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A review and meta-analysis of anti-ribosomal P autoantibodies in systemic lupus erythematosus

May Y. Choi^a, Rachael D. FitzPatrick^b, Katherine Buhler^a, Michael Mahler^c, Marvin J. Fritzler^{a,*}

^a Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB T2N4N1, Canada

^b Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada

^c Inova Diagnostics, San Diego, CA, United States of America

ABSTRACT

The discovery of autoantibodies to ribosomal proteins (anti-RibP) dates back more than fifty years when antibodies to ribosomes were identified in systemic lupus erythematosus (SLE) sera. Over the years, anti-RibP autoantibodies have been the subject of extensive study and became known as a highly specific biomarker for the diagnosis of SLE and were associated with neuropsychiatric SLE (NPSLE), lupus nephritis (LN) and hepatitis (LH). As demonstrated by studies on cultured human cells and of murine models, there is evidence to suggest that anti-RibP may have a pathogenic role in LN and NPSLE. Despite a wealth of evidence, in comparison to other SLE autoantibodies such as anti-Sm and anti-dsDNA, anti-RibP has not been included in classification criteria for SLE. A significant challenge is the variability of assays used to detect anti-RibP, including the antigens and diagnostic platforms employed. This may account for the marked variation in frequencies (10–47%) in SLE and its association with clinical and demographic features reported in SLE cohorts. We performed a systematic literature review and meta-analysis to help clarify its prevalence, various clinical and serological associations in SLE based on the different RibP antigens and assay platforms used.

1. Background

1.1. History of anti-ribosomal P

The discovery and description of autoantibodies directed against ribosomal P proteins (RibP) can be traced to the reports elucidating the biochemistry of ribosomes in the 1960s [1,2] and then to the 1970s when the prokaryote analogs of human ribosomal proteins were published [3,4]. By 1979, the primary structure of RibP homologs in the brine shrimp (*Artemia salina*) were published [5] and three RibP, termed P0, P1, and P2 with molecular weights of 38, 19, and 17 kDa, respectively, were eventually identified [6–8]. These phosphoproteins are largely localized to the cytoplasm of eukaryotic cells and comprise a pentameric multimolecular complex containing two P1-P2 heterodimers bound to the C-terminus of a single P0 molecule [3]. The RibPs are key components of the 60S ribosomal subunit and are located in a macromolecular domain referred to as the ribosomal stalk in eukaryotes [3]. These proteins are phosphorylated by a variety of protein kinases and are thought to be involved in regulating the elongation step of protein synthesis, although their exact function remains uncertain [3].

In the seminal 1965 report of Sturgill and Carpenter [1], a flocculation technique employing bentonite particles coated with rabbit and rat liver ribosomes was used to detect anti-ribosome autoantibodies in SLE sera. In this report, with the exception of one patient with low antibody titres, all 8 SLE patients with anti-ribosomal antibodies had

cytoplasmic indirect immunofluorescence (IIF) staining of tissue substrates. The relationship between antibodies to ribosomes and the cytoplasmic IIF staining was confirmed when incubation of sera with ribosomes prior to IIF absorbed the cytoplasmic staining. In 1979, Miyachi and Tan used extracts of rabbit thymus acetone powder and a fractionated cytoplasm preparation to identify ribosomal autoantibodies by double immunodiffusion [9]. In 1985 a report from Elkon, et al. [7] became an inflection point, leading to future studies that further elucidated anti-RibP antibodies, becoming the topic of reviews [8,10,11] and two meta-analyses [12,13]. This review and meta-analysis focusses on three aspects of anti-RibP autoantibodies: 1) methods used to detect them, 2) the prevalence in SLE, and 3) the clinical and serological associations with anti-RibP.

1.2. Methods to detect anti-RibP antibodies

Historically, autoantibodies to ribosomes were detected by IIF on rodent liver or kidney cryopreserved substrates. Later, HEP-2 cells were reported to produce a cytoplasmic and occasional nucleolar staining pattern that corresponded to the cellular location of the ribosome and RibP autoantigens [1,9,14,15,33]. The IIF staining of HEP-2 cells is represented by the AC-19 ANA pattern espoused by the International Consensus on Autoantibody Patterns (ICAP: https://anapatterns.org/view_pattern.php?pattern=19). Nevertheless, even early studies [14] showed that IIF was unreliable as a screening test to detect anti-RibP

* Corresponding author.

E-mail address: fritzler@ucalgary.ca (M.J. Fritzler).

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autoantibodies. For example, in a cohort of 345 anti-RibP positive sera identified by addressable laser bead immunoassay (ALBIA), the sensitivity of IIF on HEp-2 cell substrates was < 30% [33,15]. Reliance on IIF screening to detect anti-RibP antibodies is also limited because some jurisdictions do not report cytoplasmic IIF staining patterns [16]. Given the clinical relevance of cytoplasmic antibodies such as anti-RibP and other cytoplasmic autoantigen targets, the ICAP Committee recommended that the terminology be changed from ANA to anti-cellular antibodies (ACA) [16]. Other reports of SLE patients with negative ANA by conventional IIF assays but positive anti-RibP antibodies by solid phase immunoassays (SPIA) have been published including one patient with severe manifestations of anti-phospholipid syndrome and lupus nephritis (LN) [17]. In patients with monospecific anti-RibP antibodies, confirmatory serology may not be done if the ANA IIF is negative resulting in a delay in diagnosis and treatment. This emphasizes the clinical importance of detecting anti-RibP antibodies in ANA negative SLE patients and also reporting the broader spectrum of ACA [18].

To add specificity to the IIF screen, double immunodiffusion (DID), immunoblot (IB) [14,19–21], radioimmunoassay [22] and counter-immunoelectrophoresis [14] were initially used and then largely replaced by more sensitive and higher throughput solid-phase assays such as enzyme-linked immunosorbent assays (ELISA) [19,23,24], line immunoassays (LIA) [24] and ALBIA [10,25], which have achieved increasing use in clinical and research laboratories (reviewed in [10,24]).

The clinically relevant epitope targeted by human anti-RibP autoantibodies has been the subject of considerable investigation over the past two decades (reviewed in [11,26,27]). The C-terminal 22 amino acid peptide (C22) was convincingly shown to be a key conserved epitope on all three RibP (P0, P1, P2) proteins targeted by human autoantibodies [28]. Hence, anti-RibP immunoassays have utilized the C22 peptide as well as the three RibP antigens (P0, P1, P2), either as individual proteins or in various combinations [10,29] (Table 1). These analytes have been derived from purified native ribosomes, synthetic or recombinant polypeptides, or a multiple-peptide construct [20,30]. However, because of intra- and inter-molecular epitope spreading, it is likely that linear and conformational epitopes located in a variety of RibP domains are also B cell targets [28]. When the technical and clinical accuracy of various anti-RibP assays including ELISA systems and ALBIA was compared [10], the assays were reported to perform well (93–100% technical sensitivity), however, there was poor correlation between assays, even between those using the same antigen, as

well as significant differences in clinical sensitivity for SLE (12–24%). As discussed in more detail elsewhere, this may have been related to technical characteristics of the different assays or the antigen selection.

It has been shown that epitopes recognized by anti-RibP proteins in SLE patients are not only localized to the C-terminal sequence, but some are also located within the N-terminal sequence of P1 or P2 [31]. IB assays and ELISAs that used the approach of testing antibodies to all three RibP antigens, rather than the individual C22 peptide, have resulted in higher sensitivity in several studies [10,32–34] although this may come at the expense of decreased sensitivity (10). In a cohort of 130 SLE patients, 39 (30%) patients were anti-RibP positive when an ELISA containing all three native RibP (P0, P1, P2) antigenic proteins was utilized, while 15 (38.5%) were also positive on an ELISA coated with the C22 peptide [32]. However, it needs to be considered that sensitivity and specificity are always linked and the observed differences might mostly be due to cut-off selection. Therefore, receiver operating characteristics (ROC) analyses might provide better insights into the performance of those assays. Additional studies are needed to compare anti-RibP assays using large patient cohorts such as the SLE International Collaborating Clinics on SLE (SLICC) [18].

1.2.1. The prevalence of anti-RibP antibodies in SLE

The reported prevalence of anti-RibP antibodies in SLE ranges from 10 to 47% (Table 2) and this variability is known to be dependent on the immunoassay employed, ethnicity or regional differences, cohorts studied (i.e. inception vs. cross-sectional), and the age of disease onset (reviewed in [10,32,39]). In a large international study of 947 SLE patients from 11 centres, the anti-RibP immunoassay, which used ELISA plates coated with the three recombinant proteins demonstrated that 21.3% of patients had a positive test [10]. This Rib-TriPlex assay (see Table 1), which purportedly included conformational epitopes, was shown to have a fivefold increased sensitivity compared to the C22 peptide using in ELISA in another study [35].

With regard to demographic factors impacting on anti-RibP autoantibodies, when an immunoassay using all three RibP peptides was used, Chinese cohorts had the highest prevalence (35%) while a cohort from a Canadian center had the lowest frequency (8%) of anti-RibP antibodies [36]. However, when two additional homogeneous Caucasian (Poland and Berlin, Germany) SLE patient cohorts and two additional homogeneous Asian (Japan and Shanghai, China) SLE patient cohorts were studied, no statistical difference in anti-RibP prevalence

Table 1
Anti-RibP assays, their target antigens, sensitivity and specificity in SLE.

Assay	Ribosomal Antigen	Sensitivity %	Specificity %
Indirect Immunofluorescence	Cytoplasmic	<10	40–60
Immunodiffusion	P0, P1, P2	<20	N/A
Immunoblot	P0, P1, P2	N/A	N/A
Radioimmunoassay	P0, P1, P2	N/A	N/A
Counter-immunoelectrophoresis	Purified ribosome proteins	N/A	N/A
ELISA			
Varelisa (Phadia)	C22, P0, P1, or P2	16–18	100
Varelisa RibP profile (Phadia)	C22 and recombinant RibP proteins: P0, P1, and P2 in separate wells	24	100
Ribosomal P (MBL)	Recombinant ribosomal P0	12–20	95–100
Euroimmun	Purified ribosomal P proteins from a native source	12	100
Inova Diagnostics	Synthetic peptide comprising the 22C-terminal amino acid	14–18	100
Dr. Foeke Laboratorien GmbH	Synthetic peptide comprising the 22C-terminal amino acid	23	99
Rib-TriPlex assay (Laboratory Developed Test)	P0, P1, P2 recombinant proteins in molar ratio of the native heterocomplex	21	99
LIA			
RecomLine ENA/ANA IgG (Mikrogen GmbH)	Recombinant P0 antigen	N/A	N/A
INNO-LIA™ ANA Update	Synthetic peptide	12	100
ALBIA			
QUANTA Plex Rib0-P (Inova Diagnostics)	Purified C22 amino acid	10–18	100
Fluoroenzyme immunoassay			
EliA RibP	Recombinant P0, P1, P2	14	100

References: [10, 29, 33–38].

Abbreviations: ALBIA, addressable laser bead immunoassay; ELISA, enzyme linked immunoassay; LIA, line immunoassay; N/A, not available; P0, P1, P, ribosome phosphoproteins; ribP, ribosomal P proteins.

Table 2
Prevalence of anti-RibP antibodies in SLE.^a

Study Author year	Assay	Antigenic Target	SLE Frequency (%)	Association with anti-RibP
Sturgill and Carpenter, 1965 [1]	Bentonite-rat or rabbit liver ribosome flocculation IIF assay	Whole rabbit or rat liver ribosome proteins	8/33 (24%)	● Correlated with cytoplasmic IIF and high titers of anti-DNA
Bonfa and Elkon, 1986 [14]	WB, CIE, IIF	Purified rabbit thymus and canine spleen ribosomes	7/59 (12%) by WB	● All patients with psychosis had anti-RibP autoantibodies
Bonfa et al., 1987 [48]	IB, CIE, RIA	Canine-spleen and rat-liver for IB. Canine spleen and rabbit thymus for CIE. Synthetic peptide of C22 of the <i>Artemia salina</i> ribosomal P2 (17 kDa)	18/20 (90%) with SLE psychosis	● Associated with SLE psychosis and titers increased during active psychosis
Elkon et al., 1985 [7]	IB, CIE, RIA	38, 19, 17 kDa proteins isolated from canine, rat, and chicken livers	9 out of > 50 (5–10%)	● Not examined
Elkon et al., 1989 [49]	ELISA	Highly purified recombinant P2-beta-galactosidase fusion protein	24/120 (20%)	● Associated with anti-Sm antibodies.
Gordon et al., 1990 [50]	ELISA	Recombinant fusion protein of 38 kDa P0 protein	17/86 (20%)	● Not examined
Sato et al., 1991 [41]	IB, DB	Purified rat liver 80S ribosomes	37/89 (42%)	● A higher frequency of anti-RibP was seen during active SLE compared to non-active.
Van Dam et al., 1991 [51]	ELISA, IB	Synthetic peptide of C22 peptide and rat liver P0, P1, P2	10%	● Associated with skin symptoms and anti-DNA levels
Teh et al., 1993 [40]	ELISA	Synthetic peptide P0, P1, P2	61/303 (20%)	● More frequent in Malaysian Chinese patients (38%) compared to Caucasians (13%) and Afro-Caribbean (20%)
Arnett et al., 1996 [42]	ELISA	Recombinant human P2	66/384 (17%)	● More frequent in Chinese American patients (36%) compared to Caucasians, African Americans, Hispanic Americans, Greeks and Bulgarians. This was influenced by certain MHC class II alleles.
Press et al., 1996 [52]	ELISA	Recombinant fusion protein	16/79 (20%) juvenile-onset SLE	● Associated with lupus psychosis and/or depression
Hoffman et al., 2004 [46]	LIA	Synthetic peptide	14/56 (25%) juvenile-onset SLE, 22/194 (11.3%) adult-onset SLE	● Associated with active SLE psychosis and disease activity
Mahler et al., 2006 [36]	ELISA	Three recombinant RibP proteins combined at the molar ratio of the native heterocomplex, P0(P1/P2)	201/947 (21%)	● More frequent in pediatric-onset compared to adult-onset with inverse association with renal involvement.
Mahler et al., 2012 [37]	ELISA	Synthetic peptide of C22	77/333 (23%)	● Frequency and clinical associations were centre dependent
Pisoni et al., 2015 [47]	ELISA	Purified protein from bovine and/or rabbit thymus	8/30 (27%) juvenile-onset SLE, 6/92 (7%) adult-onset SLE	● Associated with malar rash, renal disorder, and inversely associated with serositis
Pradhan et al., 2015 [53]	ELISA	Ribosomes	17/60 (28%) NPSLE, 12/60 (20%) non-NPSLE	● Frequency (18.2–29.0%) was centre dependent
Massardo et al., 2015 [54]	ELISA and IB	Recombinant P0 protein by IB	15/133 (11%)	● Associated with younger age and decreased C3 and C4 levels
Arinuma et al., 2018 [55]	ELISA	Synthetic peptide of C22	23/55 (42%) of NPSLE	● Higher frequency observed in juvenile-onset compared to adult-onset SLE
Mei et al., 2018 [56]	LIA	P0 individual	168/470 (36%)	● Associated with alopecia and non-discoid skin rash
Kang et al., 2019 [57]	ELISA	Recombinant human P2 polypeptide	28/79 (35%) of LN	● Associated with mood disorders

Abbreviations: C3/C4, complement proteins 3 and 4; C22, carboxy terminal 22 amino acid peptide; CIE, counter immunoelectrophoresis; DB, dot blot; ELISA, enzyme-linked immunosorbent assay; IB, immunoblot; IIF, indirect immunofluorescence; IP, immunoprecipitation; LIA, line immunoassay; LN, lupus nephritis; NPSLE, neuropsychiatric systemic lupus erythematosus; RibP, ribosomal P; SLE, systemic lupus erythematosus; SLEDAI-2 K, Systemic Lupus Erythematosus Disease Activity Index-2000; WB, western blot

^a Studies selected based on historical and novel findings. For a complete list of studies refer to Supplemental Document 1.

between these racial groups (for the Asian group versus the Caucasian (Odds Ratio (OR) 1.3 [95% Confidence Interval (CI): 0.7-2.4]) was observed [36]. In a more recent multicentre study of 333 SLE patients from Canada, Germany and Israel that utilized an ELISA employing the C22 synthetic peptide, the prevalence was highest among the Canadian cohort (29%) [37]. The discrepancy of 8% vs. 29% in Canadian patients from these two studies suggests that differences in method of detection in combination with differences in regional practice and ethnicity has a significant impact of results.

Other studies that did not use the C22 peptide continued to demonstrate SLE patients of Asian descent have the highest prevalence of anti-RibP antibodies. In an international study using an ELISA with synthetic P0, P1 and P2 peptides, a higher prevalence of anti-RibP in SLE was reported in Malaysian Chinese patients (38%) and less frequently in Caucasians (13%) and Afro-Caribbean (20%) SLE patients [40]. In another cohort of 89 Japanese SLE patients with active disease, the prevalence was even higher at 42% using ribosomes purified from rat liver in immunoblot and dot blot assays [41]. Using an ELISA assay employing a recombinant human P2 antigen, one multi-ethnic cohort study of 394 SLE including Caucasians, African Americans, Hispanic Americans, Chinese Americans, Greeks and Bulgarians, the frequency of anti-RibP was highest in the Chinese group (36%) and lowest in Bulgarian group (6%) [42]. This study also examined the relationship between anti-RibP and MHC class II alleles in different ethnicities and found that the frequency of anti-RibP in certain ethnic groups may be influenced by certain MHC class II alleles, particularly HLA-DQB1*0602 and DQB1*0302 [43-45].

Anti-RibP antibodies have been reported to be more common among pediatric-onset SLE than adult-onset SLE [39,46,47], however, the differences may also be attributable to the use of different assays and RibP antigens. In a multi-centre comparison study between 30 pediatric and 92 adult SLE patients, the pediatric patients were found to have a higher prevalence of anti-RibP antibodies compared to adults (26.7% vs 6.5%; OR 5.21 [95% CI: 1.6-16.5]) using an ELISA coated with purified bovine and/or rabbit thymus ribosomal proteins [47]. In a study of a larger cohort of 108 pediatric and 260 adult SLE using an ELISA and Western blot, the frequency of anti-RibP in pediatric-onset SLE was 42% compared to 7.7% in adult-onset disease [39]. In an adult multi-centered study, anti-RibP antibodies were more common in younger patients (mean age 33.9 vs. 45.3 years) compared to adults ($P < .0001$) [37].

1.2.2. Association of anti-RibP with other autoantibodies

Anti-RibP and anti-dsDNA autoantibodies are often found in the same sera. As early as 1965, Sturgill and Carpenter also reported that anti-RibP antibodies were correlated with high titres of anti-DNA antibodies [1]. Anti-dsDNA are well characterized as SLE biomarkers and depending on the assay and the cohort of SLE studied are present in up to 98% of patients [58,59]. Review of the literature suggests that up to 70% of anti-RibP positive SLE sera are also positive for anti-dsDNA [38]. This close association of two antigenic targets that have no apparent physiological association (i.e. not part of a macromolecular complex) was suggested to be related to a key epitope in the C-terminal hydrophobic cluster of RibP proteins that cross-reacted with anti-dsDNA autoantibodies [60]. This apparent dsDNA mimetic region is rich in aromatic residues which may mimic the cyclic bases or the pentose sugar backbone of DNA [60]. Interestingly, this is not the first report of polypeptide mimetics of dsDNA [61]. For example, it has been reported that anti-NMDA/NR2 autoantibodies cross-react with dsDNA [62], an autoantibody reported in 13.7% of NPSLE [63]. The key NMDA/NR2 DNA mimetic, DWDYS/DWEYS, is expressed on the extracellular domain of the NMDA/NR2A subunit, providing an intriguing explanation for the pathogenic role of anti-NMDA/NR2 autoantibodies in NPSLE [64]. Perhaps this also has some bearing on reports that cytotoxic anti-dsDNA antibodies target the RibP proteins expressed on the surface of a variety of cell types [61,65]. In a study of 8 SLE patients,

one had anti-dsDNA antibodies that cross-reacted with the C-terminal peptide of RibP proteins [66]. Of note, mice that were immunized with either the C22 peptide or bovine RibP produced both anti-RibP and anti-dsDNA autoantibodies [66]. However, the independence of anti-RibP and anti-dsDNA autoantibodies demonstrated by their lack of cross-reactivity was observed by inhibition studies showing that absorbing the anti-ribosomal reactivity did not alter anti-DNA reactivity and conversely incubation of sera with purified DNA did not alter reactivity with RibP [67]. In the future, it would be of interest to observe the frequency of sera positive for anti-dsDNA, anti-NMDA/NR2, and anti-RibP autoantibodies.

1.2.3. Meta-analysis of clinical associations of anti-RibP in SLE

To elucidate the claims of the prevalence, clinical and serological correlations of anti-RibP autoantibodies we performed a meta-analysis.

1.3. Inclusion criteria

Included published studies that met the following criteria: (a) study evaluating the association of anti-RibP antibody with clinical manifestations NPSLE, LN, LH and disease activity of unselected adult SLE patients, (b) observational study with anti-RibP antibody status available, (c) study with sufficient data available to calculate the OR with 95% CI. Studies must also indicate the techniques and the antigen sources used for anti-RibP detection. Studies were excluded in any of the following cases: (a) not human study; (b) not original study; (c) study not pertaining to the clinical features of SLE; (d) children with SLE.

1.4. Literature search

Studies were identified in a literature search using PubMed, EMBASE, and Web of Science databases, restricted to the English language. The search was from inception to April 25, 2019. The search strategies contained subject headings and keywords for 'lupus', 'SLE' or 'systemic lupus erythematosus', combined with 'autoantibodies to ribosomal P', 'anti-ribosomal P', 'anti-ribosomal P antibodies', or 'anti-ribosomal P autoantibodies'. The references in the identified or related articles were then manually reviewed to search other relevant citations.

1.5. Study selection

Two review authors (MC, KB) independently screened articles for inclusion by title or abstract and full text if necessary. Disagreements were resolved by consensus or discussion with a third reviewer (MF).

1.6. Data extraction

Two review authors (MC, KB) extracted relevant data from included studies into an Excel spreadsheet. Clinical associations of anti-RibP that were reported and type of assays and antigens were extracted by one author (MC) and confirmed by a second (KB); with disagreements resolved through discussion.

1.7. Risk of bias

The methodological quality of included trials was independently assessed by one review author (KB) using the components from the tools created by the CLARITY group at McMaster University (www.evidencepartners.com/) to assess risk of bias in Cohort studies and case-control studies and cross-sectional surveys [68-70]. Studies were rated as having a "definitely low risk," "probably low risk", "unclear risk", "probably high risk" or "definitely high risk" of bias across the applicable domains. One additional domain included assessment of whether blinding of clinical characteristics was used when testing for anti-RibP.

1.8. Statistical analysis

All studies with data on the clinical features examined were included in the forest plots. For categorical variables the pooled OR was calculated and a forest plot was generated. Several smaller studies were missing data for one or more of the variables needed to calculate the OR. For studies with data missing from only one variable, 0.5 was added to all four variables and the modified values were used to estimate the OR for inclusion in the forest plot and pooled estimate; studies missing values for more than one variable were excluded from the analysis. For the disease activity index, the pooled weighted mean difference (WMD) was calculated and shown using a forest plot comparing patients with and without anti-RibP. Three of the studies reporting disease activity reported the median rather than the mean. For two of these studies (Massardo et al. 2002 [71] and 2015 [54]), the mean was estimated using the median and the range following the method suggested by Hozo et al. [72]; the third paper (Li et al. [73]) did not report the range and the mean could not be estimated. A fourth study [37] reported the mean, but the standard deviation (SD) was calculated using the range and the above method.

All analyses were stratified by antigenic target as the primary question. The results were also stratified by the testing method (DID, IB, etc.) to further explore how this impacted the relationship of anti-RibP with the various clinical variables. As several disease activity indexes were used across the studies, disease activity was stratified by index. A random effects model was used in all analyses to reflect the variety of effects likely between patients. Heterogeneity was assessed using the I^2 value and Q statistic. The risk of publication bias was assessed using a funnel plot. All analyses were performed with Stata 14.1 (StataCorp LLC, College Station, TX, USA) and statistical significance was assessed at $\alpha = 0.05$ level. Forest plots for publication were generated using Matplotlib 2.2.2 [109], Pandas 0.25.0 [110] and NumPy 1.14.2 [111]

2. Results

2.1. Search results and description of included studies

From 669 unique records, we identified 62 observational studies including >6752 patients that met our inclusion criteria (Fig. 1). A list of studies meeting the inclusion criteria are listed in Supplemental Document 1.

2.2. Overall meta-analysis

The pooled OR of each SLE manifestation is shown in Table 3. Most of the studies examined NPSLE (combined) ($n = 60$) and LN ($n = 24$). All of the SLE features including NPSLE (combined), LN, and hepatitis were significantly associated with anti-ribosomal P, the strongest association being with hepatitis (pooled OR 8.44 (95% CI: 3.16, 22.53)). For disease activity, there were 11 studies, but four different disease severity indexes were used so we did not provide an overall pooled OR. There was a wide variation of heterogeneity between SLE features. LN had the highest heterogeneity ($I^2 = 63.4\%$) followed by NPSLE (combined) ($I^2 = 49.6\%$). As such, we re-examined each SLE feature stratified by antigenic target and assay. NPSLE was also stratified by CNS, depression and psychosis. Different disease activity indexes were also examined separately. The number of antigenic targets (5–10) and assays (3–7) used varied among different SLE features. The most common antigenic target and assays used were C22 peptide and ELISA, respectively (Figs. 2 and 3). For more detailed forest plots with study level data, see Supplemental Figs. 1–3.

2.3. Neuropsychiatric SLE – CNS, depression, psychosis

The pooled OR of anti-RibP positive NPSLE (combined) was 1.95 (95% CI: 1.52–2.50) with considerable heterogeneity ($p \leq .001$,

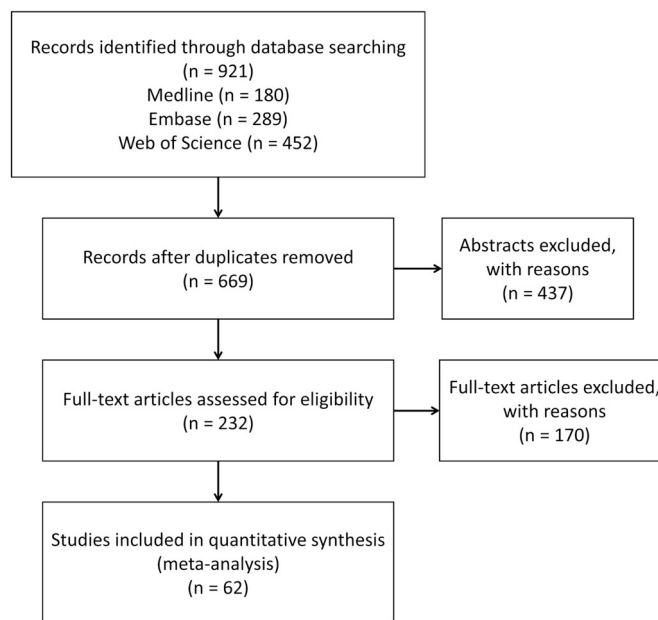


Fig. 1. Flow Chart of Literature Search.

$I^2 = 49.6\%$). When NPSLE (combined) was stratified by antigenic target, P0, P1, P2 (pooled OR 2.11 (95%CI: 1.27–3.51), $I^2 = 0.0\%$) and ribosomes (pooled OR 2.09 (95%CI: 1.35–3.26), $I^2 = 32.1\%$) remained significantly associated with anti-RibP with reduced heterogeneity. The C22 peptide-based assays had a pooled OR of 2.16 (95%CI: 1.34–3.47), however, there was significant heterogeneity ($I^2 = 58.1\%$) among different studies that used this peptide. The remaining studies either did not report the antigen ($n = 6$) or an antigen was reported but there weren't enough studies that used it to draw any meaningful conclusions. When stratified by assay, there was still significant heterogeneity. ELISA was the most common assay that was used and it had the lowest heterogeneity only when it was combined with IB ($I^2 = 37.3\%$) with a pooled OR of 1.63 (95%CI:1.00–2.66).

When NPSLE was stratified by specific NPSLE features, CNS involvement, depression, and psychosis remained associated with anti-RibP. In particular, the association with anti-RibP became stronger when stratified by psychosis (pooled OR 3.08 (95%CI: 1.94–4.87)) and depression (pooled OR 3.03 (95%CI: 1.32–6.95)). Heterogeneity was also reduced when stratified by psychosis and CNS involvement, but there was greater heterogeneity with depression.

When CNS disease was stratified by antigenic target, complete ribosomes remained significant (pooled OR 2.17 (95%CI: 1.18–3.99)) with heterogeneity of 39.9%. In contrast, the C22 peptide was not significantly associated with CNS disease and there was only one study that used P0, P1, and P2. When it was stratified by assay, ELISA alone remained associated with anti-RibP (pooled OR 1.97 (95%CI: 1.14–3.38)) with heterogeneity of 43.7%.

For depression, the C22 peptide was associated with anti-RibP (pooled OR 3.96 (95%CI: 1.17–13.47)), however, there was significant heterogeneity ($I^2 = 68.6\%$). Of note, P0, P1, P2 based immunoassays have not been used in serological studies of SLE-related depression. The ELISA method of detecting anti-RibP was significantly associated with anti-RibP (pooled OR 6.23 (95%CI: 2.08–18.62)), but it also had significant heterogeneity ($I^2 = 57.0\%$).

For psychosis, the C22 peptide was associated with anti-RibP (pooled OR 3.99 (95%CI: 2.42–6.58)) with no heterogeneity. Ribosome based assays were not significant and P0, P1, P2 based immunoassays were not examined. ELISA was associated with anti-RibP positivity (pooled OR 3.28 (95%CI: 1.67–6.43)) with low heterogeneity ($I^2 = 17.2\%$).

Table 3
Pooled Odds Ratio for anti-Ribosomal P Antibodies: Study heterogeneity, and number of antibody target or assays examined stratified by SLE manifestation.

SLE manifestation	Studies (N)	Pooled OR (95%CI)	I ² (%)	Antibodies or Combination of Targets (N)	Assays or Combination of Assays (N)
NPSLE (Combined)	60	1.95 (1.52, 2.50)	49.6**	10	7
CNS	18	1.64 (1.23, 2.18)	16.6	8	7
Depression	12	3.03 (1.32, 6.95)	64.0*	6	3
Psychosis	14	3.08 (1.94, 4.87)	23.7	5	5
Lupus Nephritis	24	1.55 (1.13, 2.14)	63.4**	7	8
Hepatitis	8	8.44 (3.16, 22.53)	28.8	5	5
Disease Activity	11	-	-	7	4
SLEDAI	6	2.47 (1.13, 3.81)	0.0	-	-
SLEDAI-2 K	2	4.76 (-0.19,9.72)	92.9	-	-
MEX-SLEDAI	1	2.75 (0.91, 4.59)	-	-	-
ECLAM	2	1.14 (0.52, 1.75)	42.0	-	-

For heterogeneity, ** means $p < .001$, * means $p < .01$.

Abbreviations: CI, confidence interval; CNS, central nervous system; ECLAM, European Consensus Lupus Activity Measurement; I², heterogeneity; N, number; OR, odds ratio; NPSLE, neuropsychiatric systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLEDAI-2 K, Systemic Lupus Erythematosus Disease Activity Index-2000; MEX-SLEDAI, Mexico- Systemic Lupus Erythematosus Disease Activity Index.

2.4. Lupus nephritis

LN had the highest overall heterogeneity (I² = 63.4%). Stratification by antigenic target revealed that only P0, P1, P2 based immunoassays remained significantly associated with anti-RibP with low heterogeneity (I² = 13.7%). The C22 peptide assays demonstrated a trend towards an association with anti-RibP and it had no heterogeneity. There were 4 studies that did not report antigenic targets with significant heterogeneity (I² = 87.3%). For detection methods, ELISA alone (pooled OR 2.04 (95%CI: 1.23–3.40)) and ELISA with DID and IB (pooled OR 5.19 (95%CI: 1.10–24.43)) were strongly associated with anti-RibP. ELISA alone had significant heterogeneity (I² = 64.9%), as well as when combined with DID and IB (I² = 71.4%).

2.5. Lupus hepatitis

Out of all the SLE features, LH had the strongest association (pooled OR 8.44 (95% CI: 3.16-22.53)) with anti-RibP and lowest heterogeneity (I² = 28.2%). There were very few studies to stratify by antigenic targets and assays. Nevertheless, ribosomes had the strongest association (OR 12.70 (95%CI: 2.13-75.60)) with no heterogeneity. Similar to LN, ELISA alone was significantly associated with anti-RibP, but only when combined with DID and IB did it also have low heterogeneity (I² = 6.1%).

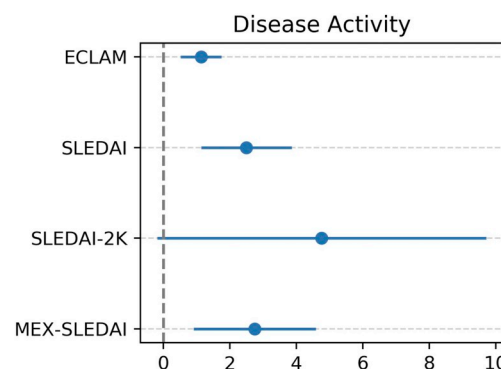


Fig. 3. Forest plot for disease activity stratified by disease activity indices. Abbreviations: ECLAM, European Consensus Lupus Activity Measurement; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLEDAI-2 K, Systemic Lupus Erythematosus Disease Activity Index-2000; MEX-SLEDAI, Mexico- Systemic Lupus Erythematosus Disease Activity Index.

2.6. Disease activity

Disease activity was stratified by disease activity index. The most commonly used index was the Systemic Lupus Erythematosus Disease

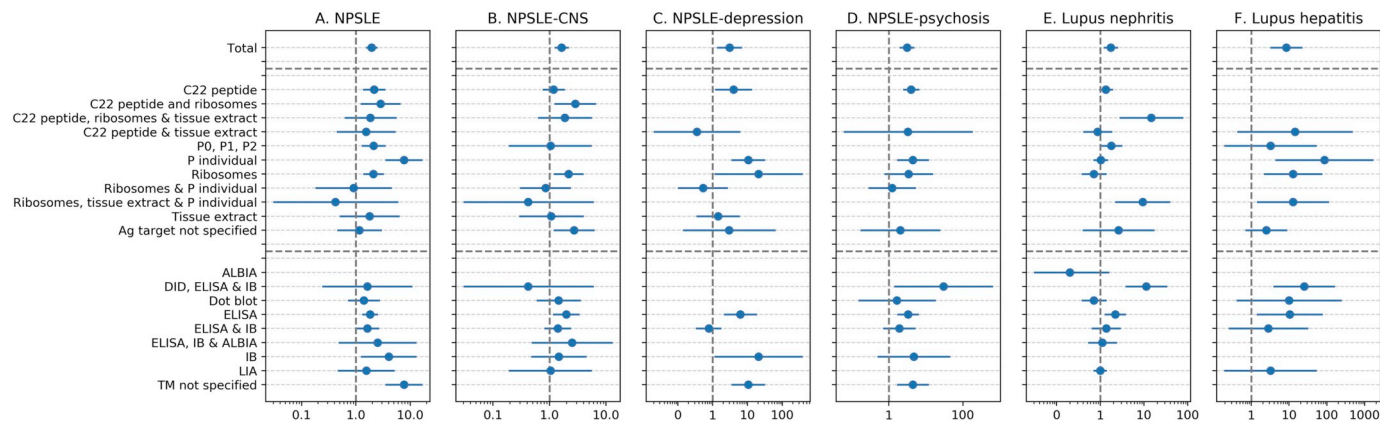


Fig. 2. Forest plots for combined NPSLE (A), CNS (B), depression (C), psychosis (D), lupus nephritis (E), and hepatitis (F), stratified by antigenic target and testing method. Abbreviations: Ag, antigen; ALBIA, addressable laser bead immunoassay; C22, carboxy terminal 22 amino acid peptide; CNS, central nervous system; DID, double immunodiffusion; ELISA, enzyme-linked immunoassay; IB, immunoblot; LIA, line immunoassay; LN, lupus nephritis; NPSLE, neuropsychiatric systemic lupus erythematosus; TM, testing method.

Activity Index (SLEDAI). For studies that used SLEDAI, the pooled weighted mean difference in score was +2.47 (95%CI: 1.13-3.81) for patients who were anti-RibP positive compared to anti-RibP negative patients. There was no significant heterogeneity between these studies. There were too few studies in European Consensus Lupus Activity Measurement (ECLAM), SLEDAI-2000 (SLEDAI-2 K) and Mexico-SLEDAI (MEX-SLEDAI) to confidently assess pooled results.

2.7. Risk of bias

Overall, there was low risk of bias in most studies (Supplemental Figure 4). The exceptions fell into three categories: controls were not appropriately matched to cases; the source population was not representative of unselected SLE patients; and an overall lack of description of the methods, leaving uncertainty as to the risk of bias. Of the case-control studies, 3/10 [51,53,74] did not match the case and controls on basic demographic data such as age and sex. Similarly, of the cohort studies, 4/10 [42,57,75,76] did not match patients based on age and sex. An additional seven studies [41,48,77–81] did not adequately describe the matching process used and we were unable to determine if there is a risk of bias. Seven studies [45,55,57,78,82–84] were deemed not representative of an unselected cohort of SLE patients; the patients for these studies were mostly selected from hospital or inpatient settings, but three studies looked exclusively at SLE LN patients [57,82,83]. In nineteen other studies, it could not be determined whether the sample would be representative of an unselected SLE cohort. It was more common for the description of the methods to be limited (leaving uncertainty about their risk of bias) than for them to describe outright bias. There were nine studies whose methods were so limited that it was not possible to assess their risk of bias across multiple components [41,48,77,79,80,85–88]. We also found that LN studies had publication bias (data not shown).

3. Discussion

The role of anti-RibP as a biomarker for SLE has been unclear since its discovery in the 1960's in part due to the conflicting evidence for its association with a variety of SLE features. We performed the largest systemic review and meta-analysis on anti-RibP in SLE to date including over 60 studies on NPSLE, LN, LH, and disease activity. Anti-RibP was associated with NPSLE, LN and LH, and higher SLEDAI scores. Under different representations of NPSLE, anti-RibP was also significantly associated with CNS lupus, depression, and psychosis. There was large variation in the type of antigenic targets and assays used in these studies, which contributed to the heterogeneity between studies particularly for NPSLE (combined) and LN. In general, studies that used an ELISA with all three of the P0, P1 and P2 ribosomal proteins combined with another test such as DID and/or IB, demonstrated a positive association with anti-RibP with low heterogeneity.

There have been two smaller meta-analyses, published in 2006 and 2015, on anti-RibP and SLE [12,13]. Shi et al. [13] included 16 cohort studies and also found that anti-RibP was associated with NPSLE (OR 2.72 (95%CI: 1.68-4.42)), hepatitis (OR 5.35 (95% CI: 2.56-11.20)), but not renal disease (OR 1.06 (95% CI: 0.75-1.49)). The authors attributed high heterogeneity ($I^2 = 48\%$, $p = .03$) among renal studies to publication bias, which we also found. Shi et al. [13] also found that anti-RibP was significantly associated with malar rash, oral ulcers, photosensitivity and anti-dsDNA antibody, with OR values of 2.05 (95%CI: 1.42–2.92), 1.49 (95%CI:1.05–2.13) and 1.44 (95%CI:1.08–1.91), respectively. These features were not examined in our meta-analysis.

To properly examine the relationship between anti-RibP and anti-dsDNA in our meta-analysis, we needed individual level data which was not available. About a third of our studies reported the prevalence of anti-dsDNA and the majority of these studies were on LN so there may have been reporting bias. In general, most suggested there was a weak or insignificant association between these two antibodies. This is

contrary to other studies that have suggested that the relationship between anti-RibP and renal disease is confounded by the remarkably high association between anti-RibP and anti-dsDNA [8,13]. Titers of anti-RibP antibodies also fluctuated with flares and remission in LN, but this was also correlated with the levels of anti-dsDNA antibodies [86]. Therefore, a combination of anti-dsDNA and anti-RibP, not anti-RibP alone, appear to be most strongly associated with renal involvement in SLE [81,89,90]. A future meta-analysis or study of a large SLE cohort examining the relationship of anti-dsDNA with anti-RibP is needed in NPSLE, LN and hepatitis. As anti-dsDNA is reputed as a biomarker of disease activity (reviewed in [59]), it is interesting that anti-RibP was also associated with higher disease activity, particularly when using SLEDAI. This is also consistent with our findings that anti-RibP was associated with particularly severe manifestations of SLE i.e. NPSLE and LN.

It remains unclear whether anti-RibP is an important biomarker for NPSLE. IIF staining of RibP has also been reported in the hippocampus, cingulate cortex, and primary olfactory piriform brain regions, findings that are compatible with the cell membrane localized antigen [91]. In murine models used to study the immunopathogenic potential of anti-RibP autoantibodies, intra-cerebroventricular injection of anti-RibP antibodies gave rise to olfactory impairment and depression-like behavior [91–93], observations supporting their association with NPSLE in humans. A meta-analysis involving 1537 SLE patients contributed by 14 research teams demonstrated that anti-RibP antibodies had low sensitivity (26%) and specificity (80%) for NPSLE, was not helpful in differentiating between different presentations of NPSLE and has limited diagnostic value for NPSLE [12]. In our meta-analysis, we demonstrate the choice of antigenic targets and assays make a difference. P0, P1, and P2 proteins have increased sensitivity but at the expense of lower specificity for SLE compared to the C22 peptide [10,32,35,36].

The diagnosis of NPSLE is challenging in part because it encompasses a wide range of clinical manifestations. The American College of Rheumatology proposed a standard nomenclature for 19 neuro-psychiatric syndromes associated with SLE [94]. By focussing on specific NPSLE phenotypes, we demonstrated that the association with anti-RibP differs between phenotypes. In addition, in CNS and psychosis, the C22 peptide demonstrated strong association and no heterogeneity. On the other hand, depression, which can be non-specific and difficult to distinguish between primary depression and depressive symptoms related to NPSLE, heterogeneity remained high when C22 peptide based assays were used.

Other reasons why the controversial relationship exists between NPSLE and anti-RibP have been proposed. In a follow-up study of the SLE International Cooperating Clinics (SLICC) inception cohort, Hanly et al. clarified that the association of anti-RibP was linked primarily to clinically distinct NP events attributed to SLE that occurred around the time of diagnosis [95]. This may explain why cross-sectional studies fail to find an association between anti-RibP and NPSLE.

In a rare disorder such as NPSLE with severe morbidity and mortality [96–99] it may be advantageous to have a more sensitive antigen to allow for earlier detection and treatment. If initial testing with C22 peptide is performed and is negative on a patient highly suspicious for NPSLE, the clinician might consider repeating the test using P0, P1, and P2 or ribosomes. If it was performed on ELISA, we showed that there is usually high heterogeneity unless combined with another assay. It is also important to note that NPSLE contains a diverse range of manifestations, some of which can be non-specific (e.g. depression) and difficult to distinguish from other conditions that can mimic NPSLE or cause neuropsychiatric disturbances in SLE condition [100,101]. Almost half of the NPSLE studies that reported their antigenic target, used C22 peptide which we found performed better in NPSLE-associated psychosis. Given the variability of antigenic performances with different NPSLE phenotypes, it is not unexpected that when all of the phenotypes are combined, the results are mixed.

The strongest association with anti-RibP was observed with lupoid

hepatitis (LH). Anti-RibP may have a direct pathogenic role as these antibodies have been reported to penetrate live human hepatoma tissue culture cells resulting in morphological changes [102] and in vivo and in vitro anti-RibP effectively inhibited protein synthesis [102,103], albeit with a variable time-course effect. This variation was ascribed to the different epitopes targeted by the respective antibodies as evidenced by studies showing that IIF of HEP-G2 cells (human liver cell line) showed a weaker cytoplasmic staining pattern when autoantibodies specifically directed against the C-terminal 22 amino acid region of RibP were used compared to those directed against all three RibP proteins [102]. A cell surface membrane protein on human neuroblastoma cells, hepatoma cells, and to a lesser extent human fibroblasts, that may be closely related to P0 has been described and could offer a potential Fc-independent mechanism for anti-RibP internalization into target cells [65,92,102,104]. Liver dysfunction has been reported in up to 50% of SLE patients [105] and there are many causes including drugs, viruses and autoimmune hepatitis. It is estimated that SLE itself is associated with hepatitis in 28–42% of patients with no other obvious causes of liver dysfunction [106]. The presence of anti-RibP has also been associated with more severe forms of hepatitis [107]. Distinguishing LH and autoimmune hepatitis is also important as it has implications of prognosis, complications, and therapy.

In summary, biomarkers are essential in the diagnosis and prognosis of multi-system diseases such as SLE, where disease manifestations are extremely complex and heterogeneous. Several classification criteria for SLE have been developed including the newest European League Against Rheumatism/American College of Rheumatology classification criteria that requires a positive ANA as an entry criterion and also gives points for the presence of anti-dsDNA, anti-Sm and anti-phospholipid antibodies [108]. Anti-RibP has never been included in any SLE classification criteria and we argue that this decision should be re-evaluated. We have demonstrated in the largest systematic review and meta-analysis of anti-RibP that it is associated with various severe manifestations of SLE and is particularly strong for hepatitis and NPSLE, both of which can be challenging to diagnose. We've clarified that the previous controversies around NPSLE may be related to use of different antigenic targets and assays. We saw more consistent results with the use of ribosomes and P0, P1, and P2 as antigenic targets and with ELISA combined with one or more assays.

4. Future studies

- International standardization of laboratory tests of anti-RibP antibodies is needed.
- Longitudinal studies, including a SLE inception cohort, very early SLE and post-diagnosis SLE, using validated anti-RibP assays are needed
- It was noted that there are a paucity of studies of anti-RibP in pediatric SLE.

5. Limitations

We apologize to authors whose articles were not retrievable from on-line digital libraries and archives.

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Disclosures

MJF is a consultant to Inova Diagnostics, (San Diego, CA, USA) and Werfen International (Barcelona, Spain).

MM is an employee of Inova Diagnostics, a company that manufactures and sells.

autoantibody assays.

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