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Diagnosis and Risk Stratification in Patients with Anti-RNP Autoimmunity

Maria F. Carpintero[1], **Laisel Martinez**[2], **Irina Fernandez**[1], **Aaron Calev Garza Romero**[1], **Carolina Mejia**[1], **YunJuan Zang**[1], **Robert W. Hoffman**[3], and **Eric L. Greidinger**[1,2]

[1]Division of Rheumatology, University of Miami Miller School of Medicine, Miami, FL

[2]Miami VA Medical Center, Miami, FL

[3]Eli Lilly and Company, Indianapolis, IN

Abstract

Introduction—Anti-RNP autoantibodies occur either in Mixed Connective Tissue Disease (MCTD) (with a frequently favorable prognosis), or in systemic lupus (SLE) cases with aggressive major organ disease. It is uncertain how to assess for the risk of severe disease in anti-RNP+ patients.

Methods—Following IRB-approved protocols, clinical data and blood was collected from patients with known or suspected anti-RNP autoimmunity and normal controls in a cohort study. Samples were screened for parameters of immune activation. Groups were compared based on clinical diagnoses, disease classification criteria, disease activity, and specific end-organ clinical manifestations.

Results—97% of patients satisfying Alarcon-Segovia MCTD criteria also met SLICC SLE criteria, while 47% of the anti-RNP+ SLE patients also met MCTD criteria. Among SLICC SLE patients, MCTD criteria were associated with reduced rates of renal disease (Odds Ratio 4.3, 95% confidence interval 1.3–14.0), increased rates of Raynaud's Phenomenon (OR 3.5, 95% c.i. 1.3–9.5), and increased serum BCMA, TACI, and TNFa levels. Circulating immune markers and markers of Type I Interferon activation were not effective at distinguishing clinical subgroups.

Conclusions—Among anti-RNP patients, the question of MCTD versus SLE is not either/or: most MCTD patients also have lupus. MCTD classification criteria (but not a broad set of immune markers) distinguish a subset of SLE patients at reduced risk for renal disease.

Keywords

Systemic Lupus Erythematosus; Inflammation; Classification Criteria; Mixed Connective Tissue Disease

Address correspondence to: Dr. Eric L. Greidinger, Division of Rheumatology, University of Miami, 1120 N.W. 16th Street (D4-10), Miami, FL 33136; phone 305 243-6884; fax 305 243-7414; egreidinger@med.miami.edu.

Introduction

Anti-RNP responses have been associated with different prognoses: generally mild in MCTD, but associated with severe disease including nephritis in SLE [1–3]. In lupus, anti-RNP antibody responses are linked temporally with disease expression [4], while in MCTD the loss of anti-RNP antibodies has been linked to clinical remission [2]. A clinician faced with a patient with anti-RNP autoantibodies may thus be seeking clues regarding whether a benign or an aggressive course of disease is likely.

A plausible but untested idea would be to consider patients with “pure” MCTD to be likely to have a mild course and for patients meeting SLE criteria to be at risk for more aggressive disease, conceivably because SLE might involve the activation of more dangerous immunostimulatory pathways. Both the recently reported European MCTD cohort and the Norwegian national MCTD registry exclude patients with other rheumatic connective tissue diseases, including SLE [5,6]. However, the extent to which patients satisfying MCTD criteria may also satisfy SLE criteria (or vice-versa) has not been reported since the introduction of the 2012 SLICC SLE criteria.

Immune processes that contribute to lupus susceptibility and lupus activity have become increasingly well-defined. Innate immune activation leading to Type I Interferon secretion has been identified, and is now being targeted in human trials [8]. Roles in lupus pathogenesis for autoreactive B and T cells have been articulated, and agents targeting these are also being developed [9,10]. Some upregulated inflammatory markers in MCTD compared to healthy controls have been documented [11,12]. Studies comparing MCTD and SLE patients have found increases in IgG, IL-10 and TNFa levels in the MCTD patients, with generally similar patterns of interferon gamma, IL-2, and IL-4 production [13–15]. We have suggested that differences in immune activation, such as preferential activation of TLR3 versus TLR7, could account for differences in disease expression between MCTD and RNP+ lupus [16].

We thus screened a cohort of anti-RNP+ patients for immune markers, and assessed these for relevance. Disease activity and many circulating markers did not differ between groups by MCTD criteria. However, the patients meeting MCTD criteria had lower rates of renal disease, higher rates of Raynaud’s Phenomenon, and trends toward increased B cell activation. Thus, MCTD shares many immune pathways with SLE, and often co-exists with SLE, but MCTD clinical criteria identify patients at lower risk for renal disease.

Methods

Subjects

Patients seen at our center between 2006 and 2011 and normal controls were recruited following IRB-approved protocols. Patients were characterized as RNP+ if they had a positive clinical laboratory anti-RNP test, and/or anti-RNP antibodies were identified in our lab by ELISA or immunoblot [17]. Subject data was obtained by structured interview, physical exam, laboratory screening and review of medical records [18]. SLE classification was by 1997 ACR and 2012 SLICC criteria [7,19]. The Alarcon-Segovia MCTD criteria set

was chosen because it has performed as well as others in identifying patients with MCTD [6,7,18,20], and does not include pulmonary or renal manifestations (facilitating association studies with renal and pulmonary outcomes). Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) scores were calculated using clinical data from dates of blood collection [21]. De-identified data from a previously reported cohort of University of Missouri RNP+ patients was used following IRB-approved protocols [18].

Laboratory and imaging studies were performed based on the clinical judgment of patients' managing physicians. Interstitial lung disease (ILD) was identified by radiologist documentation of fibrosis or ground glass opacities consistent with ILD on chest radiograph and/or high resolution chest CT [22]. Pulmonary hypertension was diagnosed by right heart catheterization studies following WHO guidelines [23]; possible pulmonary hypertension was designated if transthoracic echocardiogram showed right ventricular systolic pressure >40mmHg [24]. Lung disease not otherwise specified was designated for patients who did not meet criteria for ILD, pulmonary hypertension, or possible pulmonary hypertension yet had DLCO values less than 60% predicted without proportionate restrictive or obstructive spirometry indices, and in whom no alternative explanation emerged after pulmonology consultation. Patients were classified as having renal disease (following the SLICC criteria) if they had greater than 0.5 grams per day of proteinuria, cellular casts in their urine, or glomerulonephritis confirmed by renal biopsy. Raynaud's phenomenon was designated if patients gave a history of cold-induced color changes in the digits following the validated picture-question format of Maricq and colleagues [25].

Serum assays for dsDNA, SSA, SSB, and Smith antibodies were performed by clinical laboratory ELISA. Serum B Cell Activating Factor Belonging to the TNF Family (BAFF), BAFF Receptor (BAFFR), Transmembrane Activator and CAML Interactor (TACI), B-Cell Maturation Antigen (BCMA), Interferon-gamma (IFN-g), Tumor Necrosis Factor-alpha (TNF-a), soluble CD40 Ligand (sCD40L), e-selectin, Neutrophil Gelatinase-Associated Lipocalin (NGAL), p40 subunit of Interleukin-12 (IL-12p40), p70 form of Interleukin-12 (IL-12p70), Interleukin-17A (IL-17A), -17F (IL-17F), dimers of these (IL-17A/F), and Interleukins -21 (IL-21), -22 (IL-22), -23 (IL-23), and -33 (IL-33), were measured by ELISA in duplicate following the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Type I Interferon Gene Signature

Total RNA was isolated from whole blood using the QIAamp RNA Blood Mini Kit (Qiagen, Santa Clarita, CA) and from other cell preparations using the RNeasy Plus Mini Kit (Qiagen); cDNA was synthesized with the SuperScript First-Strand Synthesis kit using random hexamers (Invitrogen, Carlsbad, CA).

Gene expression was measured for 79 of the 88 patients for whom sufficient RNA could be extracted from whole blood, plus 8 normal controls. Whole blood from 22 additional healthy blood donors was used to establish normal expression ranges. Samples mRNAs all passed quality control standards, were analyzed using Affymetrix U133 Plus 2.0 arrays, and were compared after normalization using Robust Multichip Average algorithm.

Five gene Type I Interferon Signature Scores were calculated following the Medimmune algorithm regarding the expression of IFI27, IFI44, IFI44L, RSAD2, and IFI6 in study subjects relative to the 22 normal controls [26,27]. Additional markers of Type I Interferon induction were also compared between study groups (Supplement 1). With the exception of interferon-inducible genes, analyses were confined to circulating immune markers, based on the hypothesis that relevant subgroups of anti-RNP autoimmunity patients could be distinguished immunologically.

Statistical analyses

Data was analyzed using Prism 5.0 software (GraphPad Software, San Diego, CA). For categorical data, Odds Ratios were calculated and Fisher's Exact test was used. For quantitative data, the D'Agostino & Pearson omnibus normality test was used to determine whether a t test or a non-parametric test (Mann-Whitney) would be appropriate. Subjects with missing data were censored from individual analyses. For assays in which results for an individual patient were below the lower limit of quantitation for the assay, result levels one significant digit less than the established lower limit of quantitation for the assay were assigned. When all subjects in a group had results below the lower limit of detection for an assay, other groups were compared to this group using the Wilcoxon one group test, set to the value of the lower limit of detection of the assay.

Results

Anti-RNP patients often meet classification criteria for both SLE and MCTD

In total, 37/38 (97%) patients that satisfied MCTD criteria met SLICC SLE criteria, while 37/79 (47%) of RNP+ patients that satisfied SLICC SLE criteria met MCTD criteria. Anti-RNP antibodies were present in 81/88 patients, including 42 with clinical SLE that did not meet MCTD criteria (33/42 met ACR SLE criteria, 42/42 met SLICC SLE criteria); 18 patients with clinical SLE that also met MCTD criteria (16/18 met ACR SLE criteria; 18/18 met SLICC SLE criteria); 20 patients with MCTD clinically (all met MCTD criteria); and one patient with polymyositis and Raynaud's Phenomenon who did not meet criteria for SLE or MCTD (Table 1). The SLE and MCTD subgroups were similar regarding age, disease duration, sex, and ethnicity distribution (Table 1).

Among the 20 MCTD patients, the rate of satisfying lupus criteria was high for the ACR criteria (14/20, 70%), and even higher for the SLICC criteria (19/20, 95%). Manifestations leading to lupus classification included: ANA+ (19/20, 95%), synovitis (16/20, 80%), leukopenia (11/20, 55%), photosensitivity (11/20, 55%), serositis (10/20, 50%), and other lupus autoantibodies (7/20 dsDNA+, 4/20 Sm+, and 3/20 anti-cardiolipin Ab+, cumulatively 9/20, 45%). Of the patients meeting MCTD criteria, the prevalence of these other lupus autoantibodies did not differ significantly between those in the clinical lupus and in the clinical MCTD groups. Anti-dsDNA antibodies trended modestly toward higher prevalence and higher serum levels in the SLE patients who did not meet MCTD criteria compared to the patients with clinical MCTD (Table 1). Alopecia accounted for 4 of the 5 cases that met SLICC not ACR criteria; hypocomplementemia accounted for the other case.

Using the SLICC SLE criteria set, only 1/38 patients in our cohort would have been eligible for inclusion into a “pure” MCTD registry like the European and Norwegian MCTD cohorts (6,7). Only 6/38 (16%) MCTD patients in our cohort did not satisfy ACR SLE criteria; of these 6, one met 1980 preliminary classification criteria for Systemic Sclerosis, and another met the 2013 Classification Criteria for Systemic Sclerosis [28,29].

Clinical relevance of MCTD classification in SLE patients

We explored whether MCTD criteria were clinically meaningful in patients meeting SLE criteria (Table 2). Contrary to the assumption that MCTD is milder than “classic” SLE, disease activity as measured by SLEDAI was comparable between each of the subgroups (RNP+ and RNP-; clinically classified as SLE or MCTD) in our cohort (Table 2). The mean number of ACR SLE Classification Criteria satisfied by the patients was also comparable between the SLE and MCTD groups.

MCTD criteria were associated with less frequent renal disease. The odds ratio of renal disease (satisfying the SLICC lupus criteria for renal involvement) was 4.3 (95% confidence interval 1.3–14.0) for more renal disease in subjects who did not meet MCTD criteria compared to those who did (Table 2).

In contrast to the association between MCTD criteria and the rate of renal disease in this cohort, lung disease was similarly frequent in all of the clinical subgroups studied (Table 2). This continued to be the case when the analysis was performed on patients with pulmonary hypertension/possible pulmonary hypertension or ILD.

To assess the relevance of MCTD criteria to Raynaud’s Phenomenon, we first reclassified patients using a modified Alarcon-Segovia criteria set excluding Raynaud’s, that required one fewer clinical manifestation to meet the criteria threshold (Table 3). The odds ratio was 3.5 (95% confidence interval 1.3–9.5) for more Raynaud’s in patients satisfying the modified Alarcon-Segovia criteria.

Analysis of a historical cohort

All 41 MCTD patients from a previously reported Missouri cohort met Alarcon-Segovia criteria [18]. Of these, 28/41 (68%) met ACR SLE criteria and 30/41 (73%) met SLICC SLE criteria. Four of the 11 patients not meeting SLICC criteria and one of the two additional patients not meeting ACR SLE criteria met 1980 preliminary criteria for Systemic Sclerosis; three more of the patients not meeting SLICC criteria additionally met the 2013 Systemic Sclerosis Criteria. Thus, using the most current SLE and scleroderma classification criteria, only 4/41 (10%) of Missouri MCTD patients would be eligible for inclusion into a “pure” MCTD registry.

An additional 13 RNP+ patients (with clinical diagnoses of lupus) also had data available to review from this Missouri cohort. None of these patients met MCTD criteria, but all met SLICC SLE criteria. Renal disease was present in 5/13 (38%). In contrast, renal disease was present in only 4/41 (10%) of the Missouri MCTD patients (Fisher’s Exact $p = 0.028$). Thus, in the Missouri cohort, the Odds Ratio was 5.8 for more renal disease in patients not meeting MCTD criteria (95% confidence interval 1.3–26.5).

Immune markers in SLE and MCTD patients

Levels of serum markers of inflammation, T cell activation, B cell activation, and endothelial activation were assessed by ELISA in Miami patients with available sera (5 RNP − SLE, 32 RNP+ SLE, 13 SLE meeting MCTD criteria, and 13 MCTD). Mean levels were largely similar between these patient groups, and often elevated elevated compared to normal controls (Table 4). Trends toward increased BAFF (Mann-Whitney p = 0.023) and TNFa (Mann-Whitney p = 0.022) in patients clinically diagnosed with MCTD compared to RNP+ patients that did not satisfy MCTD criteria were noted. When we compared all 37 patients that did not meet MCTD criteria to all 26 that did, we noted that MCTD patients had slightly higher levels of B cell activation-associated factors BCMA (2.4 ± 0.6 vs 2.0 ± 0.7 ng/ml, Mann-Whitney p = 0.0077) and TACI (0.055 ± 0.050 vs 0.029 ± 0.033 ng/ml, Mann-Whitney p = 0.017) more so than BAFF (2.2 ± 2.3 vs 1.3 ± 0.7 ng/ml, p = 0.055). TNFa levels also trended higher in SLICC SLE patients meeting MCTD criteria compared to those who did not (23.6 ± 19.5 vs 15.3 ± 16.6 pg/ml, Mann-Whitney p = 0.014).

Expression levels relative to normal controls of interferon inducible gene mRNAs in whole blood cells were determined in cohort patients with available samples (Table 4). Each of the disease subsets showed markedly increased five gene type I interferon signature scores compared to normal controls, but the individual disease subsets could not be distinguished from each other. No differences between MCTD and non-MCTD disease subgroups (but clear differences compared to healthy controls) were noted with a broader panel of interferon-inducible genes (Supplement 1) [27].

Associations with Individual End-Organ Clinical Manifestations

The presence of renal disease trended weakly toward increased SLEDAI (11.6 ± 6.1 in patients with renal disease versus 8.8 ± 5.4 in patients without renal disease, Mann-Whitney p = 0.057), but excluding the SLEDAI renal elements yielded scores of 9.1 ± 5.2, essentially identical to the subjects without renal disease. Increased circulating BAFF Receptor levels were noted in patients without SLICC renal disease (Wilcoxon p = 0.016), but none of the other markers studied showed even modest trends with versus without SLICC renal disease (Table 5).

Patients with lung disease did not differ in SLEDAI compared to patients without lung disease (Table 5). Soluble e-selectin levels were increased (14.6 ± 6.0 ng/ml versus 10.4 ± 4.7 ng/ml, t test p = 0.009) in patients with lung disease compared to without; the patients with high e-selectin levels were those with pulmonary hypertension/possible pulmonary hypertension (16.8 ± 8.4 ng/ml, p = 0.001 versus patients without lung disease). The ILD subset showed a trend toward increased levels of BAFF (4.1 ± 3.8 ng/ml, p = 0.021 versus patients without lung disease).

Patients with Raynaud’s Phenomenon showed trends toward lower levels of IL-17A compared with patients without RP with tested samples (Table 5). Increased e-selectin levels were not seen in RP.

Five gene IFN signature scores were not appreciably different between patients with versus without renal disease (8.0 ± 3.8 versus 8.8 ± 3.5, p = 0.5), lung disease (8.6 ± 2.9 versus 8.3 ± 4.0, p = 0.8), or Raynaud's (9.1 ± 3.5 versus 7.1 ± 3.6, p = 0.09).

Discussion

Among RNP+ patients who meet SLE criteria, also meeting MCTD criteria is not uncommon and is associated with less risk of renal disease. Among RNP+ patients in referral rheumatology practice meeting MCTD criteria, meeting SLE criteria (especially the SLICC criteria) is extremely common. Subtle immune differences favoring increased B cell activation (increased BCMA, and TACI) exist in patients meeting MCTD criteria, despite the fact that SLEDAI disease activity levels, other circulating immune marker levels, and type I interferon signature markers in peripheral blood are largely indistinguishable between the MCTD and non-MCTD SLE subsets. The association of BCMA with plasma cell survival and TACI with increased plasma cell differentiation could account for reported frequent lack of efficacy of rituximab therapy in anti-RNP autoimmunity [30–32]. Increased plasma cell numbers have been reported in MCTD patients compared to controls [33]. We have recently reported that in comparison to SLE patients, MCTD patients have lower serum levels of anti-RNP peptide-specific IgM despite similar levels of serum IgG [34]. Notably, TACI has been reported to help drive class switch recombination [35].

Lung disease is common in RNP+ SLE in our cohort regardless of MCTD criteria status. Our murine model of anti-RNP autoimmunity has shown links between TLR3-driven inflammation and lung disease, without showing similar links to renal disease or to Raynaud's-like vasculopathy [36,37]. Thus, anti-RNP antibodies may identify a risk for TLR3 activation regardless of the presence of lupus-associated TLR7 activation. Other MCTD-like manifestations such as Raynaud's may be TLR3-independent. Further study is needed to determine the extent to which the apparent protection from nephritis seen in patients meeting MCTD criteria is mediated by increased TLR3 activation or other factors.

Limitations of this work include the use of single center cohorts of modest size; the assessment of an arbitrary (though extensive) set of circulating markers of immune activity; and concerns regarding effects of multiple comparisons on determining the p value thresholds at which statistical significance should be set. The small number of cases of pulmonary disease necessitated the use of a broadly inclusive definitions that do not account for the potential for diverse underlying disease processes (such as, for example, NSIP versus UIP).

The inability to demonstrate dramatic differences in immune markers between patient groups meeting and failing to meet MCTD criteria, despite notable differences in clinical manifestations in these groups (including the fibrotic manifestations of MCTD such as acrosclerosis and pulmonary fibrosis), suggests that factors unmeasured in the current study may play key roles in distinguishing these groups, and may be better understood by assessment of organ-specific or cell-type-specific markers.

Given that nearly half of the RNP+ patients in our cohort met classification criteria for both SLE and MCTD, it is likely that MCTD patients have been and will be enrolled in past and future observational and treatment trials of SLE unless specifically excluded. Further studies are needed to assess how patients with concomitant MCTD and SLE compare clinically and immunologically to the apparently less common condition of MCTD in the absence of SLE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographics

N= 88	**SLE (RNP−) N = 7**	**SLE (RNP+) N = 42**	**SLE (MCTD+) N = 18**	**MCTD N = 20**	**Other N = 1**
Age (years)	41 ± 7	36 ± 11	38 ± 11	39 ± 12	31
Disease Duration (months)	99 ± 97	65 ± 63	59 ± 67	50 ± 59	55
Female	7 (100%)	37 (88%)	18 (100%)	20 (100%)	1 (100%)
Hispanic	6 (86%)	23 (55%)	12 (67%)	12 (60%)	0
White	4 (57%))	9 (21%)	7 (39%)	5 (25%)	0
Black	1 (14%)	18 (43%)	6 (33%)	6 (30%)	1
Native	2 (28%)	6 (14%)	2 (11%)	4 (20%)	0
>1 Race	0	9 (21%)	3 (17%)	5 (25%)	0
Anti-dsDNA Antibodies Present	3 (43%)	28 (67%)	11 (61%)	7 (35%)*	0
Anti-dsDNA Antibody Levels (EU/ml)	92 ± 88	646 ± 1111	185 ± 258	56 ± 38†	--

Patients are designated as SLE, MCTD, or other based on the judgment of their treating rheumatologists. RNP− and RNP+ denote results of anti-RNP antibody assays. The SLE patients denoted as MCTD+ fulfill Alarcon-Segovia MCTD classification criteria. Numerical variables are presented as mean ± standard deviation; categorical variables are presented as number (percentage) satisfying listed criteria.

*Fisher's Exact p = 0.03 versus SLE patients not meeting MCTD criteria.

†Mann-Whitney p = 0.07 versus SLE patients not meeting MCTD criteria.

Table 2

Relevance of MCTD Criteria to SLE Subgroups: Renal Disease

N= 86	**SLE (RNP−) N = 7**	**SLE (RNP+) N = 42**	**SLE (MCTD+) N = 18**	**MCTD N = 19**
SLEDAI	7.0 ± 5.6	10.0 ± 6.0	10.4 ± 6.6	8.3 ± 4.0
# ACR SLE criteria	4.4 ± 1.1	4.8 ± 1.6	5.2 ± 1.6	4.3 ± 1.2
SLICC renal (17/49 (35%) vs 4/37 (11%), p = 0.012)*	3 (38%)	14 (33%)	3 (17%)	1 (5%)
Any Lung Disease	1 (14%)	14 (34%)	7 (39%)	8 (42%)
Pulmonary hypertension/possible pulmonary hypertension	1 (14%)	6 (18%)	6 (33%)	4 (21%)
Pulmonary Fibrosis	0	2 (6%)	4 (22%)	2 (11%)

Clinical subgroups and data presentation are as in Table 1, restricted to patients meeting SLICC SLE classification criteria. SLEDAI = mean SLEDAI-2k scores (see methods).

ACR SLE criteria = number of criteria from the 1997 revision of the ACR SLE Classification Criteria satisfied. SLICC renal = patients meeting any of the criteria from the SLICC SLE Classification Criteria for renal manifestations of SLE. Any Lung Disease, pulmonary hypertension/ possible pulmonary hypertension, and pulmonary fibrosis are as described in the Methods.

* The statistical test shown for SLICC renal compares all 49 patients without MCTD (by Alarcon-Segovia criteria) to all 37 patients with MCTD, using Fisher's Exact test.

Table 3

Relevance of MCTD Criteria (Excluding Raynaud's) to SLE Subgroups: Raynaud's Phenomenon

N= 86	**SLE (RNP−) N = 7**	**SLE (RNP+) N = 37**	**SLE (MCTD+) N = 23**	**MCTD N = 19**
SLEDAI	7.0 ± 5.6	9.0 ± 6.2	10.4 ± 6.6	8.3 ± 4.0
Raynaud's Phenomenon (26/44 (59%) vs 35/42 (83%), p = 0.018)*	6 (86%)	20 (54%) (vs 35/42 (83%), p = 0.007)**	17 (74%)	18 (90%) (vs 43/67 (64%), p = 0.009)***

Clinical subgroups, data presentation, and SLEDAI are as in Table 2. Raynaud's Phenomenon = patients with Raynaud's Phenomenon as described in the Methods. Statistical comparisons by Fisher's Exact test shown are

* between patients with and without MCTD as in Table 2,

** between RNP+ patients with and without MCTD by Alarcon-Segovia criteria, and

*** between patients with clinical diagnoses of SLE or MCTD.

Table 4

Immune Markers in Clinical Subgroups of SLICC SLE+ Patients

N= 61 plus normals	**SLE (RNP−) N = 5**	**SLE (RNP+) N = 32**	**SLE (MCTD+) N = 13**	**MCTD N = 11**	**Normal N = 8**
SLEDAI	9.0 ± 5.2	11.3 ± 5.9	11.5 ± 7.0	9.0 ± 3.8	--
BAFF (ng/ml)	1.5 ± 0.8 p = 0.019 vs normals	1.3 ± 0.7 p = 0.012 vs normals	2.6 ± 3.2 p = 0.012 vs normals	1.6 ± 0.5 p = 0.002 vs normals p = 0.023 vs SLE (RNP+)	0.8 ± 0.4
BAFFR (ng/ml)	Not Detected (<0.1)	0.16 ± 0.30	0.10 ± 0.03	0.24 ± 0.35	Not Detected (<0.1)
BCMA (ng/ml) All Non-MCTD: 2.0 ± 0.7 All MCTD: 2.4 ± 0.6 p = 0.0077	1.9 ± 0.6	2.0 ± 0.7 p = 0.0009 vs normals	2.4 ± 0.6 p < 0.0001 vs normals	2.4 ± 0.6 p = 0.0003 vs normals	1.4 ± 0.3
TACI (ng/ml) All Non-MCTD: 0.033 ± 0.029 All MCTD: 0.055 ± 0.050 p = 0.017	0.03 ± 0.01	0.03 ± 0.03 p = 0.014 vs normals	0.06 ± 0.04 p = 0.009 vs normals p = 0.008 vs SLE (RNP+)	0.04 ± 0.07	Not Detected (<0.02)
IFNg (pg/ml)	11 ± 16	58 ± 130 p = 0.014 vs normals	18 ± 40	25 ± 39	6 ± 8
TNFa (pg/ml) All Non-MCTD: 15.3 ± 16.6 All MCTD: 23.6 ± 19.5 p = 0.014	14.4 ± 10.4	15.4 ± 17.5 p = 0.009 vs normals	23.5 ± 23.7 p = 0.0013 vs normals	23.8 ± 14.2 p = 0.003 vs normals p = 0.022 vs SLE (RNP+)	5.0 ± 2.6
sCD40L (pg/ml)	1140 ± 560	3500 ± 5130 p = 0.0025 vs normals	3570 ± 3730	7370 ± 6880	1430 ± 530
IL-12p40 (pg/ml)	27.1 ± 17.1	67.7 ± 155.9	16.3 ± 5.8	42.9 ± 60.6	29.3 ± 32.4
IL-12p70 (pg/ml)	8.6 ± 9.7	26.1 ± 87.6	8.6 ± 14.6	18.8 ± 36.0	9.6 ± 9.1
IL-23 (pg/ml)	515 ± 626	3224 ± 16886	1327 ± 4351	5442 ± 10760	196 ± 152
IL-17A (pg/ml)	Not Detected (<1.4)	20.2 ± 42.4 p < 0.0001 vs SLE (RNP−)	10.3 ± 25.6	3.9 ± 5.9	2.0 ± 1.9
IL-17AF (pg/ml)	31.1 ± 26.0	33.0 ± 52.1	46.2 ± 77.8	47.6 ± 49.9	Not Detected (<19.5)
IL-17F (pg/ml)	165 ± 174	338 ± 974 p = 0.0002 vs normals	239 ± 299 p = 0.016 vs normals	689 ± 918 p = 0.016 vs normals	Not Detected (<39)
IL-21 (pg/ml)	18.3 ± 10.7	15.5 ± 11.2	Not Detected (<13.6)	17.1 ± 8.5	Not Detected (<13.6)
IL-22 (pg/ml)	Not Detected (<32)	33 ± 7	32 ± 3	35 ± 10	Not Detected (<32)
IL-33 (pg/ml)	59 ± 72	199 ± 1009	106 ± 334	438 ± 852	Not Detected (<12.4)
E-selectin (ng/ml)	13.4 ± 11.0	11.5 ± 5.0 p = 0.005 vs normals	10.3 ± 5.4	13.2 ± 6.6 p = 0.01 vs normals	6.0 ± 2.8

N= 61 plus normals	**SLE (RNP−)** **N = 5**	**SLE (RNP+)** **N = 32**	**SLE (MCTD+)** **N = 13**	**MCTD** **N = 11**	**Normal** **N = 8**
NGAL (ng/ml)	19.0 ± 23.6	18.1 ± 14.1 p = 0.003 vs normals	17.1 ± 12.4	16.6 ± 5.9 p = 0.003 vs normals	7.8 ± 4.7
mRNA expression analysis	N = 3	N = 21	N = 9	N = 10	N = 8
5-Gene IFN Signature Score	10.0 ± 4.3 p < 0.0001 vs normals	8.6 ± 3.1 p < 0.0001 vs normals	7.9 ± 4.9 p = 0.0009 vs normals	8.0 ± 3.4 p < 0.0001 vs normals	0.7 ± 0.2

Clinical subgroups, data presentation, and SLEDAI are as in Table 3, restricted to patients with available sera allowing immune marker testing. Immune markers tested are as described in the Methods. Normals = study subjects with no known inflammatory rheumatic disease. The Student's t test or the Mann-Whitney test are used as described in the Methods. Shading in the table identifies analytes for which differences between clinical SLE and MCTD subgroups were identified.

Table 5

End Organ Manifestations and Immune Markers

	Renal Dz+ (N = 18)	Renal Dz− (N = 43)	Lung Dz+ (N = 19)	Lung Dz− (N = 42)	RP+ (N = 43)	RP− (N = 18)
SLEDAI	11.6 ± 6.1	8.8 ± 5.4	9.4 ± 5.7	9.5 ± 5.8	8.8 ± 5.0	11.2 ± 6.8
BAFFR	Not Detected (<0.1)	0.18 ± 0.31 p = 0.016	0.09 ± 0.002	0.18 ± 0.31	0.14 ± 0.19	0.18 ± 0.39
e-selectin	12.0 ± 6.9	11.6 ± 5.6	14.6 ± 7.4	10.4 ± 4.7 p = 0.009	11.2 ± 6.1	12.8 ± 5.6
IL-17A	18.8 ± 45.5	11.4 ± 27.3	8.3 ± 18.9	16.0 ± 38.2	7.1 ± 18.2	29.2 ± 52.6 p = 0.015
BAFF	1.7 ± 2.4	1.6 ± 1.2	2.5 ± 2.6	1.3 ± 0.5	1.8 ± 1.9	1.3 ± 0.5
BCMA	1.9 ± 0.5	2.2 ± 0.8	2.3 ± 0.9	2.0 ± 0.6	2.2 ± 0.7	1.9 ± 0.6
TACI	0.04 ± 0.03	0.04 ± 0.04	0.05 ± 0.05	0.04 ± 0.03	0.04 ± 0.04	0.04 ± 0.04
TNFa	16.2 ± 13.3	19.5 ± 19.8	24.1 ± 21.7	16.0 ± 15.9	19.6 ± 16.9	16.1 ± 21.1

Clinical subgroups, data presentation, SLEDAI, and immune markers are as in Table 4. Renal Dz+ = renal disease as in Table 2; Renal Dz− = not fulfilling renal disease criteria as in Table 2. Lung Dz = lung disease as in Table 2. RP = Raynaud's Phenomenon as in Table 3. The Student's t test or Mann Whitney test are used as described in the Methods. Only immune markers from Table 4 with trends toward between-group differences, or with trends toward differences between patients segmented on the basis of renal, lung, or Raynaud's manifestations are shown. Shading identifies analytes and clinical subsets where differences were identified.