Supplementary materials

Distinct anti-MDA5 antibody trajectories associated with long-term outcomes in 6-month survivors of anti-MDA5 dermatomyositis with interstitial lung disease

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Running Title: Anti-MDA5 antibody trajectories in anti-MDA5+DM-ILD

Table of Contents

Material and Methods in Details

LCMM process

1.1 Normalization of values of anti-MDA5 antibodies

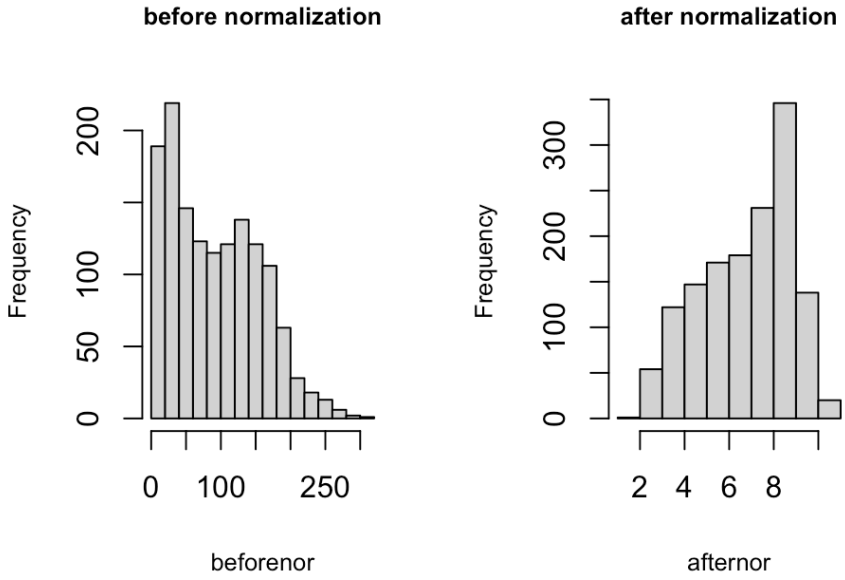

Figure S1 The Box-Cox transformation was used to normalize the values of anti-MDA5 antibodies.

1.2 STEP 2 Determine the optimal number of classes

To determine the optimal number of classes, we constructed a mixed effects model with latent classes K=1-10 using lcmm function with quadratic specification of time from the 'lcmm' package for R. The BIC and sizes of each latent class are presented in Table S1. The model with two classes was identified with a lower BIC and clinically consistent.

Table S1. BIC and classes percentages for models with 1 to 10 classes.

G	loglik	npm	AIC	BIC	SABIC	entropy	%class1	%class2	%class3	%class4	%class5	%class6	%class7	%class8	%class9	%class10
1	-7245.51	7.00	14505.02	14531.13	14508.93	1.00	100.00									
2	**-7175.08**	**11.00**	**14372.16**	**14413.19**	**14378.30**	**0.72**	**25.65**	**74.35**								
3	-7160.53	15.00	14351.06	14407.01	14359.44	0.64	28.25	11.04	60.71							
4	-7149.11	19.00	14336.22	14407.09	14346.83	0.61	15.91	12.66	19.16	52.27						
5	-7148.57	23.00	14343.13	14428.93	14355.98	0.49	15.91	9.09	22.40	31.17	21.43					
6	-7148.54	27.00	14351.07	14451.78	14366.15	0.46	15.91	9.42	22.40	38.64	0.00	13.64				
7	-7127.44	31.00	14316.89	14432.52	14334.20	0.60	13.64	0.97	22.08	6.49	40.91	10.39	5.52			
8	-7141.65	35.00	14353.30	14483.85	14372.85	0.59	15.91	6.82	21.43	35.71	19.48	0.00	0.00	0.65		
9	-7133.45	39.00	14344.91	14490.38	14366.69	0.55	15.26	0.65	22.08	6.82	40.58	13.96	0.00	0.65	0.00	
10	-7141.64	43.00	14369.28	14529.67	14393.30	0.52	16.56	6.82	21.43	42.86	11.69	0.00	0.00	0.65	0.00	0.00

1.3 STEP 3 Refine the model

We further refined and compared models using the favored K=2 with different parameters: (i) using time, quadratic time^2 and cubic time^3; (ii)normalizing transformation of anti-MDA5 antibodies (Figure S1); (iii) using linear and beta splines. Model performances (BIC) and subjects' distributions in the latent classes were reported in the Table S2. Model with unnormalized anti-MDA5 antibodies, cubic time effects and beta splines had lowest BIC and was used in the following analysis.

Table S2. BICs and subjects' distributions in each latent class. Model with different parameters of data (anti-MDA5 antibodies normalized), time effects and splines

Normalization of anti-MDA5 Ab titers	spline	time	loglik	npm	AIC	BIC	SABIC	entropy	%class1	%class2
No	linear	linear	-7346.65	9	14711.30	14744.87	14716.33	0.01	0.00	100.00
No	linear	Quadratic	-7175.08	11	14372.16	14413.19	14378.30	0.72	25.65	74.35
No	linear	Cubic	-7344.96	13	14715.92	14764.41	14723.18	0.72	48.38	51.62
No	beta	linear	-7017.23	11	14056.46	14097.49	14062.61	0.63	74.03	25.97
No	beta	Quadratic	-6862.06	13	13750.13	13798.62	13757.39	0.62	45.78	54.22
No	beta	Cubic	-6781.73	15	13593.46	13649.42	13601.84	0.67	48.38	51.62
Yes	linear	linear	-2415.95	9	4849.90	4883.47	4854.93	0.75	81.82	18.18
Yes	linear	Quadratic	-2243.44	11	4508.88	4549.91	4515.02	0.68	47.08	52.92
Yes	linear	Cubic	-2160.73	13	4347.46	4395.95	4354.72	0.72	50.32	49.68
Yes	beta	linear	-2333.52	11	4689.03	4730.06	4695.17	0.62	76.62	23.38
Yes	beta	Quadratic	-2179.33	13	4384.65	4433.14	4391.91	0.61	45.13	54.87
Yes	**beta**	**Cubic**	**-2105.72**	**15**	**4241.43**	**4297.38**	**4249.81**	**0.68**	**47.73**	**52.27**

1.4 STEP 4 Model adequacy assessments

Model adequacy was determined by assigning each patient to a trajectory class based on the posterior probability. The average of the maximum posterior probability assignments (APPA) and odds of correct classification (OCC) for each class were presented in Table S3.

Table S3. APPA and OCC in Latent classes.

Description	Number of classes	BIC	Relative entropy	Class-specific parameters	Class 1	Class 2
Random Cubic	2	4297.38	0.68	n of patients (%)	143 (48.31)	153 (51.69)
Beta				Average posterior probability assignment (APPA)	0.914	0.891
				Odds of correct classification (OCC)	10.855	7.964

1.6 STEP 5 Graphical presentation

Graphical presentation approaches with the use of mean trajectory plots with 95% predictive intervals for each class and individual level 'spaghetti plots' were presented in Figure 2.

Table S4: Baseline characteristics of patients with anti-MDA5 antibody-positive dermatomyositis within 6 months(n=525).

Characteristics	Survivors (n=394)	Deceased (n=131)	P value
Age, years	49.33 ± 11.68	58.64 ± 9.18	<0.001
Sex, female (n, %)	275 (69.8)	76 (58.0)	0.01
PaO_2/FiO_2 (mmHg)			
≥300	318 (80.7)	30 (22.9)	
≥ 200, and, < 300	58 (14.7)	34 (26.0)	<0.001
< 200	18 (4.6)	67 (51.1)	
Laboratory examinations			
Lym ($\times 10^9$/L)	1.02 ± 0.67	0.74 ± 0.50	<0.001
ESR (mm/h)	30.83 ± 21.74	34.95± 21.86	0.06
SF (ng/ml)	686.15 (280.4, 1315.8)	1404.9 (600.8, 2709.1)	<0.001
LDH (U/L)	328.99 ± 272.47	487.32 ± 210.62	<0.001
MDA5 titers (RU/mL)	152.36 ± 47.95	173.47 ± 49.87	<0.001
Treatment			
Jak inhibitors (n, %)	366 (92.9)	126 (96.2)	0.18
Others*	153 (38.8)	91 (69.5)	<0.001
Anti-fibrosis drugs	117 (29.7)	49 (37.4)	0.10
Comorbidity			
Spontaneous pneumomediastinum and/or pneumothorax	25 (6.3)	36 (27.5)	<0.001
Infection	136 (34.52)	87 (66.4)	<0.001

*, Other treatments, including tacrolimus (FK506), rituximab (RTX), methotrexate (MTX), cyclophosphamide (CTX), cyclosporine (CsA), abatacept, IL-6 inhibitors, and zanubrutinib, either alone or in combination. Lym, lymphocytes; ESR, erythrocyte sedimentation rate; SF, serum ferritin; LDH, lactate dehydrogenase; CK, creatine kinase; MDA5, melanoma differentiation-associated protein 5.

Table S5. Baseline characteristics of anti-MDA5 antibody-positive dermatomyositis patients between survived within 6 months.

Characteristics	All survivors (n=394)	Eligible survivors (n=310)	Unavailable survivors (n=84）	*P value
Age, years	49.33 ± 11.68	49.26 ± 11.57	49.63 ± 12.11	0.08
Sex, female (n, %)	275 (69.8)	215 (69.4)	60 (71.4)	0.71
PaO2/FiO2 (mmHg)				
≥300	318 (80.7)	244 (78.7)	72 (88.1)	0.15
≥ 200, and, < 300	58 (14.7)	50 (16.1)	8 (9.5)	
< 200	18 (4.6)	16 (5.2)	2 (2.4)	
Laboratory examination				
Lym (×109/L)	1.02 ± 0.67	1.0 ± 0.66	1.09 ± 0.71	0.26
ESR (mm/h)	30.83 ± 21.74	31.6 ± 21.62	27.93 ±22.03	0.17
SF (ng/ml)	686.15 (280.4, 1315.8)	743.7 (300.43, 1414.0)	516.15 (199.95, 1013.68)	0.05
LDH (U/L)	328.99 ± 272.47	332.6 ± 297.6	314.95 ± 137.57	0.61
CK (U/L)	49 (28, 77)	51 (28.0, 81)	44.5 (27.25,73.0)	0.28
Anti-MDA5 antibodies titers (RU/mL)	152.4 ± 47.9	154.8 ± 49.2	143.87 ± 42.17	0.07
Treatment (n, %)				
JAK inhibitors	366 (92.9)	289 (93.2)	77 (91.7)	0.62
Others#	153 (38.8)	119 (38.4)	34 (40.5)	0.74
Anti-fibrosis drugs	117 (29.7)	97 (31.3)	18 (21.4)	0.08
Comorbidity (n, %)				
Spontaneous pneumomediastinum and/or pneumothorax	25 (6.3)	19 (6.1)	6 (7.1)	0.74

*P value indicated comparisons between eligible survivors and Unavailable survivors. #Other treatments, including tacrolimus (FK506), rituximab (RTX), methotrexate (MTX), cyclophosphamide (CTX), cyclosporine (CsA), abatacept, IL-6 inhibitors, and zanubrutinib, either alone or in combination. Lym, lymphocytes; ESR, erythrocyte sedimentation rate; SF, serum ferritin; LDH, lactate dehydrogenase; CK, creatine kinase; MDA5, melanoma differentiation-associated protein 5.

Table S6. Factors associated with achieving LDA by univariable and multivariable COX regression model.

Characteristics	HR (univariable) with 95%CI	P value	HR (multivariable) with 95%CI	P value
Age ≥55 years	1.02 (0.74-1.41)	0.92		
Female	1.52 (1.07-2.17)	0.02	1.39 (0.91-2.13)	0.13
PaO_2/FiO_2 (mmHg)				
⩾300	1.0 (refer)			
⩾ 200, and, < 300	0.82 (0.53-1.28)	0.39		
< 200	1.46 (0.81-2.64)	0.21		
Lymphocyte counts ≥ 0.5×109/L	0.86 (0.59-1.26)	0.44		
SF ≥1000 ng/ml	0.50 (0.36-0.71)	<0.001	0.69 (0.45-1.04)	0.08
LDH ≥300 U/L	0.75 (0.55-1.02)	0.07	0.82 (0.57-1.17)	0.28
Anti-MDA5 antibody titers ≥150 RU/mL	0.7 (0.52-0.95)	0.02	0.81 (0.57-1.14)	0.23
Maximum dosage of MP (mg/d)	1.0 (0.998-1.00)	0.91		
Treatment with immunoglobulin	1.12 (0.82-1.53)	0.46		
Reduction in ΔMDA5@3m ≥33%	2.41 (1.62-3.59)	<0.001	1.96 (1.33-2.90)	<0.001

LDA, Low disease activity; ΔMDA5@3m was defined as the relative changes of anti-MDA5 antibody levels within the first three months.

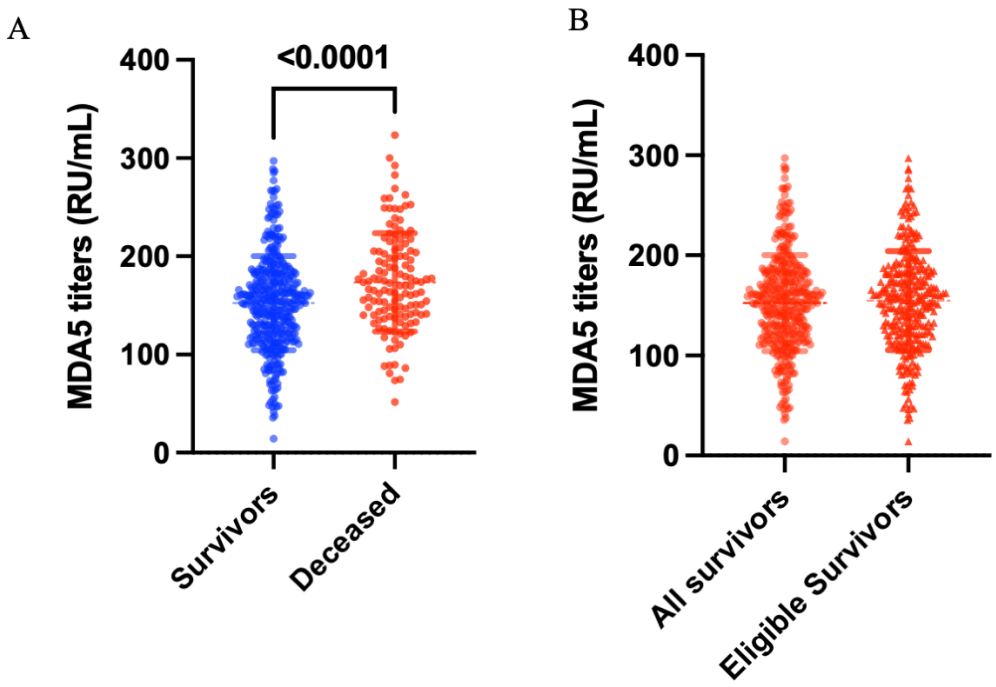

Figure S2. The levels of anti-MDA5 antibody in the inception cohort of anti-MDA5+ DM-ILD. (A) The levels of anti-MDA5 antibody between survivors and deceased patients within 6 months. (B) The levels of anti-MDA5 antibody between all survivors and eligible survivors in trajectory class.

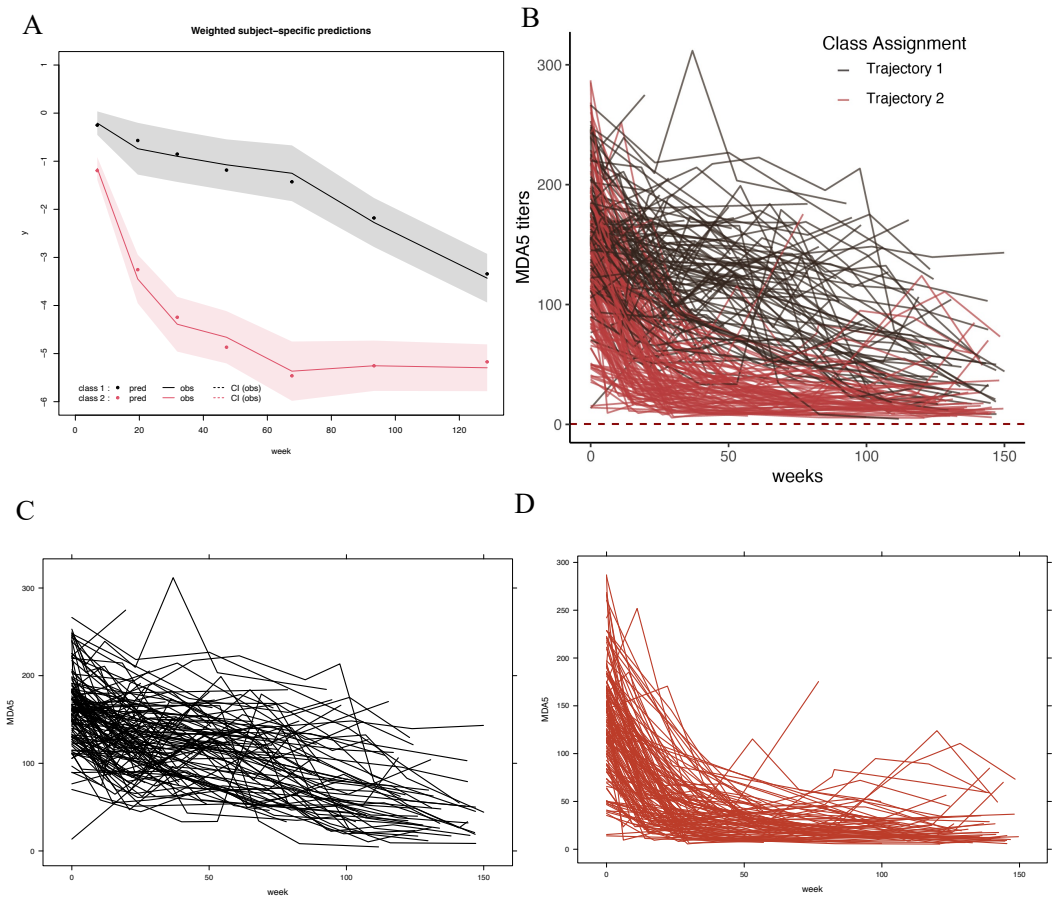

Figure S3. Two trajectories of anti-MDA5 antibody levels in the survivors of anti-MDA5+ DM-ILD within 6 months excluding patients using rituximab and immunoadsorption and plasm exchange (n=278). (A) Trajectory plots with average and 95% predictive intervals of anti-MDA5 antibody levels for each cluster. The x axis represents time, and the y axis represents the level of normalized anti-MDA5 titers converted by 'lcmm' package. (B) Overall survival curve of seroconversion between 'MDA5 slow seroconvertors', and 'MDA5 fast seroconvertors'. (B) Individual level 'spaghetti plots' showed changes of original anti-MDA5 antibody levels of each patient in all trajectories and in Trajectory 1 (C), and Trajectory 2 (D).

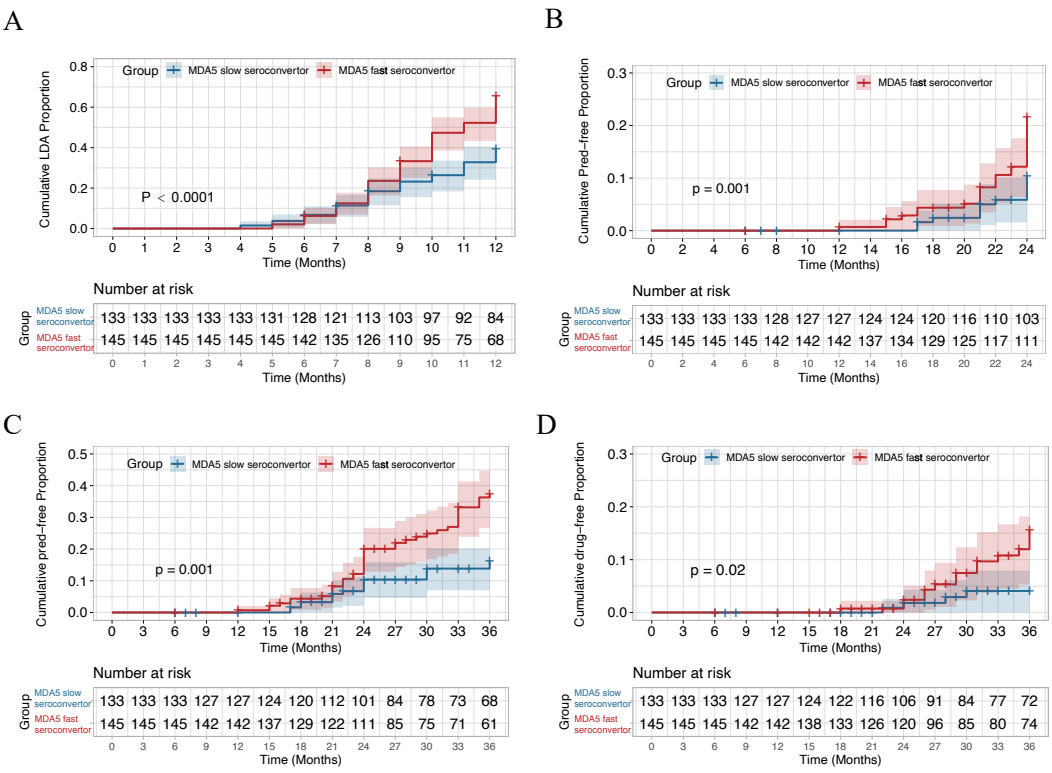

Figure S4. The long-term outcomes between 'MDA5 slow seroconvertors' and 'MDA5 fast seroconvertors' excluding patients using rituximab and immunoadsorption and plasm exchange (n=278). (A-D) Comparison of LDA at 12 months (A); Pred-free remission at 24 months (B); Pred-free remission (C) and drug-free remission (D) at 36 months in Kaplan Meier curves.

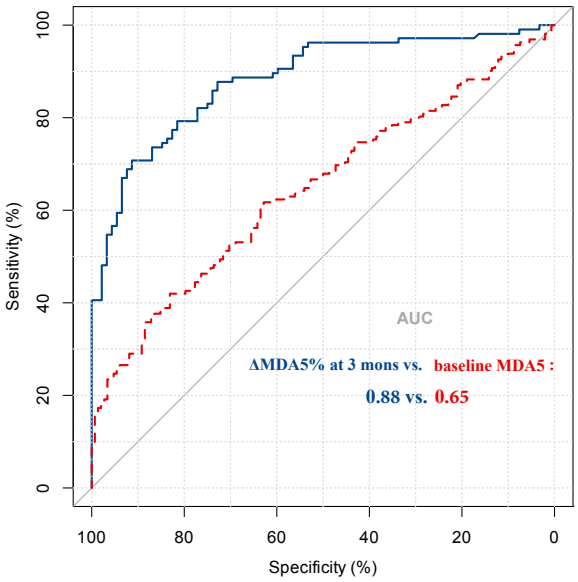

Figure S5. Receiver operating characteristic (ROC) curves of ΔMDA5@3m and baseline anti-MDA5 antibody for trajectory classification in eligible patients excluding those with rituximab and immunoadsorption and plasm exchange.

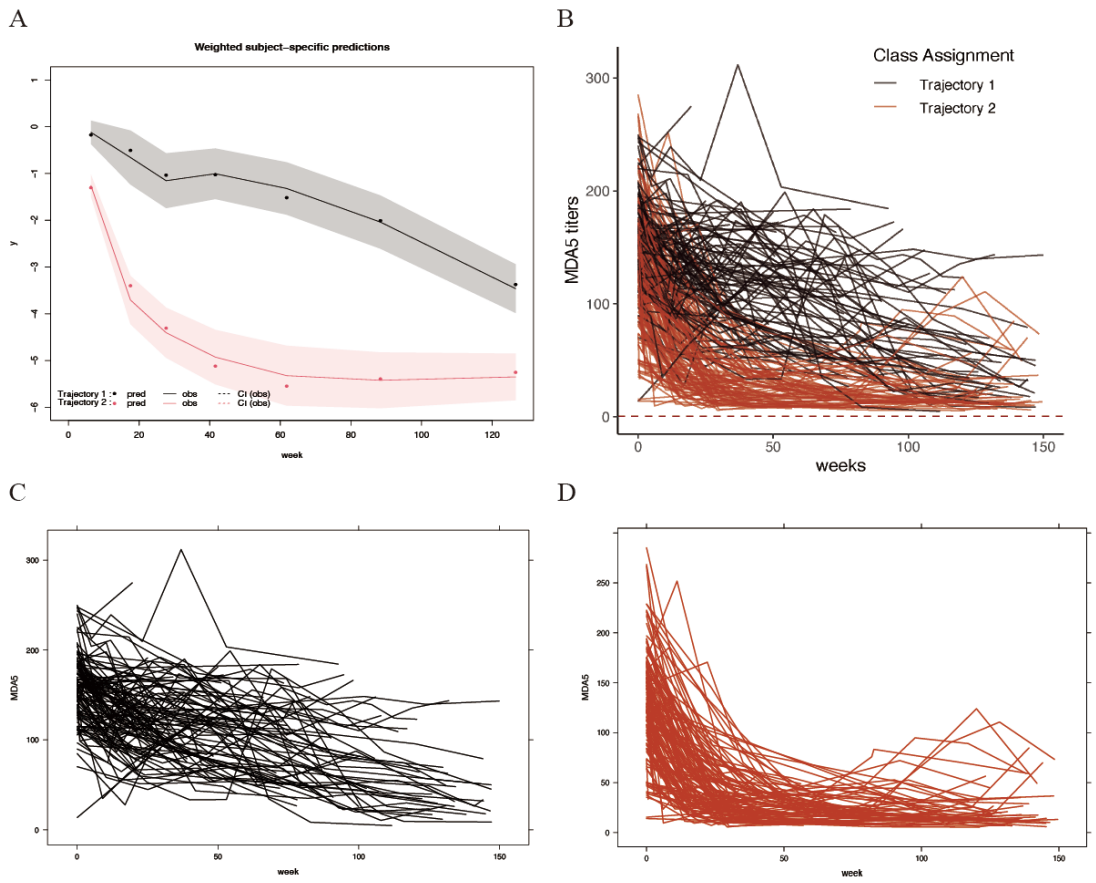

Figure S6. Two trajectories of anti-MDA5 antibody levels in the survivors of anti-MDA5+ DM-ILD within 6 months between January 2020 and June 2024 (COIVD-19 era) (n=258). (A) Trajectory plots with average and 95% predictive intervals of anti-MDA5 antibody levels for each cluster. The x axis represents time, and the y axis represents the level of normalized anti-MDA5 antibody titers converted by 'lcmm' package. (B) Overall survival curve of seroconversion between 'MDA5 slow seroconvertors', and 'MDA5 fast seroconvertors'. (B) Individual level 'spaghetti plots' showed changes of original anti-MDA5 antibody levels of each patient in all trajectories and in Trajectory 1 (C), and Trajectory 2 (D). Trajectory 1 and Trajectory 2 represented the slow seroconvertors and fast seroconvertors, respectively.

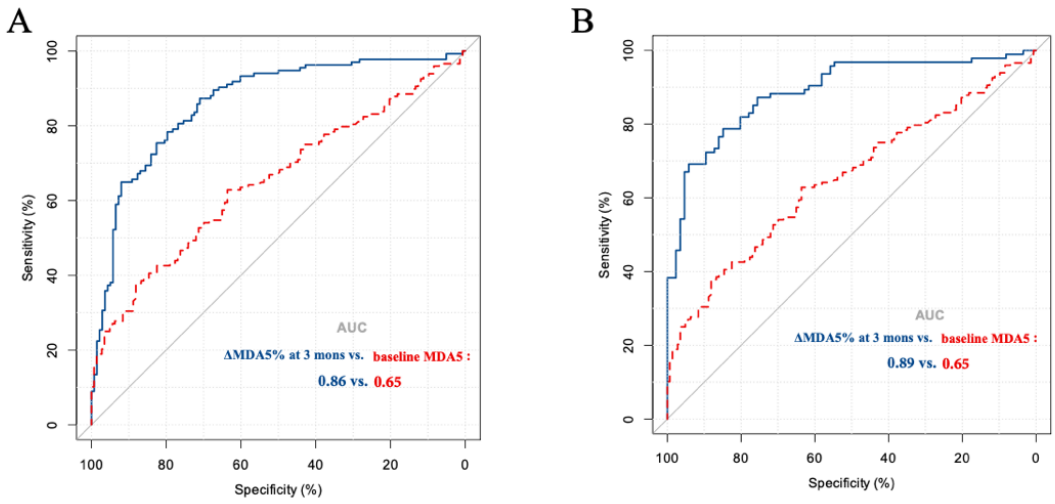

Figure S7. Sensitivity analyses of the potential clinical values of ΔMDA5@3m and anti-MDA5 antibody level at baseline excluding ΔMDA5@3m imputation. (A, B) Receiver operating characteristic (ROC) curves of ΔMDA5@3m and anti-MDA5 antibody level at baseline for trajectory classification in the eligible survivors (A) and in the eligible survivors excluding those with rituximab and immunoadsorption (B).