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Differential immunoglobulin class-mediated responses to components of the U1 small nuclear ribonucleoprotein in Systemic Lupus Erythematosus and Mixed Connective Tissue Disease

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Summary

Objective—To determine whether patients with Systemic Lupus Erythematosus (SLE) and Mixed Connective Tissue Disease (MCTD) possess differential IgM-and IgG-specific reactivity against peptides from the U1 small nuclear ribonucleoprotein particle (U1 snRNP).

Methods—The IgM- and IgG-mediated responses against 15 peptides from subunits of the U1 snRNP were assessed by indirect ELISAs in sera from patients with SLE and MCTD and healthy individuals (n = 81, 41 and 31, respectively). Additionally, 42 laboratory tests and 40 clinical symptoms were evaluated to uncover potential differences. Binomial logistic regression analyses (BLR) were performed to construct models to support the independent nature of SLE and MCTD. Receiver Operating Characteristic (ROC) curves corroborated the classification power of the models.

Results—We analyzed IgM and IgG anti-U1 snRNP titers to classify SLE and MCTD patients. IgG anti-U1 snRNP reactivity segregates SLE and MCTD from non-disease controls with an accuracy of 94.1% while IgM-specific anti-U1 snRNP responses distinguish SLE from MCTD patients with an accuracy of 71.3%. Comparison of the IgG and IgM anti-U1 snRNP approach with clinical tests used for diagnosing SLE and MCTD revealed that our method is the best classification tool of those analyzed ($p \leq 0.0001$).

Conclusions—Our IgM anti-U1 snRNP system along with lab tests and symptoms provide additional molecular and clinical evidence to support the hypothesis that SLE and MCTD may be distinct syndromes.

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Keywords

Systemic Lupus Erythematosus (SLE); Mixed Connective Tissue Disease (MCTD); immunoglobulin M (IgM); U1 small nuclear ribonucleoprotein particle (U1 snRNP); auto-immune disorders; classification criteria

Introduction

Systemic Lupus Erythematosus (SLE) and Mixed Connective Tissue Disease (MCTD) are systemic autoimmune disorders with overlapping clinical manifestations that possess aberrant immune responses against common auto-antigens[1, 2, 3–6]. Despite its description as an independent auto-immune disease[7], the classification of MCTD as distinct from SLE remains controversial due to the high number of common clinical features between SLE and MCTD patients[7–13]. Nevertheless, the concept of MCTD has been reported as a useful definition in clinical practice[3, 11–13], and clinical and serological features segregate the two illnesses[14–16]. The American College of Rheumatology (ACR) has created universal classification parameters for SLE[14]; however, four different criteria sets exist for MCTD patients, with the Alarcón-Segovia criteria being the most widely accepted[17].

Currently, there is no single test with sufficient specificity and sensitivity to discriminate between SLE and MCTD[2, 4, 15, 18–20], which has hampered the identification of MCTD as a separate syndrome. A positive diagnosis by any set of criteria requires a patient to exhibit at least four clinical symptoms and/or tests out of those included in each list, which can take years to develop[2, 14]. Moreover, traditional laboratory tests are performed with numerous commercially available kits that can vary in principle and cut-off values, which may alter the final results and diagnoses[4, 15, 18, 21, 22]. These and other factors complicate proper diagnosis of these two closely related and overlapping illnesses.

Previous investigations have demonstrated that SLE and MCTD patients often exhibit 1000-fold greater auto-reactivity to subunits of the U1 small nuclear ribonucleoprotein particle (snRNP) than to any other cellular component [23, 24]. The U1 snRNP is an RNA-protein complex that is responsible for pre-mRNA processing and is composed of 10 proteins (U1–70K, U1A, U1C and seven Smith antigen (Sm) proteins)[25, 26, 27]. In general, previous studies aimed at finding biomarkers for SLE and MCTD have focused on IgG-specific responses to nuclear components, including the U1 snRNP; however, some studies have revealed differential IgM reactivity for nuclear components in SLE and MCTD patients[24, 29–31]. Yet, the potential use of the IgM response as a molecular tool to classify SLE and MCTD patients has not been fully explored.

To determine whether SLE and MCTD represented distinct disorders and test whether the two patient groups can be segregated, we evaluated the IgG- and IgM-specific responses of patients with SLE and MCTD and healthy individuals against 15 different U1 snRNP peptides (named P1–15) by indirect enzyme-linked immunosorbent assays (ELISAs). Interestingly, we observed higher IgG-based reactivity for U1 snRNP peptides in individuals with SLE or MCTD compared to healthy individuals, but elevated IgM responses in SLE patients compared to those with MCTD and healthy adults. The IgM response to two peptides, P4 and P10 (P4/P10), exhibited 71.3% accuracy in segregating between these two autoimmune disorders ($p \leq 0.05$). In summary, these data support the notion that SLE and MCTD are, indeed, distinct disorders and highlight the potential clinical use of the IgM anti-U1 snRNP system as a molecular tool to assist in the classification of SLE and MCTD patients.

Methods

Collection and preparation of sample sera

Sera were obtained from whole blood of 122 patients previously diagnosed with SLE (n=81) or MCTD (n=41) and 31 healthy individuals. Samples were collected following the Institutional Review Board (IRB) accepted protocols of the University of Miami (IRB numbers: 200307-24 and 200402-86) and Florida International University (IRB number: 040308-00). SLE and MCTD patients (collectively referred to here as "ill" or "patient group") were clinically diagnosed according to the American College of Rheumatology (ACR) criteria[14] and the Alarcón-Segovia criteria[17], respectively, along with clinician judgment. The laboratory tests in this study were commercially performed by Quest Diagnostic Incorporated and their positive values are included in Table 2. Details of the flare or remission period in these SLE and MCTD patients were not recorded at the moment of whole blood collection and, therefore, disease activity for these SLE and MCTD patients has not been considered in this study.

Selection of U1 snRNP peptides

The U1 snRNP peptides included were previously reported in Somarelli *et al*.,[32] and commercially synthesized by BioMatik Corporation (Wilmington, DE, USA) The observed IgM reactivity for each of the U1 snRNP peptides was ranked from most (1) to least (15) antigenic for each disease state (Table 1).

Monitoring IgM reactivity for U1 snRNP peptides by indirect ELISAs

The indirect ELISA protocol employed to assess IgM reactivity for each peptide and sample included was previously described[32]. The average IgM derived OD value for each peptide was normalized using the average OD value of the healthy group per peptide examined and was expressed as OD% based on the following formula[33] (Supplementary data 1):

$$OD\% = \left(\frac{\overline{X}\, OD \ of \ sample \ in \ Px}{\overline{X}\, OD \ of \ control \ group \ in \ Px} \right) \times 100$$

where "$\bar{X}$ *OD of sample in* P_x" is the average OD value of the sample group (SLE or MCTD) and "$\bar{X}$ *OD of control in* P_x" indicates the average OD of the control group (healthy group) from each of the peptides included in this study (P1–P15). To evaluate the relative reactivity contributed by IgM and IgG in SLE, MCTD and healthy populations, the average OD values from IgG-specific ELISAs previously reported by Somarelli *et al*.,[32], which used the same samples and U1 snRNP peptides included in this study, were re-analyzed and converted to OD% using the equation described above[33] (Supplementary data 2).

Statistical analyses

Significant differences in IgG and IgM reactivity between patients (SLE and MTCD) and healthy groups and between SLE and MCTD individuals for each of the peptides was assessed using independent sample *t*-tests. Clinical tests and symptoms were evaluated by independent sample *t*-tests (numerical data) or Chi (X) squared tests (nominal data). Receiver Operating Characteristic (ROC) curves were generated with the PASW software package (version 18). Forward binary logistic regression (BLR) analyses using the IgM and IgG anti-U1 snRNP titers in ill (SLE and MCTD) and healthy individuals as well as SLE and MCTD patients were performed with the PASW software package (version 18). P-values ≤ 0.05 were considered statistically significant for all tests. Correlations between the IgM anti-U1snRNP peptide reactivity and IgM anti-Rheumatoid Factor (RF) antigenicity

were performed using the PASW software package (version 18). We found no significant correlation between the IgM-specific anti-U1 snRNP reactivity and IgM-mediated anti-RF activity. As a result, these analyses were not further considered in this study.

Results

IgM anti-U1 snRNP reactivity is elevated in SLE but not MCTD patients

The IgM response to U1 snRNP peptides was monitored via indirect ELISAs and reported as OD% (Figure 1A and Supplementary data 1). IgM anti-U1 snRNP titers were significantly higher in the SLE group than either the MCTD population or healthy individuals ($p \leq 0.05$). In fact, in many instances, IgM responses to U1 snRNP peptides in MCTD patients were equal to or below those exhibited by healthy individuals (P3, P4 and P9–P15 in Figure 1A). The discrimination capacity of IgM-anti-U1 snRNP peptide ELISAs was assessed by ROC curve analysis and indicates that IgM reactivity for P1 and P13 provides significant power to classify SLE and MCTD patients; however, none of the IgM responses were sufficient to discern SLE and MCTD from non-disease controls with statistical significance (Figures 1C–D and Supplementary data 3).

SLE and MCTD patients exhibit an elevated IgG response for U1 snRNP peptides

As previous studies have reported [32, 34–38], the IgG-mediated reactivity for each of the U1 snRNP peptides was significantly higher in both SLE and MCTD populations than in the healthy group; however, IgG reactivity does not differ between the two autoimmune disorders (Figure 1B). ROC curve analyses on IgG anti-U1 snRNP titers per peptide ascertain their individual ability to discern between patients (SLE and MCTD) and healthy individuals and between SLE and MCTD patients (Figure 1C – D, respectively; and Supplementary data 4). As previously reported[32], all but IgG anti-P2 responses were capable of significantly discriminating SLE and MCTD from healthy individuals with IgG anti-P4 being the best ($p \leq 0.05$); however, none of the IgG anti-U1 snRNP titers had a statistically significant ability to classify SLE and MTCD patients (Figure 1D).

Differential auto-immune responses and symptoms are observed in SLE and MCTD patients

We found that SLE and MCTD patients exhibit significantly different IgM anti-U1 snRNP reactivity ($p \leq 0.05$) despite similar IgG-mediated antigenicity for the same peptides (Figures 1A – B). To further support the idea that SLE and MCTD represent distinct auto-immune illnesses, statistical analysis of 42 standard laboratory tests were performed with blood samples from the SLE and MCTD patient cohort. These analyses revealed that 11 out of the 42 clinical tests were significantly different in SLE and MCTD patients ($p \leq 0.05$) (Table 2). Specifically, differences were observed in tests designed to detect nuclear auto-antigens (RNP, Sm, SCL70, dsDNA, elevated DNA), renal function (creatine phosphokinase levels, renal proteinuria, renal hematuria) and immune system components (C3 and C4 complement levels) ($p \leq 0.05$). These findings support the idea that SLE and MCTD represent distinct autoimmune manifestations, with specific antigenic targets and antibody class reactivities.

Similarly, statistical assessment of 40 clinical symptoms from patients in our SLE and MCTD cohort indicated that 16 out of the 40 clinical characteristics evaluated were significantly different between SLE and MCTD patients (Table 3). Most of the significantly different clinical manifestations involved the skin and joints of these patients; however, our data also confirmed that neuropsychiatric disorders and problems in the circulatory system were also significantly different between the two groups. Once again, the fact that clinical

symptoms differ in SLE and MCTD populations supports the hypothesis that these maladies may be clinically distinct.

Antibody class reactivities for U1 snRNP peptides segregate among SLE, MCTD and healthy individuals

The IgM and IgG responses for all U1 snRNP peptides were combined in a BLR to determine which peptide and auto-antibody combinations might provide the highest segregation between patient (SLE and MCTD) and healthy populations. These analyses revealed that the combined IgG-specific response for P2, P4, P5, P10 and P13 has the greatest capacity to discern between sick and healthy individuals with an overall accuracy of 94% ($p \leq 0.05$) (Figure 2A) where the probability of correctly predicting a patient with either SLE or MCTD is higher than that for correctly predicting a healthy individual (96.7% and 83.9%, respectively).

Additional BLRs were performed with the individual IgG and IgM reactivities for each U1 snRNP peptide to assess which peptide and Ig class combination significantly discriminates between SLE and MCTD patients. These analyses indicated that only the combined IgM response for P4 (U1C) and P10 (U1A) significantly discriminate between SLE and MCTD patients, with an overall accuracy of 71.3% ($p \leq 0.05$) (Figure 2B). Remarkably, most of the classification power derives from the proper classification of SLE patients (95.1%) rather than proper grouping of MCTD patients (24.4%) (Figure 2B). Consequently, our data demonstrate that by first combining the IgG reactivity for P2, P4, P5 and P10 and then the titers for IgM anti-P4/P10, we can achieve an overall accuracy of 73.9% at discriminating among SLE, MCTD and healthy groups.

Comparing the power of IgM anti-P4/P10 with conventional clinical tests

To determine the classification power of our proposed IgM-specific P4/P10 ELISA-based system, ROC curves were used to compare our system with eight conventional clinical tests. The individual IgM reactivities for P1 and P13 were also included in the ROC curves analyses because they discriminate between SLE and MCTD (Figure 1D). The 11 laboratory tests that significantly differ between SLE and MCTD patients were performed only in a small portion of each sub-population (Table 2). As a result, not all tests could be analyzed due to the reduced sample size. Instead, eight of the most frequently-used laboratory tests that are part of the classification criteria to diagnose SLE or MCTD were included in the ROC curve analysis (FANA titers, dsDNA ELISA, elevated serum DNA titers and positive results for RNP, Sm, SSA, SSB and SCL-70)[4, 15–16, 21–22]. When using the subset of individuals for whom clinical test results were available (SLE = 59 and MCTD = 24), the IgM anti-P4/P10 titers and IgM anti-P1 reactivity displayed the greatest discrimination capacity to classify SLE and MCTD patients ($p \leq 0.05$) (Figure 3 and Supplementary data 3). ROC curves confirmed that among the conventional tests evaluated, elevated DNA and positive results for Sm are the third and fourth best at significantly segregate SLE and MCTD ($p \leq 0.05$).

Improving the discriminatory capacity of IgM anti-P4/P10 titers

BLR analyses were performed to assess whether the combination of the IgM anti-P4/P10 system and any of the eight laboratory tests employed to diagnose SLE or MCTD (FANA titers, dsDNA ELISA, elevated serum DNA titers and positive results for RNP, Sm, SSA, SSB and SCL-70)[4; 15–16; 21–22] might provide greater capacity to distinguish between these syndromes. The individual IgM reactivities for P1 and P13 were considered in this BLR analysis because they showed a significant ability to classify SLE and MCTD patients ($p \leq 0.05$) (Figure 1D). BLR analyses indicated that the combination of the IgM-based reactivity for P4/P10 and an elevated DNA assay represent the best combination of variables to

segregate SLE from MCTD when compared with IgM anti-P4/P10, -P1, or-P13 and any single laboratory test examined ($p \leq 0.0001$) (Figure 3 and Supplementary data 3). None of the other clinical test combinations improved the power of discrimination between SLE and MCTD patients over that exhibited by the individual tests alone ($p \leq 0.05$). Our analyses also suggest that, when combined with the standard elevated DNA test, the IgM response against P4/P10 may be useful in enhancing the current segregation of SLE from MCTD.

Discussion

Despite the fact that MCTD was described as a distinct rheumatic syndrome in 1972[7], placement of this disorder as a separate auto-immune illness remains controversial. Opinions are divided regarding classification of MCTD as a separate malady due to the number of auto-antigens and clinical symptoms that show overlap with SLE[1–13]. The immune responses of SLE and MCTD patients for overlapping 'self' antigens coupled with the diversity of commercially available clinical tests with differing protocols, reagents and cut-off values have impeded the development of standard and uniform assays to segregate these syndromes[2, 4, 6, 21]. With the exception of a few studies[24, 29–31], most investigations have focused on IgG-mediated reactivity toward specific antigens as potential molecular tools to differentiate between SLE and MCTD patients[34–38]. Given that SLE and MCTD patients are characterized by elevated blood titers of multiple Ig classes, including IgM[24, 39–40], we hypothesized that IgM responses to a number of U1 snRNP peptides may allow us to increase the present discrimination between SLE and MCTD and provide additional molecular evidence to claim the independent nature of these two disorders.

Our data indicate that the combined IgM reactivity for fragments of U1C (P4) and U1A (P10) is capable of classifying SLE and MCTD patients with an accuracy of 71.3% (Figure 2B), a value higher than previously reported peptide-based immunoassays that have been used to segregate these disorders[21]. These findings are in concordance with previous reports, which revealed a preponderance of IgM anti-U1 snRNP antibodies in SLE, but not MCTD patients[24, 30]. Therefore, our work is congruent with prior investigations and demonstrates the potential utility of differential Ig class responses as a classification tool for SLE and MCTD. The current work also provides molecular evidence to support the distinct etiology of these syndromes.

The binomial analyses identified combinations of laboratory tests and/or peptide reactivities that significantly discern between these maladies. Interestingly, the IgM anti-P4/P10 ELISA-based system provided the greatest capacity to segregate between SLE and MCTD disorders than eight other conventional laboratory tests ($p \leq 0.0001$) (Figure 3). Additionally, we revealed that the combination of IgM anti-P4/P10 antigenicity with the elevated DNA test segregated 79.8% of SLE and MCTD patients, even in the smaller subset of patients for whom clinical test results were available (n = 59 for SLE and n = 24 for MCTD) (Figure 3). It is not surprising that the dsDNA test contributes to the differentiation of these diseases given that antibodies against DNA have been detected in approximately 70% of SLE patients and shows 95% specificity for this disorder (16; 18). Yet, the fact that the dsDNA test alone exhibits a lower ability to segregate SLE and MCTD patients (66.4%) than the IgM anti-P4/P10 system (73.1%), indicates the significant contribution of our ELISA-based system in discerning between these two maladies (Figure 3).

We delineated a total of 16 out of 40 clinical manifestations that differed significantly between SLE and MCTD patients (Table 3). On average, MCTD patients exhibited hand/joint swelling and muscle weakness with 25% higher frequency than SLE patients. Similarly, malar and discoid rashes were found to be more prevalent in the SLE than the MCTD group (46% and 10% versus 13% and 0%, respectively). These findings are in

concordance with previous studies that reported these clinical manifestations as key features in SLE or MCTD patients[19–20]. Evidence of mental illness was also found to be 32% higher in MCTD than SLE patients. Although we cannot rule out selection bias of the clinicians diagnosing these disorders, our results obtained from a subset of SLE and MCTD patients suggest that the immune response of SLE patients seems to be directed to skin areas on the face while those suffering from MCTD appear to develop a more systemic immune response that attacks the skin, joints and muscles throughout various parts of the body. Furthermore, these findings highlight specific clinical manifestations that appear to differ between SLE and MCTD patients and should be considered as clinical evidence that they may be distinct diseases.

Overall, this study further highlights the current challenges in developing quantitative tests for the classification of SLE and MCTD and therefore the recognition of MCTD as a separate entity[4,2,6,21]. Here, we describe a novel approach based on differential antibody class (IgM and IgG) responses as a mechanism to discriminate between SLE and MCTD patients with better accuracy than conventional laboratory tests currently employed as part of the classification criteria to diagnose these syndromes. In addition, our data revealed contrasting frequencies of clinical symptoms characterizing these auto-immune syndromes whereby SLE patients showed a concentrated auto-immune manifestation directed to skin areas on the face while those suffering from MCTD developed more systemic immune responses that attack the skin, joints and muscles throughout various parts of the body. Consequently, our results provide further evidence to support the fact that there are molecular and clinical aspects of SLE and MCTD to indicate that these diseases are, indeed, two distinct autoimmune syndromes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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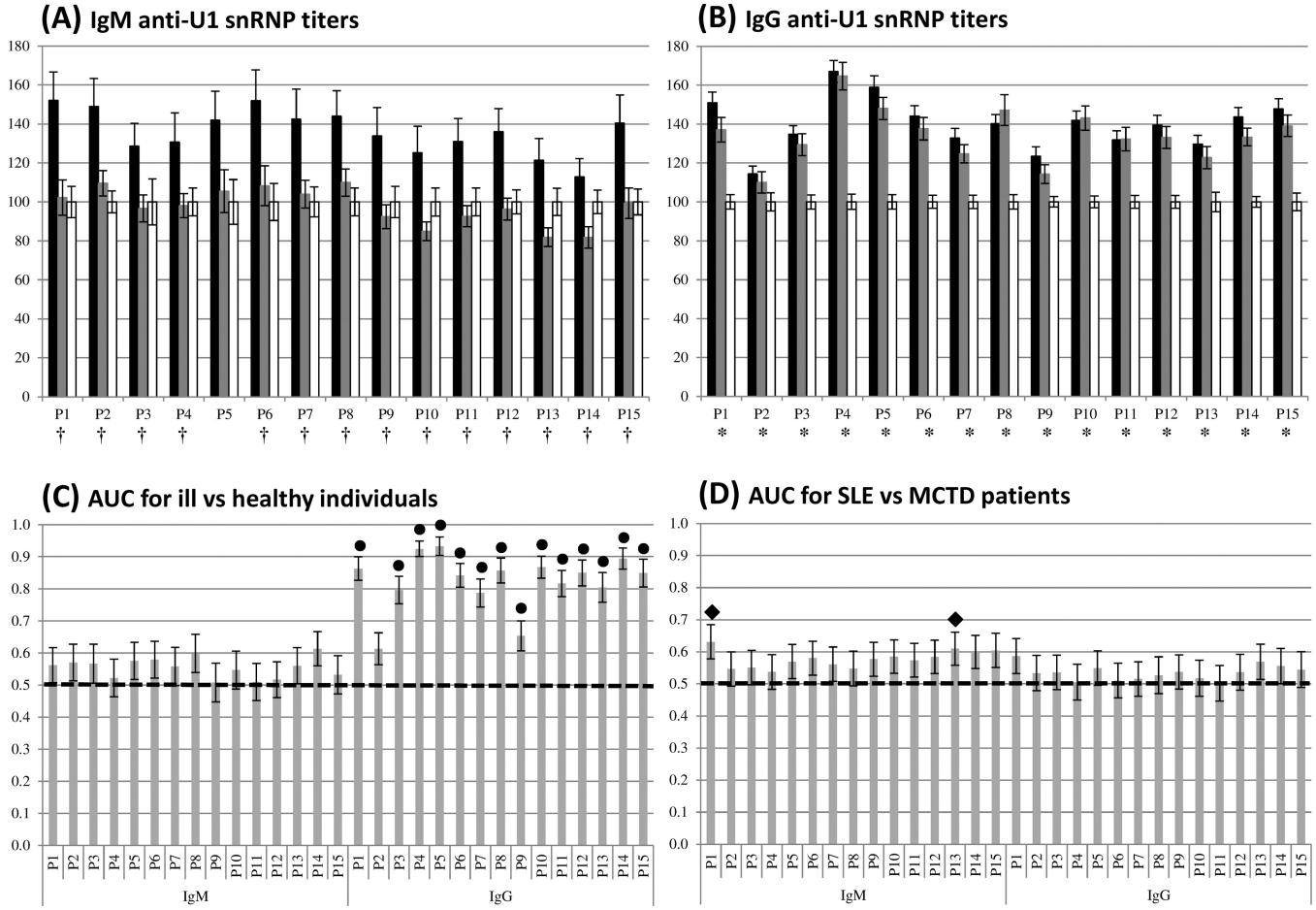
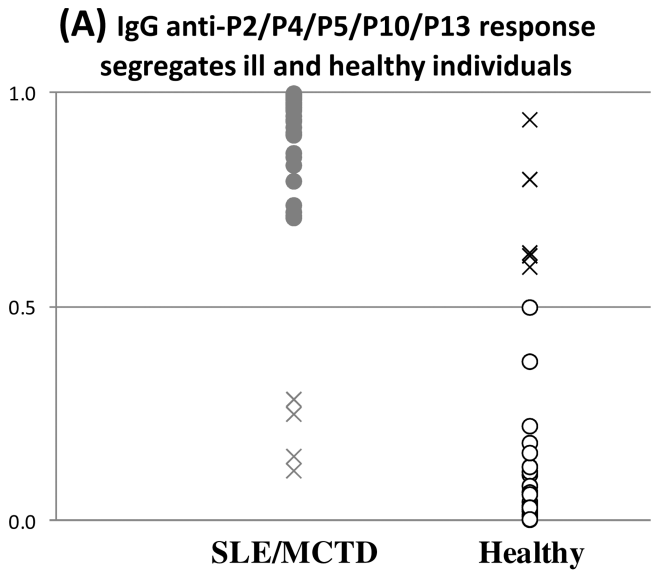
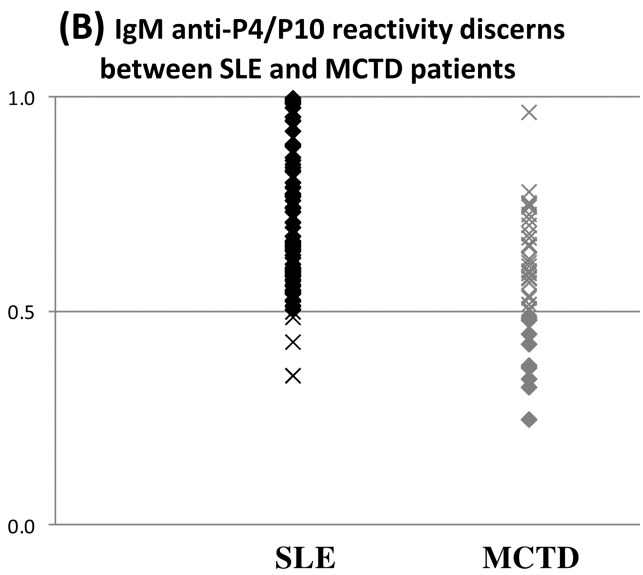


Figure 1. Contrasting IgM-specific anti-U1 snRNP peptide responses observed in SLE and MCTD patients

(A) and **(B)** represent the average percent optical density (OD%) values for the IgM class and IgG classes, respectively. Peptide number and OD% are on the x and y axes, respectively. The black, gray and white bars symbolize the average OD% of SLE, MCTD and healthy groups, respectively. (†) and (*) indicate significantly different OD% between SLE and MCTD as well as patients and healthy populations, respectively ($p \leq 0.05$). **(C)** and **(D)** correspond to the area under the curve (AUC), derived from ROC curves, for ill (SLE and MCTD) vs. healthy individuals as well as SLE vs. MCTD patients, respectively. Peptide number per Ig class and their AUC values are indicated on the x and y axes, respectively. (●) and (♦) symbolize significantly different AUC between patients and healthy individuals as well as SLE and MCTD patients, respectively ($p \leq 0.05$). The dotted lines in **C** and **D** indicate the cut-off value (0.5). Black bars in all graphs represent standard error of the mean.



	SLE/MCTD	Healthy
n	122	31
TP	118	26
FN/FP	4	5
Accuracy	96.7%	83.9%
Overall accuracy = 94.1%		



	SLE	MCTD
n	81	41
TP	77	10
FN/FP	4	31
Accuracy	95.1%	24.4%
Overall accuracy = 71.3%		

Figure 2. Identification of a two-step ELISA system for classification of SLE, MCTD and healthy individuals

(A) The combination of IgG-mediated anti-P2/P4/P5/P10/P13 provides the best segregation between SLE and MCTD and non-disease controls. The distribution of Ill (SLE and MCTD) and healthy individuals and the predicted combined IgG-mediated reactivity are represented on the x and y axes, respectively. Gray and white circles indicate true positives (TP). **(B)** Combined IgM-anti-P4/P10 can classify SLE and MCTD patients. The distribution of SLE and MCTD patients' combined IgM anti-P4/P10 predicted values are on the x and y axes, respectively. Black and gray diamonds indicate true positive (TP) samples for SLE and MCTD patients, respectively. The crosses represent false negatives (FN) or false positives (FP). Predicted values were obtained using binomial logistic regression (BLR) with a cut-off of 0.5 ($p \leq 0.05$).

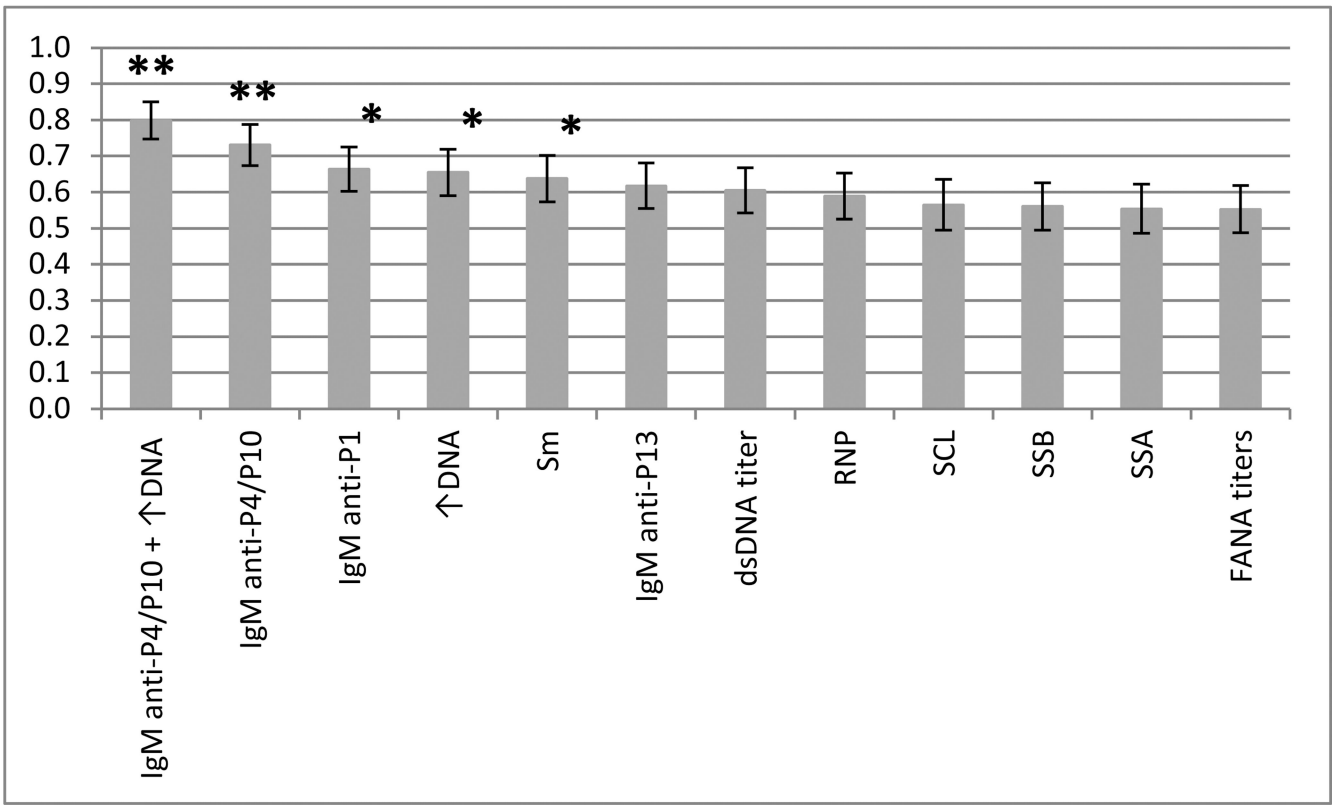


Figure 3. Area under the curve analysis reveals the classification power of IgM anti-P4/P10

Receiver operating characteristic (ROC) curves were generated using peptide antigenicities or laboratory tests. The columns in the graph represent the area under the curve (AUC) on the y axis for each variable tested. The bars on top of each column indicate standard error of the mean. FANA titers, dsDNA, ↑DNA (elevated serum DNA) and positive results for RNP, Sm, SSA, SSB and SCL are clinical tests used during SLE and MCTD diagnosis. The "IgM anti-P4/P10" indicates the combined IgM anti-P4/P10 titer while "IgM anti-P4/P10 + ↑DNA" represents the combination of the IgM anti-P4/P10 ELISA and the elevated DNA assay. The "*" and "**" indicate significant differences in classifying SLE and MCTD with *p* values of ≤ 0.05 and ≤ 0.0001, respectively.

Table 1

Overview of U1 snRNP peptides used in the study

Peptide number	U1 snRNP protein	Peptide region (amino acid range)	Peptide sequence	Rank observed IgM reactivity	
				SLE	*MCTD*
1†*	U1A	196–203	PPAQPLSE	1	6
2†*	Sm E	63–70	EIHSKTKS	3	2
3†*	Sm F	46–53	NTEEYIDG	12	9
4†*	U1C	90–97	GMMPAPHM	11	8
5*	U1-70K	337–344	PDGPDGPE	6	4
6†*	Sm B	83–90	EGPPPKDT	2	3
7†*	Sm G	1–8	MSKAHPPE	5	5
8†*	Sm D3	20–27	CETNTGEV	4	1
9†*	Sm F	77–84	EEEEDGEM	9	12
10†*	U1A	112–119	KPKSQETP	13	13
11†*	Sm D2	14–21	EELQKREE	10	11
12†*	Sm D1	22–29	GTQVHGTI	8	10
13†*	U1A	178–185	GQIPPGAM	14	14
14†*	U1-70K	325–332	APPDDGPP	15	15
15†*	U1C	66–73	PFSAPPPA	7	7

The peptide designation, region and sequences as well as the U1 snRNP protein column displayed in this table (first four columns) were previously published by Somarelli et al. (2011). The observed IgM antigenicity (columns 5 and 6, from left to right) was ranked from 1 to 15 where “1” represents the peptide with the highest IgM antigenicity and “15” indicates the peptide with the lowest IgM antigenicity.

Daggers (†) indicate IgM peptide antigenicities that significantly differ between SLE and MCTD patients while the asterisks (*) represent IgG reactivities for U1 snRNP peptides that significantly differ between ill (SLE and MCTD) and healthy individuals ($p \leq 0.05$).

Table 2

Clinical tests evaluated in SLE and MCTD patients

	Clinical test name	Definition of positive test	SLE		MCTD		*P* value
			Positive	*Total*	*Positive*	*Total*	
SLE/MCTD specific	Fluorescence antinuclear Abs titers	> 1:320 IU/ml	76.56%	64	90.63%	32	0.3860
	Fluorescence antinuclear Abs pattern	Homogenous, mixed or speckled pattern	86.30%	73	85.71%	35	0.4430
	IgG anticardiolipin positive	> 10 GPL U/ml	23.53%	68	23.81	21	0.8480
	IgM anticardiolipin positive	> 10 GPL U/ml	6.06%	66	18.18%	22	0.0870
	Rheumatoid factor titer by latex	> 14 IU/ml	8.33%	12	20.00%	5	0.0870
	IgM anti-rheumatoid factor Abs by ELISA	> 20 IU/ml	41.86%	43	38.10%	21	0.8480
	IgM anti-rheumatoid factor Abs titer	> 20 IU/ml	20.93%	43	23.81%	21	0.9040
	RNP positive	> 20 EU/ml	84.00%	75	100%	40	0.0080
	Sm positive	> 20 EU/ml	60.27%	73	28.21%	39	0.0010
	SSA positive	> 20 EU/ml	58.11%	74	47.22%	36	0.2820
	SSB positive	> 20 EU/ml	21.62%	74	11.11%	36	0.1800
	SCL 70 positive	> 20 EU/ml	1.45%	69	16.13%	31	0.0040
	Elevated serum DNA titer	≥ 10 IU/ml	64.10%	78	29.73%	37	0.0010
	Anti-dsDNA positive	5–9 IU/ml >5 SD when	49.35%	77	27.03%	37	0.0010
	IgG anti U1-70K Abs*	compared to healthy group >5 SD when	37.84%	37	55.00%	20	0.2130
	IgG anti SmB/B' Abs*	compared to healthy group >5 SD when	44.12%	34	50.00%	20	0.7700
	IgG anti SmD Abs*	compared to healthy group	73.53%	34	63.16%	19	0.7260

Clinical test name	Definition of positive test	SLE		MCTD		*P* value
		Positive	***Total***	***Positive***	***Total***	
Anemia	Hematocrit range < 35%	25.00%	80	22.50%	40	0.7630
Hemolytic anemia	Positive for anemia and hemolysis	3.23%	62	8.33%	12	0.4120
White blood count	> 10.8/mm	8.77%	57	6.06%	33	0.2780
Leukopenia	White blood count <3.0/mm	11.54%	78	12.50%	40	0.8780
Lymphopenia	Total lymphocyte count < 1500/mm	36.71%	79	42.5%	40	0.5400
Thrombocytopenia	Platelet count < 100,000	10.13%	79	2.50%	40	0.1370
Thrombocytosis	Platelet count > 600,000	5.13%	78	2.56%	39	0.5180
Creatine phosphokinase positive	<165 U/L	91.07%	56	74.29%	35	0.4770
Creatine phosphokinase elevated	>165 U/L	7.02%	57	25.71%	35	0.0120
Serum creatinine	> 1.07 mg/dL	10.53%	76	5.26%	38	0.4500
Renal cellular cast	Abnormal cellular cast on urinalysis	10.67%	75	2.56%	39	0.1280
Renal proteinuria	Defined by multiple creatine ratio criteria[†]	40.85%	71	13.51%	37	0.0040
Renal hematuria	5 red blood cells per high power field[‡]	18.92%	74	2.70%	37	0.0180
Creactive protein	> 1.0 mg/dl	20.83%	72	18.18%	33	0.4850
Elevated C reactive protein	>10 mg/dl	23.61%	72	20.59%	34	0.7290
Low C3 complement	< 90mg/dl	41.77%	79	15.00%	40	0.0030
C3 complement level	90 – 180 mg/dl	56.96%	79	82.50%	40	0.0001
Low C4 complement	< 10 mg/dl	48.10%	79	30.77%	39	0.0730
C4 complement level	16 – 47 mg/dl	44.30%	79	69.23%	39	0.0001

	Clinical test name	Definition of positive test	SLE		MCTD		*P* value
			Positive	***Total***	***Positive***	***Total***	
General	Erythrocyte sedimentation rate	0 – 20 mm/hr	57.89%	79	52.27%	40	0.8530
	Elevated erythrocyte sedimentation rate	>20 mm/hr	56.48%	76	55.00%	40	0.8710
	IgG anti-rheumatoid factor Abs	> 20 IU/ml	41.86%	43	42.86%	21	0.9400
	IgG anti-rheumatoid factor Abs titer	> 20 IU/ml	41.86%	43	38.10%	21	0.8480
	Immunoglobulin isotypes for RF factor	Presence of IgM and/or IgG antibodies	79.07%	43	76.19%	21	0.5300
	Lymphocyte absolute value	850 – 3900 cells/uL	74.14%	58	75.86%	29	0.3460

Laboratory tests that significantly differ between SLE and MCTD patients are highlighted in gray ($p \leq 0.05$). ‘Abs’, ‘IgG’ and ‘IgM’ stands for antibodies, immunoglobulin M and immunoglobulin G, respectively.

Asterisks (*) denote non-commercial laboratory tests for which positive values are recorded. All other laboratory tests listed were performed by Quest Diagnostic Incorporated, and each of their reported positive values are included. A positive value is indicated if the sample(s) exhibit values five times above the standard deviation obtained by the average value of the healthy group.

The dagger (†) defines the criteria for Renal proteinuria, which is >0.5g/24hr or >24mg/dl in random samples >2+ on urinalysis or spot urine/creatine ratio >0.2.

The double dagger (‡) indicates that a test for renal hematuria was considered positive when there was no other reason for hematuria, such as infection.

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Table 3

List of clinical symptoms observed in SLE and MCTD patients

Clinical symptom name	Definition	SLE		MCTD		*p*-value
		Positive	*Total*	*Positive*	*Total*	
Skin telangiectasia	Vascular lesions formed by dilation of small blood vessels	5%	79	15%	40	0.0650
Skin nasal/oral ulcers	Shallow and painful open sores that appear as necrotic or eroded areas on the oral mucosa	29%	79	23%	39	0.4880
Raynaud's syndrome	Vascular disorder causing periods of severely restricted blood flow to the fingers and toes	53%	80	85%	40	0.0001
History of hand swelling	Hand swelling by history	41%	81	61%	41	0.0120
Observed hand swelling	Hand swelling observed on physical exam at the date of visit	19%	81	39%	41	0.0140
Acrosclerosis	Scleroderma of the distal extremities, sometimes extending to the neck and face	4%	80	26%	38	0.0001
Skin digital pitting	Loss of skin on the tips of the fingers caused by scars and ulcers	8%	80	8%	39	0.9700
Proximal scleroderma	Skin fibrosis of the extremities proximal to the elbows or knees, or of the thorax.	3%	79	0%	39	0.3160
Skin alopecia	Hair loss condition that usually affects the scalp	58%	80	72%	39	0.1310
Malar rash	Skin rash of both cheeks joined by an extension across the bridges of the nose	46%	78	13%	39	0.0001
Discoid rash	Chronic skin problem resulting from lupus disease	10%	78	0%	39	0.0380
Skin rash	Red and swollen area on the skin	33%	78	38%	39	0.5840
Skin photosensitivity	Skin rash resulting from reaction to sunlight	57%	76	58%	38	0.8940
Skin calcinosis	Abnormal deposition of calcium salts in tissues	1%	79	5%	38	0.2000
Sicca, xerophthalmia and xerostomia	Characterized by dry eyes (xerophthalmia) and dry mouth (xerostomia)	49%	81	66%	41	0.1330
Erosive inflammatory arthritis	Synovitis with joint erosions	43%	23	45%	20	0.9200
Lymphadenopathy	Swollen or enlarged lymph nodes	24%	79	20%	40	0.6180
Fever	Increase in body temperature above the normal range (98–100°F)	22%	78	15%	40	0.7780
Proximal muscle weakness	Malfunction of muscle fibers resulting in weakness	29%	76	49%	39	0.0360

Clinical symptom name	Definition	SLE		MCTD		*p*-value
		Positive	*Total*	*Positive*	*Total*	
Myositis	Inflammation of muscle tissue	6%	79	27%	33	0.0020
Myalgia	Tenderness or pain in the muscles	54%	80	48%	40	0.8970
Morning stiffness	Joint and muscle stiffness present upon awakening	53%	73	64%	36	0.3000
Swelling of three or more joints	Multiple joints swelling	42%	78	63%	40	0.0380
Joint tenderness	Sensitivity to touch or pressure on fat pad, tendon attachment, ligament, muscle and/or skin	35%	79	69%	39	0.0010
Joint swelling	Intra-articular effusion, synovial thickening, and periarticular soft tissue inflammation	29%	78	63%	40	0.0010
Symmetric swelling	Swelling occurs in the same joint on both sides of the body	27%	78	64%	39	0.0001
Rheumatoid nodule	Includes subcutaneous nodules over bony prominences, extensor surface or in juxta articular regions	6%	77	10%	40	0.5000
Arthralgia	Joint pain	82%	79	84%	38	0.7950
Neuropathy	Any type of nerve disorders	31%	77	28%	40	0.6810
Seizure	A sudden attack of pain, of a disease, or of certain symptoms	4%	80	0%	40	0.2150
Psychosis	Any mental disorder characterized by personality disintegration and loss of contact with reality	3%	79	0%	39	0.3160
Neuropsychiatric disorder	Evidence of mental illness	19%	78	51%	39	0.0001
Hypomotility in cine deglutition esophageal	Decreased motility of the esophagus (usually results in difficulty swallowing or increased acid reflux)	41%	78	58%	40	0.0890
Pulmonary fibrosis	Development of excess fibrous connective tissue in the lungs	22%	45	23%	26	0.6330
Pleuritic pain or rubbing heard	Inflammation of membrane that enfolds both lungs	37%	78	23%	39	0.1250
Pericarditis	Inflammation of the pericardium	30%	73	18%	39	0.1610
Avascular necrosis	Cellular death of bone components due to interruption of the blood supply	3%	74	0%	33	0.3040
Clotting	Thick, viscous, or coagulated mass in blood stream.	1%	75	12%	34	0.0160
Myocardial infarction	Partial or complete occlusion of one or more of the coronary arteries resulting in myocardial injury	0%	73	11%	35	0.0030

Clinical symptom name	Definition	SLE		MCTD		*p*-value
		Positive	*Total*	*Positive*	*Total*	
Stroke	Brain hemorrhage or lack of blood flow	6%	81	2%	41	0.3570

Clinical manifestations that differ significantly between SLE and MCTD patients are highlighted in gray ($p \leq 0.05$). Neuropathy, seizure and psychosis symptoms were diagnosed in the absence of offending drugs or known metabolic derangements.