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Impact of mycophenolate mofetil on recombinant zoster vaccine immunogenicity in systemic sclerosis: a prospective observational study

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ABSTRACT

Background:

To evaluate mycophenolate mofetil (MMF) impact on humoral, cell-mediated, and cytokine/chemokine responses to the recombinant zoster vaccine (RZV) in systemic sclerosis (SSc) patients.

Methods:

In this prospective observational study, 16 SSc patients (8 receiving stable MMF therapy and 8 not receiving immunosuppressive treatment) and 12 healthy controls (HC) received the two-dose RZV schedule. Humoral immunity was assessed by anti-glycoprotein E (anti-gE) IgG titres, cell-mediated immunity (CMI) was evaluated by intracellular cytokine staining of gE-stimulated peripheral blood mononuclear cells. Cytokine/chemokine pathways were investigated using multiplex immunoassays and composite scores.

Results:

Post-vaccination anti-gE titres were significantly lower in SSc patients than in HC [median 2328 (IQR 1520–2980) vs ≥4000mIU/mL; $p<0.0001$]. Within the SSc cohort, MMF-treated patients had lower antibody titres than untreated patients [1756 (891–2547) vs 2830 (2297–3408) mIU/mL; $p=0.03$]. In multivariable analysis, MMF treatment (β=-1465.4, 95% CI -2298 to -632; $p=0.003$) and older age (β=-33.4, 95% CI -61.9 to -4.8; $p=0.03$) independently predicted lower antibody titres. In contrast, CMI was induced similarly across groups. Humoral response-related cytokines and type I interferon score correlated positively with post-vaccination antibody titres, while inflammatory cytokine score was significantly impaired by MMF. No herpes zoster reactivation or SSc worsening occurred during the 2-year follow-up.

Conclusion:

Our data show that MMF impairs vaccine humoral response, but not cellular immune response. Since herpes zoster reactivation mainly depends on specific T cell-mediated immunity decline over time, our study suggests that MMF can be continued during RZV schedule.

Keywords: Mycophenolate mofetil, Systemic sclerosis, Recombinant zoster vaccine, Humoral immunity, Cell-mediated immunity, Cytokines, Vaccination response

Background

Patients with systemic sclerosis (SSc) are at increased risk of infections because of immune dysregulation, disease-related organ involvement, and the frequent use of immunosuppressive therapies [1]. Herpes zoster (HZ) may cause prolonged morbidity and potentially severe complications, particularly in patients receiving immunosuppressive treatment [2]. The adjuvanted recombinant zoster vaccine (RZV) has demonstrated high efficacy and an acceptable safety profile in both immunocompetent and immunocompromised populations [3]. Unlike the live attenuated vaccine, RZV induces both humoral and cell-mediated immune (CMI) responses through the recombinant varicella-zoster virus glycoprotein E (gE) antigen combined with the AS01B adjuvant system [4]. While antibody responses are readily measurable, protection against HZ is thought to rely predominantly on vaccine-induced VZV-specific CMI [5]. Consequently, evaluating both humoral and cellular immunity is essential when assessing vaccine immunogenicity, especially in patients receiving immunosuppressive therapies.

Recently, Luppino-Assad *et al.* [6] prospectively demonstrated that RZV is immunogenic, safe and not associated with worsening disease activity in patients with SSc. However, although patients receiving immunosuppressive therapies were included, the impact of individual immunomodulatory agents on vaccine-induced immune responses was not specifically addressed. This issue is clinically relevant because different drugs may differentially affect humoral and cellular immune responses, potentially influencing vaccine efficacy and the interpretation of immunogenicity studies.

Mycophenolate mofetil (MMF) is among the most frequently prescribed immunosuppressive agents for SSc, particularly in patients with interstitial lung disease and diffuse cutaneous involvement [7]. By inhibiting inosine monophosphate dehydrogenase, MMF selectively suppresses lymphocyte proliferation and can reduced vaccine-induced antibody responses [8]. We previously demonstrated a dose-dependent impairment of humoral, but not cellular, immunity following SARS-CoV-2 mRNA vaccination in patients receiving therapeutic doses of

MMF, suggesting that this drug may exert differential effects on distinct arms of adaptive immunity [9].

Based on these observations, we conducted a prospective observational study to evaluate the effect of MMF on humoral and cell-mediated immune responses following RZV in patients with SSc. In addition, we characterized vaccine-induced immune activation, generating biologically informed composite pathway scores, and explored their relationship with vaccine immunogenicity and clinical outcomes.

Methods

Study design and participants

We conducted a prospective observational study to evaluate the immunogenicity of the RZV in patients with SSc. Consecutive patients ≥18 years of age fulfilling the 2013 ACR/EULAR classification criteria for SSc [10] were prospectively enrolled at IRCCS Humanitas Research Hospital (Rozzano, Milan, Italy) between December 2023 and December 2025. Sixteen patients were included: eight receiving stable MMF therapy for at least 3 months before vaccination and eight not receiving MMF (1 on azathioprine, 7 without treatment) treatment. Twelve healthy controls (HC) were enrolled for comparison. Exclusion criteria included history of cancer, and pregnancy. All participants received the standard two-dose RZV schedule (Shingrix®, GSK) according to the manufacturer's recommendations. Blood samples were collected at baseline and 1 month after the second dose.

Assessment of vaccine-induced immunity

Humoral immunity was assessed by measuring serum anti-gE IgG concentrations using the DiaSorin LIAISON® assay (DiaSorin, Saluggia, Italy), according to the manufacturer's instructions. The cut-off for considering a serologic response, named thereafter "seroconversion" was a ≥4-fold increase from the assay lower limit of detection (150mIU/mL) in anti-gE igG titre.

Cell-mediated immunity (CMI) was evaluated in peripheral blood mononuclear cells (PBMCs). Cryopreserved PBMCs were stimulated with a gE peptide pool (gE PepMix®, JPT Peptide Technologies, Berlin, Germany; according to the manufacturer's instructions) and analysed by intracellular cytokine staining using a BD FACSCanto II flow cytometer (BD

Biosciences, San Jose, CA, USA), as previously described [6]. Vaccine-specific CMI was expressed as the frequency of activated gE-specific CD4 T cells (the cut-off for considering a positive CMI was 320 $CD4^{2+}$ cells $\times 10^6$ $CD4^+$ T cells counted).

Cytokine and chemokine profiling

Culture supernatants obtained after PBMC gE-stimulation were analysed using a commercially available Bio-Plex Pro Human Cytokine 27-plex Assay (Bio-Rad, Hercules, CA, USA) and a custom Bio-Plex Human Chemokines 6-plex Assay (Bio-Rad). Cytokines and chemokines were grouped into biologically relevant pathways: inflammatory (IL-1β, IL-6, TNF-α, IL-17), humoral (IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF), chemotactic (IL-8, MCP-1, MIP-1α, MIP-1β, RANTES and eotaxin), Th1/antiviral (IFN-γ, IL-2, IL-12, IL-15 and IP-10), fibrovascular/remodelling (VEGF, FGF and PDGF-BB), and type I interferon-IFN (CCL2, CCL8, CCL19, CXCL9, CXCL10 and CXCL11) scores. This classification integrates contemporary concepts of T functional polarization, chemokine-guided leukocyte trafficking, growth factor-mediated remodelling, and IFN pathway activation [11-14], consistent with standardized cytokine functional scoring approaches used in multiplex immunoassay platforms (Eve Technologies, Calgary, Canada). Composite pathway scores were calculated as the mean of the natural log-transformed concentrations of analytes belonging to each pathway.

Statistical analysis

Continuous variables are presented as median and interquartile range (IQR), whereas categorical variables are expressed as counts and percentages. Comparisons between two groups were performed using the Mann-Whitney U test, and comparisons among three groups using the Kruskal-Wallis test followed by Dunn's multiple-comparison test, when appropriate. Correlations between immune variables were assessed using Spearman's rank correlation coefficient. Categorical variables were compared between clinical groups using the chi-square test or Fisher’s exact test, as appropriate. Multivariable linear regression analysis was performed to identify independent predictors of vaccine immunogenicity.

Statistical analyses were performed using GraphPad Software 9.5.1 for Windows and StataSE/18.0 for Windows, and statistical significance was set at $p<0.05$.

Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Comitato Etico Territoriale Lombardia 5 (protocol ICH n°2864). All participants provided written informed consent before the enrolment.

Results

Study population

Baseline demographic and clinical characteristics of 8 SSc patients on MMF, 8 SSc patients not receiving MMF, and 12 HC completing the two-dose RZV vaccination schedule are summarized in **Table 1**. HC did not have rheumatic diseases and received RZV for ≥2 episodes of HZ. No HZ reactivation episodes were reported before the study in SSc patients.

Humoral and cellular immune responses

All participants achieved seroconversion following RZV vaccination (**Table 1**). However, post-vaccination anti-gE IgG titres were significantly lower in patients with SSc than in HCs [median 2328 (IQR 1520–2980) vs 4000 (4000–4000) mIU/mL; $P<0.0001$]. Within the SSc cohort, patients receiving MMF exhibited significantly lower antibody titres than untreated patients [1756 (891–2547) vs 2830 (2297–3408) mIU/mL; $P=0.03$], and the overall comparison among the three groups was significant ($P=0.0002$) (**Figure 1A**). No associations were observed between antibody titres and age, sex, disease subset, autoantibody profile or internal organ involvement on univariable analysis. At multivariable linear regression analysis, MMF treatment (β = −1465.4, 95% CI −2298 to −632; $P=0.003$) and older age (β = −33.4, 95% CI −61.9 to −4.8; $P=0.03$) were independently associated with lower post-vaccination antibody titres.

In contrast, vaccine-induced CMI was comparable across groups, even if the percentages of SSc patients achieving CMI and the frequency of activated gE-specific $CD4^{2+}$ T cells were lower in SSc patients on MMF (**Table 1 and Figure 1B**).

Cytokine and chemokine pathway responses

Composite cytokine and chemokine pathway scores did not differ significantly between gE-stimulated PBMCs derived from the overall vaccinated SSc cohort and HC. Within the SSc cohort, MMF-treated patients showed a lower inflammatory pathway score than untreated

patients [1.25 (0.6-1.6) vs 2.06 pg/ml (1.6-4.0), p=0.02; overall considering also HC 1.8 (0.6-2.2) p=0.06], as expected. Humoral and chemotactic scores also showed a decreasing trend in MMF-treated patients, although not statistically significant (**Figure 1C**).

Type I IFN score was not different between groups and globally increased after gE-stimulation of cultured PBMCs [in SSc 4.2 (3.6-5.0) to 4.6 (3.8-5.8), p=0.04, in HC 4.5 (3.3-5.2) to 5.4 (4.7-6.1), p=0.03].

Both humoral and type I IFN scores showed positive correlations with post-vaccination anti-gE titres (Spearman *r*=0.50, *P*=0.05; and *r*=0.40, *P*=0.03, respectively) (**Figure 1D**). Of note, in our study MMF did not affect IFN score from gE-stimulated PBMCs. Taken together these data suggest that SSc *per sè* and a high SSc disease burden do not contribute to the reduced humoral response to RZV.

No composite pathway score correlated with vaccine-induced CMI.

Clinical follow-up

During the over 2 years follow-up (median 28.5 months, IQR 22.5-29.25), no HZ reactivation was observed among patients and HC. SSc patients did not show clinically significant worsening disease activity or clinically relevant safety concerns. Immunosuppressive treatment remained stable throughout follow-up except for one patient who discontinued MMF because of pregnancy and one who initiated nintedanib for progressive interstitial lung disease.

Discussion

In this prospective observational study, we found that SSc *per sè* or SSc disease activity do not impair RZV immune responses following RZV vaccination, while MMF selectively impaired humoral, but not cell-mediated immune responses. Although all participants achieved serological response, patients receiving MMF developed significantly lower anti-gE antibody titres than untreated patients and HC, while vaccine-induced gE-specific $CD4^{2+}$ T-cell responses remained comparable across groups. Moreover, MMF treatment and older age independently predicted lower post-vaccination antibody titres, suggesting that aging and concomitant immunosuppression, rather than SSc itself, is the principal determinant of reduced humoral immunogenicity.

These findings complement the recent study by Luppino-Assad *et al.* [6]. Our data indicate that stratification according to ongoing treatment provides clinically meaningful information on vaccine immunogenicity in patients with SSc. More broadly, they reinforce the concept that vaccine responses should be evaluated according to the mechanism of action of specific immunomodulatory agents rather than considering immunosuppression as a homogeneous condition.

Despite reduced antibody production, vaccine-induced CMI was preserved in SSc patients treated with MMF. Unlike many vaccines for which antibody titres represent the principal correlate of protection, immunity against varicella-zoster virus depends predominantly on antigen-specific cell-mediated immune responses [5]. Consequently, lower antibody concentrations in MMF-treated patients should not necessarily be interpreted as reduced clinical protection. The absence of HZ reactivation during the two-year follow-up further supports the hypothesis that preserved CMI may provide meaningful protection despite reduced humoral responses.

Our results are also consistent with our previous observations following SARS-CoV-2 mRNA vaccination [9], in which therapeutic doses of MMF selectively impaired humoral but largely preserved cellular immunity. Together, these findings suggest that the differential effect of MMF on adaptive immune responses may extend across vaccine platforms and pathogens, supporting the biological plausibility of our observations.

Beyond conventional immunogenicity analyses, we explored vaccine-induced immune activation using biologically informed cytokine and chemokine composite scores. Interestingly, both humoral and type I IFN composite scores correlated positively with post-vaccination antibody titres, suggesting that integrated pathway-based biomarkers may better reflect the quality of vaccine-induced immune activation than individual cytokines. In addition, we observed that MMF-treated patients exhibited lower inflammatory pathway score, but not a lower IFN score after gE stimulation. However, these analyses should be regarded as exploratory and hypothesis-generating, and their clinical relevance requires validation in larger independent cohorts and a comparison with serum scores from which most of the published data are derived. Importantly, RZV was well tolerated, with no evidence of SSc worsening or safety concerns during follow-up. These findings are consistent with previous reports supporting the safety of RZV in patients with SSc and provide additional reassurance regarding vaccination in patients receiving MMF [6].

Several limitations should be acknowledged. First, the small sample size, the observational design, and the single-centre study limit the precision and generalizability of our findings. Second, cytokine pathway analyses were exploratory and not adjusted for multiple testing, warranting cautious interpretation. Third, immunogenicity was assessed using laboratory surrogate markers and the study was not powered to detect differences in herpes zoster incidence.

In conclusion, MMF selectively attenuates humoral but preserves cell-mediated immune responses following RZV vaccination in SSc. These findings support RZV vaccination in patients receiving MMF and suggest that assessment of vaccine-induced CMI may provide clinically relevant information beyond antibody titres alone. Furthermore, cytokine/chemokine pathway profiling may represent a promising approach for characterizing vaccine-induced immune responses, although its role requires confirmation in larger prospective studies.

List of abbreviations: ACR/EULAR: American College of Rheumatology / European Alliance of Associations for Rheumatology; CCL: Chemokine (C-C motif) ligand; CD: Cluster of differentiation; CI: Confidence interval; CMI: Cell-mediated immunity; CXCL: Chemokine (C-X-C motif) ligand; FGF: Fibroblast growth factor; gE: Glycoprotein E; GM-CSF: Granulocyte-macrophage colony-stimulating factor; HC: Healthy controls; HZ: Herpes zoster; INF: Interferon; IgG: Immunoglobulin G; IL: Interleukin; IQR: Interquartile range; MMF: Mycophenolate mofetil; mRNA: Messenger ribonucleic acid; PBMCs: Peripheral blood mononuclear cells; PDGF-BB: Platelet-derived growth factor BB; RZV: Recombinant zoster vaccine; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SSc: Systemic sclerosis; Th1: T helper cell type 1; TNF-α: Tumor necrosis factor alpha; VEGF: Vascular endothelial growth factor; VZV: Varicella-zoster virus.

Declarations:

Ethics approval and consent to participate: The study was conducted in compliance with the Helsinki Declaration and the local Ethic Committee ‘Comitato Etico Territoriale Lombardia 5’ approved the study (protocol n° ICH 2864); all participants gave written informed consent for the study.

Consent for publication: not applicable

Availability of data and materials: the data are available from the corresponding author upon reasonable request.

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

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Authors' contributions: MDS and FM had a role in conception and design of the work, and the acquisition and analysis of data. GC and EA had a role in design of the work and acquisition of data. NI had a role in acquisition, analysis, and interpretation of data. AT, NM, and CS had a role in analysis and interpretation of data. All authors drafted the work and substantively revised it. All authors agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. All authors approved the final version of the work.

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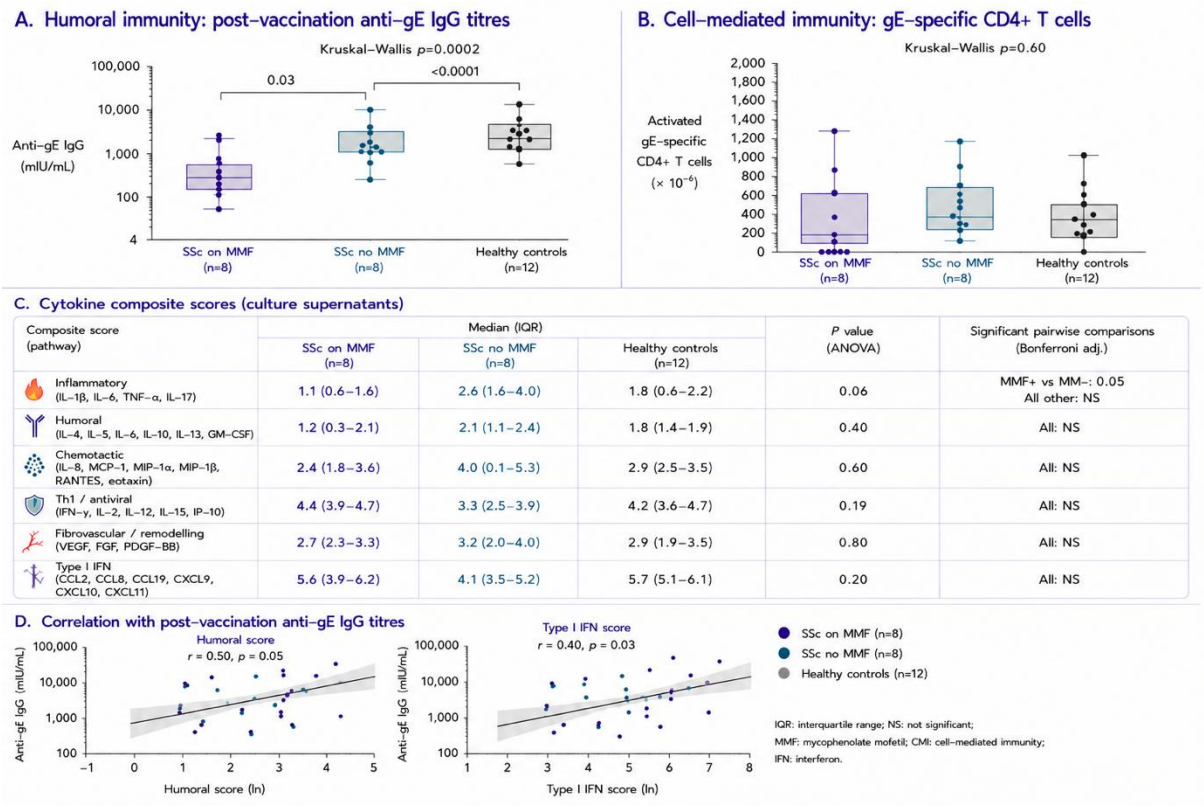
Table 1: Demographic and clinical characteristics, and vaccine immune response of the enrolled subjects.

	SSc on MMF (n=8)	**SSc no MMF (n=8)**	**Controls (n=12)**	p*
Male sex	1/8	1/8	5/12	0.1
Age (years)	55 (49-65)	73 (53-84)	61 (40-75)	0.7
Race	All Caucasian	All Caucasian	All Caucasian	NA
Disease features				
Disease duration (years)	10 (9-30)	12 (7.6-33)		0.9
Diffuse	5/8 (62.5%)	1/8 (12.5%)		0.1
Anti-centromere Ab	1/8 (12.5%)	6/8 (75%)		0.04
Anti-topoisomerase Ab	6/8 (75%)	1/8 (12.5%)		0.04
Other Ab	0	1/8 (12.5%)		>0.9

Organ involvement				
mRSS - median (IQR)	7.5 (5.75 - 10)	5 (1.5-12)		0.4
Skin ulcers	2/8 (%)	5/8 (%)		0.3
Calcinosis	2/8 (25%)	3/8 (37.5%)		>0.9
ILD	6/8 (75%)	0		0.007
pHI	1/8 (%)	2/8 (%)		>0.9
PAH	4/8 (%)	0/8 (%)		0.07
Gut	0	5/8 (62.5%)		0.02
SRC	0	0		>0.9
Immunosuppressive treatment (ever)				
None	NA	5/8 (%)		NA
Glucocorticoids	1/8 (12.5%)	0		>0.9
HCQ	2/8 (25%)	1/8 (12.5%)		>0.9
MMF	NA	1/8 (12.5%)		NA
MTX	1/8 (12.5%)	1/8 (12.5%)		>0.9
AZA	1/8 (12.5%)	0		>0.9
TCZ	1/8 (12.5%)	0		>0.9
RTX	2/8 (25%)	0		0.4
CYC	1/8 (12.5%)	0		>0.9
Vaccine response				
Seroconversion rate	100%	100%	100%	NA
CMI	3/8 (37.5%)	5/8 (62.5%)	7/12 (58.3%)	0.5

Continuous variables are presented as median (interquartile range), whereas categorical variables are expressed as counts (percentages). SSc: systemic sclerosis; MMF: mycophenolate mofetil; n: number; IQR: interquartile range; NA: not applicable; Ab: antibodies; mRSS: modified Rodnan skin score; ILD: interstitial lung disease; pHI: primary heart involvement; PAH: pulmonary arterial hypertension; SRC: scleroderma renal crisis; MTX: methotrexate; AZA: azathioprine; TCZ: tocilizumab; RTX rituximab; CYC: cyclophosphamide; CMI: cellular-mediated immunity. *: statistical comparisons were performed using one-way ANOVA for the first three rows and the Mann–Whitney U test for the comparison between MMF and no-MMF groups.

Figure 1. Humoral, cellular, and cytokine/chemokine responses to recombinant zoster vaccine in systemic sclerosis according to mycophenolate mofetil treatment.



C. Cytokine composite scores (culture supernatants)

Composite score (pathway)	Median (IQR) SSc on MMF (n=8)	SSc no MMF (n=8)	Healthy controls (n=12)	*P* value (ANOVA)	Significant pairwise comparisons (Bonferroni adj.)
Inflammatory (IL-1β, IL-6, TNF-α, IL-17)	1.1 (0.6–1.6)	2.6 (1.6–4.0)	1.8 (0.6–2.2)	0.06	MMF+ vs MM–: 0.05 All other: NS
Humoral (IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF)	1.2 (0.3–2.1)	2.1 (1.1–2.4)	1.8 (1.4–1.9)	0.40	All: NS
Chemotactic (IL-8, MCP-1, MIP-1α, MIP-1β, RANTES, eotaxin)	2.4 (1.8–3.6)	4.0 (0.1–5.3)	2.9 (2.5–3.5)	0.60	All: NS
Th1 / antiviral (IFN-γ, IL-2, IL-12, IL-15, IP-10)	4.4 (3.9–4.7)	3.3 (2.5–3.9)	4.2 (3.6–4.7)	0.19	All: NS
Fibrovascular / remodelling (VEGF, FGF, PDGF-BB)	2.7 (2.3–3.3)	3.2 (2.0–4.0)	2.9 (1.9–3.5)	0.80	All: NS
Type I IFN (CCL2, CCL8, CCL19, CXCL9, CXCL10, CXCL11)	5.6 (3.9–6.2)	4.1 (3.5–5.2)	5.7 (5.1–6.1)	0.20	All: NS

Alt text: Four-panel figure summarizing immune responses to recombinant zoster vaccine (RZV) in patients with systemic sclerosis (SSc) receiving mycophenolate mofetil (MMF; n=8), patients with SSc not receiving MMF (n=8), and healthy controls (n=12). Panel A shows post-vaccination anti-glycoprotein E (anti-gE) IgG antibody titres, with significantly lower responses in MMF-treated patients than in untreated patients and healthy controls. Panel B shows gE-specific CD4+ T-cell responses after peptide stimulation, demonstrating no significant differences between groups, consistent with preserved cell-mediated immunity. Panel C presents composite cytokine and chemokine pathway scores (inflammatory, humoral, chemotactic, Th1/antiviral, fibrovascular/remodelling, and type I interferon) as summary values for each study group together with overall and pairwise statistical comparisons. Only the inflammatory composite score is reduced in MMF-treated patients compared with untreated patients, whereas all other pathway scores are similar across groups. Panel D shows positive correlations between post-vaccination anti-gE IgG titres and both the humoral composite score and the type I interferon composite score. Values are expressed as median (IQR).