeiner B *et al.* Tocilizumab in two children with pansclerotic morphea: a hopeful therapy for refractory cases? Clin Exp Rheumatol 2017; 35(Suppl 106):211–3.

8 Lythgoe H, Baildam E, Beresford MW *et al.* Tocilizumab as a potential therapeutic option for children with severe, refractory juvenile localized scleroderma. Rheumatology (Oxford) 2018;57: 398–401.

9 Martini G, Ramanan AV, Falcini F *et al.* Successful treatment of severe or methotrexate-resistant juvenile localized scleroderma with mycophenolate mofetil. Rheumatology (Oxford) 2009;48:1410–3.

10 Mertens JS, Marsman D, van de Kerkhof PCM *et al.* Use of mycophenolate mofetil in patients with severe localized sclerodema resistant or intolerant to methotrexate. Acta Derm Venereol 2016;96:510–3.

11 Laxer RM, Zulian F. Localized scleroderma. Curr Opin Rheumatol 2006;18:606–13.

12 Martini G, Murray KJ, Howell KJ *et al.* Juvenile-onset localized scleroderma activity detection by infrared thermography. Rheumatology (Oxford) 2002;41:1178–82.

13 Arkachaisri T, Vilaiyuk S, Torok KS, Medsger TA. Development and initial validation of the localized scleroderma skin damage index and physician global assessment of disease damage: a proof-of-concept study. Rheumatology (Oxford) 2010;49:373–81.

14 Torok KS, Kurzinski K, Kelsey C *et al.* Peripheral blood cytokine and chemokine profiles in juvenile localized scleroderma: T-helper cell-associated cytokine profiles. Semin Arthritis Rheum 2015;45: 284–93.

15 Mertens JS, De Jong E, van den Hoogen LL *et al.* The identification of CCL18 as biomarker of disease activity in localized scleroderma. J Autoimmun 2019;101:86–93.

16 Fanouriakis A, Kostopoulou M, Cheema K *et al.* 2019 update of the joint European League against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of lupus nephritis. Ann Rheum Dis 2020; 79:713–23.

17 Roos N, Poulalhon N, Farge D *et al.* In vitro evidence for a direct anti-fibrotic role of the immunosuppressive drug mycophenolate mofetil. J Pharmacol Exp Ther 2007;321: 583–9.

18 Hinchcliff M, Toledo DM, Taroni JN *et al.* Mycophenolate mofetil treatment of systemic sclerosis reduces myeloid cell numbers and attenuates the inflammatory gene signature in skin. J Invest Dermatol 2018;138:1301–10.

19 Le EN, Wigley FM, Shah AA, Boin F, Hummers LK. Long-term experience of mycophenolate mofetil for treatment of diffuse cutaneous systemic sclerosis. Ann Rheum Dis 2011;70:1104–7.

20 Namas R, Tashkin DP, Furst DE *et al.* Efficacy of mycophenolate mofetil and oral cyclophosphamide on skin thickness: post hoc analyses from two randomized placebo-controlled trials. Arthritis Care Res (Hoboken) 2018;70:439–44.