ความสัมพันธ์ระหว่างแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้ และการวัดการกำเริบของโรคในผู้ป่วยโรคลูปุส

นางสาว เรวดี เดชเทวพร

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THE ASSOCIATION OF ANTI-DOUBLE-STRANDED DNA ANTIBODIES AND DISEASE ACTIVITY MEASUREMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

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เรวดี เดชเทวพร : ความสัมพันธ์ระหว่างแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้และการวัดการกำเริบของโรคในผู้ป่วยโรคลูปุส (THE ASSOCIATION OF ANTI-DOUBLE-STRANDED DNA ANTIBODIES AND DISEASE ACTIVITY MEASUREMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS) อ. ที่ปรึกษา : รศ. พญ. มนาธิป โอศิริ, อ. ที่ปรึกษาร่วม : ผศ. นพ. ยิ่งยศ อวิหิงสานนท์ ; 64 หน้า. ISBN 974-14-1981-3.

วัตถุประสงค์ เพื่อแสดงความสัมพันธ์ระหว่างแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้กับการวัดการกำเริบของโรคในผู้ป่วยโรคลูปุส

วิธีการศึกษา การศึกษาความสัมพันธ์ระหว่างแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้กับการวัดการกำเริบของโรคในผู้ป่วยโรคลูปุส โดยใช้ตัวชี้วัดการวัดการกำเริบของโรคที่ปรับปรุงไว้ใช้ในประเทศกำลังพัฒนาโดยผู้วิจัยจากประเทศเม็กซิโก

ผลการศึกษา การศึกษานี้มีผู้ป่วยโรคลูปุสจำนวน 173 ราย ผู้ป่วยร้อยละ 53.2 (92 ราย) มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นบวก (ค่า ≥1:10) และผู้ป่วยร้อยละ 46.8 (81 ราย) มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นลบ (ค่า <1:10) กลุ่มผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นบวกมีอายุเฉลี่ยในขณะทำการศึกษา 32.4±10.3 ปี ระยะเวลาของการป่วยเป็นโรค 5.3±5.7 ปี กลุ่มผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นลบมีอายุเฉลี่ยในขณะทำการศึกษา 34.0±10.4 ปี ระยะเวลาของการป่วยเป็นโรค 6.7±6.1 ปี ผู้ป่วยทั้ง 2 กลุ่มประกอบด้วยผู้ป่วยเพศหญิงมากกว่าเพศชาย การวัดการกำเริบของโรคในผู้ป่วยโรคลูปุสโดยใช้ตัวชี้วัดในการวัดการกำเริบของโรคจากประเทศเม็กซิโกมี 3 รูปแบบ: กลุ่มผู้ป่วยที่ไม่มีการกำเริบของโรคประกอบด้วย ผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นบวก ร้อยละ9.8 (9 ราย) ผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นลบ ร้อยละ 34.6 (28 ราย) กลุ่มผู้ป่วยที่น่าจะมีการกำเริบของโรคประกอบด้วย ผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นบวก ร้อยละ 21.7 (20 ราย) ผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นลบ ร้อยละ9.9 (8 ราย) กลุ่มผู้ป่วยที่มีการกำเริบของโรคชัดเจนประกอบด้วย ผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นบวกร้อยละ 68.5 (63 ราย) ผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นลบ ร้อยละ55.6 (45 ราย) ($p<0.001$) ในกลุ่มผู้ป่วยที่มีผลตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นบวก มีความสัมพันธ์กับการกำเริบของโรคลูปุสที่อวัยวะอื่นๆดังนี้ ระบบโลหิต ร้อยละ 75 ($p=0.011$) ระบบผิวหนัง ร้อยละ 40.2 ($p=0.019$) ระบบกระดูกและข้อ ร้อยละ12.0 ($p=0.047$) อย่างไรก็ตาม ไม่พบความสัมพันธ์กับระบบไตในการศึกษานี้ ($p=0.426$)

สรุป การตรวจของแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้ที่ให้ผลบวกมีความสัมพันธ์กับการวัดการกำเริบของโรคในผู้ป่วยโรคลูปุส แต่ผลตรวจที่เป็นลบสามารถพบในผู้ป่วยที่มีการกำเริบของโรคได้ ผลตรวจที่เป็นบวกมีความสัมพันธ์กับการกำเริบของโรคลูปุสที่ ระบบโลหิต ระบบผิวหนัง ระบบกระดูกและข้อแต่ไม่มีความสัมพันธ์กับระบบไต

ภาควิชา	อายุรศาสตร์	ลายมือชื่อนิสิต	เรวดี เดชเทวพร
สาขาวิชา	อายุรศาสตร์	ลายมือชื่ออาจารย์ที่ปรึกษา	
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4774770530: MAJOR MEDICINE (RHEUMATOLOGY)
KEY WORDS: SYSTEMIC LUPUS ERYTHEMATOSUS/ ANTI-DS DNA ANTIBODIES/ DISEASE ACTIVITY INDEX
REVADEE DEJTHEVAPORN : THE ASSOCIATION OF ANTI-DOUBLE-STRANDED DNA ANTIBODIES AND DISEASE ACTIVITY MEASUREMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS. THESIS ADVISOR : ASSOC. PROF. MANATHIP OSIRI, M.D., THESIS CO-ADVISOR : ASST. PROF. YINGYOS AVIHINGSANON, M.D. 64 pp. ISBN 974-14-1981 -3.

Objective: To demonstrate the association between anti-double stranded DNA antibodies (anti-dsDNA Ab) and disease activity in systemic lupus erythematosus (SLE).

Methods: A 1-year study of the association between anti-dsDNA Ab titer and the Mexican Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI) in patients with SLE was performed.

Results: One hundred and seventy-three patients with SLE were included in the study. Positive anti-dsDNA Ab (anti-dsDNA Ab ≥1:10) were detected in 92 patients (53.2%) and negative anti-dsDNA Ab (anti-dsDNA Ab < 1:10) were detected in 81 patients (46.8%). In positive Ab group, the mean age at inclusion was 32.4±10.3 years, with the mean disease duration of 5.3±5.7 years. In negative Ab group, the mean age at inclusion was 34.0±10.4 years, with the mean disease duration of 6.7±6.1years. Both positive and negative Ab groups were predominately female. Three patterns of disease activity index according to MEX-SLEDAI scoring system were observed: Nine patients (9.8%) in the positive Ab group and 28 patients (34.6%) in the negative Ab group had inactive disease. Twenty patients (21.7%) in the positive Ab group and 8 patients (9.9%) in the negative Ab group were classified as probably active disease. Sixty-three patients (68.5%) in the positive Ab group and 45 patients (55.6%) in the negative Ab group had clearly active disease ($p<0.001$). Positive anti-dsDNA Ab was associated with several organ involvements: hematological 75% ($p=0.011$), mucocutaneous 40.2% ($p=0.019$) and musculoskeletal involvement 12.0% ($p=0.047$). However, this Ab was not related to renal manifestation (55.4%) in our study ($p=0.426$).

Conclusions: Positive anti-dsDNA Ab is mostly correlated with disease activity in SLE patients whereas negative titer cannot totally exclude disease flare. Positive titer is associated with hematological, mucocutaneous and musculoskeletal involvement but it is not correlated with renal manifestation.

Department	Medicine	Student's signature	Revadee Dejthevaporn
Field of study	Medicine	Advisor's signature	
Academic year	2005	Co-advisor's signature	

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ABBREVIATIONS

SLE	=	Systemic lupus erythematosus
Anti-dsDNA Ab	=	Anti-double stranded DNA antibodies
MEX-SLEDAI	=	Mexican Systemic Lupus Erythematosus Disease Activity Index
LR	=	Likelihood ratio
Ab	=	Antibody
Abs	=	Antibodies
OPD	=	Out-Patient Department
IPD	=	In-Patient Department
ELISAs	=	Enzyme-linked immunosorbent assays
MMF	=	Mycofenolate mofetil
LN	=	Lupus Nephritis
AIHA	=	Autoimmune hemolytic anemia
CVA	=	Cerebrovacular accident
ssDNA	=	Single stranded DNA
PPV	=	Positive predictive value
NPV	=	Negative predictive value

CHAPTER I

INTRODUCTION

1.1 Background and Rationale

Systemic lupus erythematosus (SLE) is the prototypic inflammatory autoimmune disease characterized by excessive autoantibody production, immune complex formation and multi-organ system involvement with diverse clinical manifestations.[1,2,3] The etiology remains unclear but many literatures demonstrated that both genetic and environmental factors contributed to disease susceptibility.[4,5,6]

Although many effective treatments are now available for the severe manifestations and survival has improved significantly over the past 50 years, SLE remains a condition with significant morbidity and mortality and has become a chronic disease entity.[7] Permanent organ damage, either from the disease itself or from its treatment (especially corticosteroid therapy), occurs in more than 50% of the patients.[8] Corticosteroid therapy, which almost all patients with SLE will receive at some time in their disease courses, has been presumed to be one of the principal culprits and numerous studies found that longer steroid use resulted in poorer outcome.[7,9,10,11,12] Therefore, it is important to detect disease flare in SLE. It is still uncertain which immunologic parameter is the best parameter to help diagnose active SLE.

Anti-double stranded DNA Ab (anti-dsDNA Ab) is one of the immunologic parameters that have been extensively studied in SLE. However, the informative data of this Ab and SLE are still controversial.

The initial discovery of the association between anti-dsDNA Abs and disease activity in SLE has been more than 35 years already.[13] Anti-dsDNA Abs can be detected in over 70 % of SLE patients at some time in the disease course and have 95 % specificity for the disease.[14] They are strongly correlated with lupus nephritis and disease activity.[15] However, several reports described lupus nephritis in the absence of anti-ds DNA Abs, and others described persistently high titer of anti-dsDNA Abs in the

absence of renal injury.[16] Although most anti-dsDNA Abs are elevated in lupus patients with renal involvement, but little information is available on whether the titers are different in inactive and active phases of SLE. In addition, there are no data available either for Asian or Thai patients about anti-dsDNA Abs and disease activity in SLE.

Whether the anti-dsDNA Abs are truly linked to disease pathogenicity and how accurately they reflect disease activity are all questions that have been posed during past 20 years.[17]

1.2 Research Questions

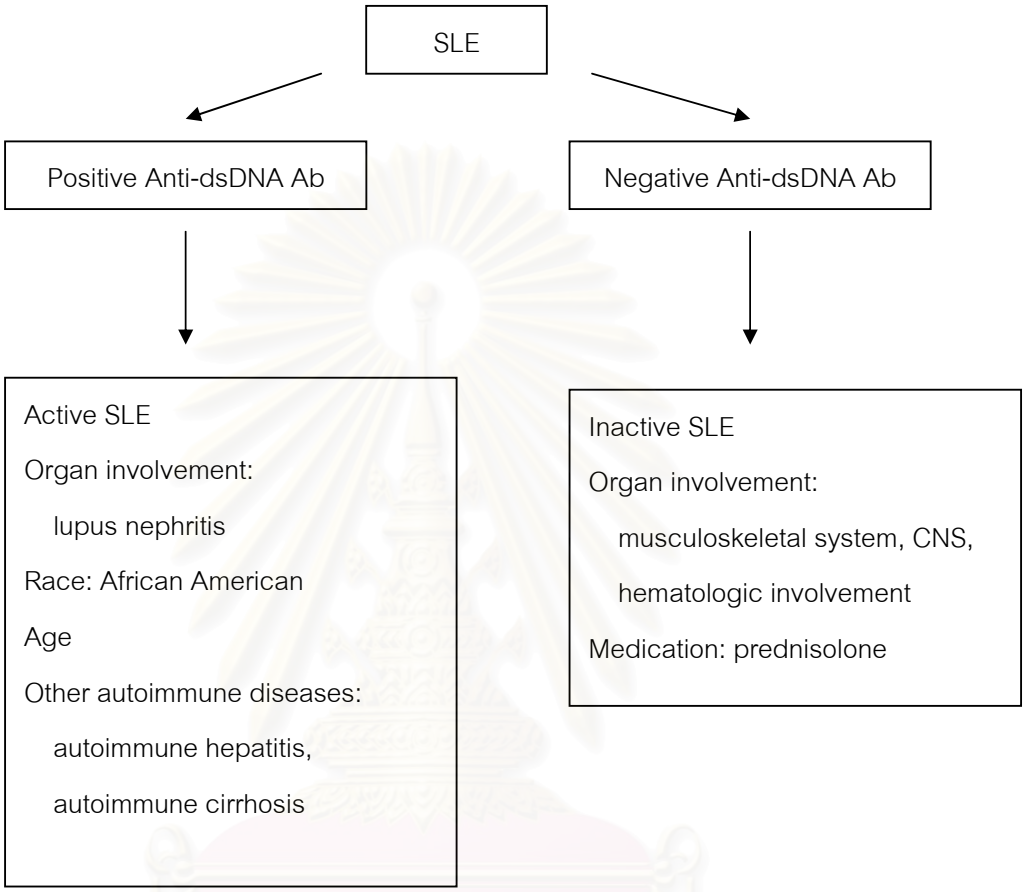
Primary question: What is the association between anti-dsDNA Abs and disease activity in SLE?

Secondary question: What is the relationship between anti-dsDNA Abs and organ involvements in SLE?

1.3 Objectives

1. To demonstrate the association between anti-dsDNA Abs and disease activity in SLE
2. To demonstrate the relationship between anti-dsDNA Abs and organ involvements in SLE.

1.4 Conceptual Framework



1.5 Research Methodology

A cross-sectional study of the association between anti-dsDNA Abs titer and the disease activity measured by using the Mexican Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI) in patients who fulfilled the 1997 American College of Rheumatology revised criteria for the classification criteria of SLE at King Chulalongkorn Memorial Hospital was performed.

1.6 Ethical Considerations

The Institutional Review Board of King Chulalongkorn Memorial Hospital approved the study, and all participants gave written informed consents.

1.7 Limitation

1. The patients with SLE who were recruited in this study may not be a good representative group for the SLE in general population in Thailand as they were just a small group and might have more severe and/or active disease.

2. Since data collection of this study was medical chart review, some medical data of SLE patients were missing and not included in the analysis.

1.8 Expected Benefit and Application

1. The association of anti-dsDNA Abs and disease activity in SLE patients.
2. The relationship of anti-dsDNA Abs and organ involvements in SLE patients.
3. The importance of anti-dsDNA Abs titer as a diagnostic marker for disease activity in SLE patients.

CHAPTER II

LITERATURE REVIEW

Systemic lupus erythematosus is a prototypic autoimmune disease characterized by the production of auto-Abs. Of these auto-Abs, anti-DNA Abs are the most characteristic of lupus. These Abs are marker of diagnostic and prognostic significance as well as mediators of immunopathologic damage characteristic of this disease.[18] One of the prognostic assessment includes disease exacerbation detection. Laboratory tests are commonly used for both diagnosis and prognostic assesssment in SLE.[19] However, there is no single serologic test that reliably measures disease activity in SLE. Anti-dsDNA Abs are utilized by more than 92 % of US rheumatologists to monitor disease activity in patients with SLE.[20]

2.1 Anti-DNA Abs

Auto-Abs to DNA were first described in the 1950s. These are the best recognized specific auto-Abs found in the patients with SLE. Auto-Abs to DNA can be divided primarily into two groups: those reactive with purine and pyrimidine bases of denatured (single stranded) DNA and those target the ribose phosphate backbone of native (double stranded) DNA.[21,22]

2.2 Measurement of Anti-dsDNA Abs

There are currently three methods commonly used by most clinical laboratories to quantitate anti-dsDNA Abs. Most of these tests measure both high-and low-avidity Abs.

1. <u>The Farr assay</u>

It is based on the precipitation of radioactively labeled DNA-anti-DNA Ab complexes in 50% saturated ammonium sulfate. This assay primarily detects immune complexes consisting of histone and anti-DNA Abs.[23] Approximately 50 to 78% of all

patients with SLE have elevated titers of anti-DNA Abs measured by this method; the titer appear to correlate closely with disease activity, especially with active proliferative nephritis.[23,24] Because this method requires the use of a radioactive antigen, its routine use has been limited. However, it is still routinely used in some laboratories because it measures high avidity anti-DNA and provides a very accurate methods of assessing dsDNA Ab levels.[17]

2. The *Crithidia luciliae* assay

It is an indirect immunofluorescent assay that makes use of the fact that basal body of this unicellular flagellate is very rich in double stranded DNA in the absence of other nuclear antigens.[25] This method, while of comparable sensitivity to the Farr assay, is more cumbersome to quantitate and the Abs detected correlate less closely with active nephritis.[26,27] It is considered to be the simplest, cheapest and specific test at the moment and most laboratories use it as a screening tool.[17]

3. The ELISA technique

This method is in routine use.[28,29] Double-stranded DNA adherent to polystyrene microwells, treated to increase their adhesiveness, serves as an antigen to capture Abs. These Abs are then quantitated using a second antiserum to human immunoglobulin conjugated to a detector enzyme. As a consequence, the ELISA technique is less prone to nonspecific reaction.[30] This method is positive in approximately 70% of patients with SLE. The IgG Ab titers correlate moderately well with active nephritis and there is a good correlation with disease activity in general.[21]

More recently other methods for detecting anti-DNA making use of immunoblotting and microarrays have been introduced.[31,32]

2.3 Properties of Anti-dsDNA Abs

The presence of anti-dsDNA Abs has been a criterion for SLE according to the 1997 American College of Rheumatology revised criteria for the classification criteria of SLE however, it is clear that not all anti-dsDNA Abs are pathogenic and certain characteristics of some anti-dsDNA Abs make them more likely to be pathogenic.[33,34]

Anti-dsDNA Abs can demonstrate different properties based on avidity that affects their usefulness as a diagnostic tool. High-affinity IgG anti-dsDNA Abs can be demonstrated in 70 to 80% of patients with SLE when their disease is active.[35] In contrast, some patients with SLE have predominantly IgM or low-avidity IgG Abs to dsDNA. These Abs are less useful diagnostically, as they can be found in association with drug-induced lupus, a variety of autoimmune diseases including rheumatoid arthritis, Sjögren's syndrome, other connective tissue diseases, chronic infection, chronic liver disease, autoimmune hepatitis, autoimmune cirrhosis and normal aging [24,36,37] ; in these instances, the Abs have no clinical significance. Furthermore, there is a significant number of anti-dsDNA Abs found in the serum of patients with myeloma protein but these patients did not develop features suggestive lupus. It is possible that lower avidity Abs are actually reacting with ssDNA fragments in the DNA preparations used as antigenic substrates.[17,21]

There are reports of patients with significant infections (e.g., septicemia) due to Escherichia coli or klebsiella organisms developing detectable anti-DNA Abs.[38] These Abs are detected by the ELISA method which can identify both low and high affinity Abs; the former being much less likely to be of pathogenic significance.

A number of properties of anti-dsDNA Abs other than avidity also affect their pathogenicity, including the isoelectric point, isotype, and idiotype. Anti-DNA Abs that are IgG1 and IgG3 isotypes, cationic charge, crossreactivity with alpha actinin and bind with high affinity correlate best with renal activity.[17,21]

2.4 Pathogenic Anti-dsDNA Abs

Some anti-dsDNA Abs are pathogenic and cause disease activity in SLE.

1. Koffler, et al, concluded that anti-dsDNA Abs have been eluted from the kidneys of both patients with lupus and murine models of the disease.[39]

2. Raz, et al, using an isolated perfused rat kidney system showed that some murine monoclonal antidsDNA Abs and some affinity purified human serum anti-dsDNA Abs could bind directly to renal glomeruli and significantly increase proteinuria.[40]

3. Madaio, et al, have shown that some murine monoclonal anti-DNA Abs when transferred to healthy strain mice can bind to capillary loops of glomeruli and cause proteinuria.[41]

4. Kalden, et al, have undertaken a series of experiments studying the effects of human monoclonal anti-dsDNA Abs in Severe Combined Immune Deficient (SCID) mice and have shown that some of these Abs have the capacity to bind exclusively to the kidney (in one case causing swelling of the glomerular basement membrane with fusion of the foot processes – early features of lupus nephritis) and in other cases to bind to the kidney and other tissues. In both cases a significant degree of proteinuria was induced.[17,42,43]

5. Isenberg, et al. concluded that whereas over 20% of the healthy relatives of patients with lupus had Abs to single stranded DNA only two out of 140 relatives had (marginally) elevated levels of anti-dsDNA Abs supporting the notion that these Abs are truly associated with the disease.[44]

2.5 Association of Anti-dsDNA Abs and Disease Activity

At present, a variety of techniques have been employed to test for anti-dsDNA Abs including Immunofluorescence against *Crithidia luciliae* and ELISA which are utilized most commonly in clinical practice. The immunofluorescent assay is regarded as most specific because of the tendency for ELISA technique to detect both low affinity and high affinity Abs and because of anti-ssDNA Abs, which can contaminate the anti-dsDNA Ab determinations and give false positive results.[15,45,46]

The problem of the correlation of anti-dsDNA Abs and disease activity in SLE is that SLE patients can show persistently elevated antidsDNA Ab levels with no evidence of disease activity.[47,48,49] or persistent clinical activity with normal anti-dsDNA Ab levels.[50]

Generally, anti-dsDNA Abs are relatively specific for SLE and this specificity making them very useful for diagnostic purpose as shown in the table 2.1

Table 2.1 Sensitivity, Specificity and Likelihood Ratio of Anti-dsDNA Abs.[51]

	Sensitivity	Specificity	Positive LR	Negative LR
SLE vs normal and other diseases	0.573	0.974	16.4	0.49
SLE: active vs inactive	0.66	0.66	4.14	0.51
Lupus nephritis:				
present vs absent	0.65	0.41	1.7	0.76
active vs inactive	0.86	0.45	1.7	0.3

Likelihood ratio = LR

2.6 Disease Activity Measurement

Several clinical indices have been proposed to measure disease activity in patients with SLE. At present, there are several validated and reliable disease activity indices available for assessing disease activity in patients with SLE. Those well established global activity indices are Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), Mexican Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI), Systemic Lupus Activity Measure (SLAM) and the British Isles Lupus Assessment Group Activity Index (BILAG).[52,53,54,55,56,57,58,59,60]

The Mexican Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI) was developed to use in the Third World countries where immunologic and complement assays are costly and/or unavailable. In a prospective study representing spectrum of disease activity, physicians scored disease activity using SLEDAI and MEX-SLEDAI; both instruments demonstrated comparable validity and responsiveness. MEX-SLEDAI was considered the least expensive instrument.[61]

2.7 Study of Anti-dsDNA Abs and Disease Activity

1. Ho, et al.[62]

The study determined the degree to which changes in anti-dsDNA Abs, as determined by *Crithidia* and ELISAs, precede or coincide with changes in SLE activity, as measured by 5 clinical indices, the physician's global assessment (PGA), Modified SLE Disease Activity Index (M-SLEDAI), Modified Lupus Actiivty Index (M-LAI), Systemic Lupus Activity Measure (SLAM), and the modified British Isles Lupus Assessment Group (M- BILAG). They concluded that a previous increase in anti-dsDNA level occurred before SLE flares, as measured by the M-SLEDAI and M-LAI only. However, during lupus flares, including the subset of renal flares, anti-dsDNA levels frequently decreased and hypothesized that this decrease in anti-dsDNA represents deposition in tissue at the time of flare.

2. Zonana-Nacach, et al.[63]

The study assessed flares in outpatients with SLE using Systemic Lupus Activity Measure (SLAM) and to determine laboratory abnormalities as predictors of disease activity. The laboratory investigation included anti-DNA, C3 and C4. They concluded that flares were frequent in patients with SLE and they occurred independently of disease duration and the time the disease had been under control. Flares were apparently predictable and were related to serologic abnormalities such as high anti-DNA, low C3 and low C4.

3. Barbara, et al.[64]

The study identified the frequency of serologic activity in the flare of clinical quiescence in a large cohort of patients with SLE followed prospectively in a single center. These serologic tests included low C3, C4, CH 50 and elevated anti-DNA Abs. They considered only the serologically active clinically quiescent (SACQ) period itself and found that there was no difference between the groups in absolute DNA Ab level, type of low complement. They concluded that there was a significant population of patients with SLE are SACQ and must be followed over time and treated only on the basis of clinical criteria.

4. Förger, et al. [65]

The study aimed to investigate the association between patterns of anti-dsDNA Ab isotypes and specific clinical manifestations. The concentration of anti-dsDNA isotypes showed a strong correlation with disease activity. There was a significant association of IgM isotype with cutaneous involvement and IgG isotype with lupus nephritis.

5. Schur, et al. [13]

Titers rise when disease is active, and usually fall (generally into the normal range) when the flare subsides. [66,67] The studies reported a tight correlation between high-titer anti-dsDNA Abs and nephritic activity, particularly in the setting of hypocomplementemia.

6. Swaak, et al. [68]

Many patients with renal exacerbations of the lupus, a sharp fall in the anti-dsDNA level usually preceded by a rise. There was an observation suggesting that the Abs were being deposited in one or more of the body's tissues.

7. Lloyd, et al. [66]

The complement depletion and raised dsDNA Abs were associated more with renal than nonrenal exacerbations in patients with lupus.

8. Ter Borg, et al. [67]

Active lupus nephritis was usually associated with high anti-dsDNA Ab levels.

9. Isenberg, et al. [69]

Anti-dsDNA Ab was correlated with renal disease activity, cardiopulmonary disease and global score but not with disease activity in the musculoskeletal system, the central nervous system or with hematological involvement.

Conclusion

Anti-dsDNA Ab is specific for SLE and useful for diagnosis. Titers of anti-dsDNA Abs are important in the management of some patients with SLE. The association between anti-dsDNA Abs and organ involvement of SLE is controversial. The utility of anti-dsDNA antibdies may be helpful in distinguishing active lupus disease from infectious complications and available information may help in caring patients in the future.

CHAPTER III

Research Methodology

Research Design

A cross sectional study was conducted between January 2005 and January 2006 at the Department of Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Consecutive cases of SLE who were admitted or followed at Rheumatology and/or Nephrology outpatients Clinics were included in the study.

3.1 Population

3.1.1 Target population

All SLE patients in Thailand.

3.1.2 Study population

All cases fulfilled the 1997 American College of Rheumatology revised criteria for the classification of SLE [70,71] , who were admitted at Department of Medicine, King Chulalongkorn Memorial Hospital or followed at Rheumatology and/or Nephrology outpatient clinics.

3.1.2 Inclusion criteria

1) All eligible patients who fulfilled the 1997 American College of Rheumatology revised criteria for the classification of SLE.
2) The patients were 18 years of age or older.

3.1.3 Exclusion criteria

1) The SLE patients who had no disease activity for at least 5 years.
2) The patients were pregnant at the study recruitment.

3.1.4 Sample Size Determination

Sample size calculation: $n = \frac{\left[Z_{\alpha/2} / \sqrt{2P(1-P)} + Z_{\beta} \sqrt{p1(1-p1) + p2(1-p2)} \right]^2}{(p1 - p2)^2}$

$$P = \frac{p1 + p2}{2}$$

$p1$ SLE patients with elevated anti-dsDNA antibodies and clinically classified as quiescence group

= 12% [72]

$p2$ SLE patients with elevated anti-dsDNA antibodies and clinically classified as flare group

= 30% [72]

$$P = \frac{(0.12 + 0.30)}{2}$$

= 0.21

$$n = \frac{\left[1.96 / \sqrt{2(0.21)(0.79)} + 0.842 \sqrt{(0.12)(0.88) + (0.30)(0.70)} \right]^2}{(0.18)^2}$$

= 81 patients per group

Sample size calculation = 2n = 2x81 = 162 patients

3.2 Methods and Materials

Data collection was informed performed through history taking, physical examination, laboratory determinations and disease activity assessment. The General information is obtained on age, gender, age at disease onset, disease duration and concurrent medication.

3.2.1 Variable Measurements

1) **Anti-dsDNA antibodies**

Venous blood samples (3-5 ml clotted blood) from each patient for anti-dsDNA Abs titers measurement are examined using standardized laboratory tests: Indirect immunofluorescence assay against *Crithidia luciliae* technique.

2) **MEX-SLEDAI**

The disease activities of all SLE patients were assessed by one physician by using the Mexican Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI) scoring system at the study recruitment.

3.2.2 Operational Definition

1) **Anti-dsDNA antibodies**

Positive anti-dsDNA antibodies titers are defined at levels $\geq$ 1:10.

2) **MEX-SLEDAI scoring system**

For the MEX-SLEDAI scoring system, disease activity is scored on a 0–32 point scale according to the clinical parameters. Patients with a MEX-SLEDAI score at least 2 points were considered active. A higher score implies greater disease activity.[61]

1. Scoring less than 2 is clearly inactive disease.
2. Scoring between 2 and 5 is categorized as probably active.
3. Scoring more than 5 is clearly active.[71,72,73,74,75]

3) **Lupus nephritis**

Evidence of lupus nephritis is defined as the presence of protenuria or evidence of microscopic hematuria or documented according to the classification of the World Health Organization as demonstrated in the table 3.1.[76,77]

Table 3.1 World Health Organization (WHO) Classification System for Lupus Nephritis

WHO Class	Histological finding
Class I	Normal glomeruli
Class II	Mesangial glomerulonephritis
Class III	Focal Proliferative glomerulonephritis
Class IV	Diffuse Proliferative glomerulonephritis
Class V	Diffuse membranous glomerulonephritis
Class VI	Advanced sclerosing glomerulonephritis

4) Proteinuria

Proteinuria is defined as presence more than 500 mg of protein in a 24-hour urine specimen collection. [73]

5) Microscopic hematuria

Microscopic hematuria defined as presence of or red cells > 5 cells per high power- field.[78]

6) Clinical activity of lupus nephritis

Clinical activity of lupus nephritis is defined by one or more of the following:

- decrease in renal function (serum creatinine >1.0 mg per deciliter
- proteinuria
- microscopic hematuria
- presence of cellular casts
- active urine sediment (hematuria or cellular casts)
- increasing proteinuria with rising levels of serum creatinine [79]

3.2.3 Outcome Mesurements

1) Anti-dsDNA antibodies level
2) Disease activity measured by MEX-SLEDAI scoring system
3) Organ involvement

- Neurological involvement
- Renal involvement
- Hematological involvement
- Musculoskeletal involvement
- Mucocutaneous involvement
- Respiratory involvement
- Cardiovascular involvement
- Gastointestinal involvement
- Constitutional involvement

3.3 Data collection

All patients were interviewed and examined with the use of a standardized data-collection instrument. Disease activity index are assessed at the time of enrollment in the study by using MEX-SLEDAI scoring system. Comprehensive medication histories are obtained through interviews with the patients and chart review. The use of corticosteroid therapy is categorized as the average daily dose.

3.4 Statistical Analysis

The data was analysed using SPSS version 13.0 for Windows. Baseline characteristics of the positive and negative Abs group were compared by Chi-square or Fisher's exact test for categorical variables and the t-test for continuous variables. All continuous variables had a normal distribution and the values were reported as mean ± SD. To compare the frequency of anti-dsDNA Ab and disease activity Chi-square test or Fisher's exact test was used and the values were reported as percentage. Differences at p value less than 0.05 were considered statistical significance.

3.4 Data Presentation

The data was presented by using table, pie chart and bar chart presentation. Data were described using mean ± SD or median (range) where appropriated and frequency (percentage) for continuous and categorical variables. Data comparing between positive and negative anti-dsDNA Abs were described by using frequency (percentage) and p value to show statistical difference.

3.5 Budget

The study was supported by grants from Faculty of Medicine, Department of Medicine, King Chulalongkorn Memorial Hospital, Chulalongkorn University.

The Anti-dsDNA antibodies 100 baht / specimen	= 18,000 baht.
The blood examination equipment including needles and syringes	= 1,000 baht.
Data collection form	= 2,000 baht.
Stationery expense and office supplies	= 5,000 baht.

CHAPTER IV

RESULTS

4.1 General Baseline Characteristics

One hundred and seventy-three patients with SLE were included in the study. The studied patients were predominantly female (164 female and 9 male). The patients' age ranged from 18 to 76 years (mean ± SD 33.1±10.4 years) and the mean ± SD age of onset was 27.2±10.4 years. The mean ± SD disease duration of the patients was 6.0±5.9 years.

At the time of anti-dsDNA Ab investigation, 127 patients (73.4%) were receiving glucocorticoids, with daily dose varied from 1.25 mg per day to 60 mg per day of prednisolone (mean 14.3±18.5 mg per day) whereas 46 patients (26.6%) were not receiving glucocorticoids. Thirty-seven patients (21.4%) were treated with anti-malarial agents, 17 patients (9.8%) with intravenous cyclophosphamide, 1 patient (0.6%) with oral cyclophosphamide, 12 patients (6.9%) with azathioprine, 8 patients (4.6%) with mycofenolate mofetil, 1 patient (0.6%) with rituximab and 1 patient (0.6%) with cyclosporin.

Positive anti-dsDNA Abs (anti-dsDNA Ab titer $\geq$ 1:10) were detected in 92 patients (53.2%) and negative anti-dsDNA Abs (anti-dsDNA Ab titer < 1:10) were detected in 81 patients (46.8%). In positive Ab group, the mean ± SD age at inclusion was 32.4±10.3 years (range 18 to 76 years), with the disease duration of 5.3±5.7 years. In negative Abs group, the mean ± SD age at inclusion was 34.0±10.4 years (range 18 to 62 years), with the disease duration of 6.7±6.1years. The percentage of female in the positive and negative Ab groups was 95.7% and 93.8% respectively. The baseline characteristics of patients with SLE in two different groups are demonstrated in table 4.1.

Table 4.1 Baseline Characteristics of Patients with Systemic Lupus Erythematosus

Characteristic	Positive AntidsDNA Ab (n=92)	Negative AntidsDNA Ab (n=81)	*P* value†
Age (years)‡	32.4±10.3	34.0±10.4	0.105
Female gender (%)	88 (95.7%)	76 (93.8%)	0.736
Age at onset (years) ‡	27.1±9.9	27.3±11.0	0.422
Duration of SLE (years) ‡	5.3±5.7	6.7±6.1	0.343
Type of patients: (%)			0.007*
- Out patient	27 (40.3%)	40 (59.7%)	
- In patient	65 (61.3%)	41 (38.7%)	
Medication: (%)			
- Prednisolone	61 (66.3%)	66 (81.5%)	0.024*
- Prednisolone dose (mg/d) ‡	11.2±16.1	17.8±20.4	0.275
- Anti-malarial agents	24 (26.1%)	13 (16.0%)	0.108
- Cyclophosphamide IV	7 (7.6%)	10 (12.3%)	0.296
- Cyclophosphamide po	0	1 (1.2%)	
- Azathiopine	5 (5.4%)	7 (8.6%)	0.407
- Mycophenolate mofetil	1 (1.1%)	0	
- Rituximab	1 (1.1%)	0	
- Cyclosporin	1 (1.1%)	0	

†*P* values were calculated with the use of Student's t-test for continuous variables and with the use of the chi-square test and Fisher's exact test for categorical variables.

* *P* value < 0.05 was considered statistically significant.

‡ Plus-minus values are means ± SD.

The Demographic features of the study population between the two 2 groups including age, age at disease onset, disease duration from the time of SLE diagnosis and gender were not significantly different. The studied patients were from OPD 67 patients and IPD 106 patients. Positive Ab group mostly derived from IPD patients while negative Ab group derived from both OPD patients (61.3% and 59.7% respectively, p=0.007*).*

The current medication the patients were receiving at the time of anti-dsDNA Abs investigation mostly consisted of glucocorticoids, anti-malarial agents and immunosuppressive agents. Sixty-one patients (66.3%) from positive Ab group and 66 patients (81.5%) from negative Ab group were receiving prednisolone (p=0.024*)*. The mean ± SD daily doses of prednisolone in positive and negative anti-dsDNA Ab groups were varied from 0 mg per day to 60 mg per day (11.2±16.1 and 17.8 ± 20.4 mg, respectively, p=0.275). The finding implied that there was a significant higher percentage of prednisolone use in negative Abs group than positive group whereas the finding implied that there was a significant higher percentage of prednisolone use in negative Abs group than positive group whereas there was no statistical significance in steroid dosage between 2 groups.

There was no statistical difference in anti-malarial agents (p=0.108), intravenous cyclophosphamide (p=0.296), oral cyclophosphamide (p=0.468), azathioprine (p=0.407), rituximab (p=0.347), and cyclosporine (p=0.347), receiving between 2 groups whereas there was a patient receiving mycofenolate mofetil in positive Abs group only (p=0.026).

4.2 Association between Anti-dsDNA Abs and Disease Activity

In patients with active disease, a tendency of positive anti-dsDNA Abs in those patients (68%) compared with inactive disease (34.6%) were observed. (Table 4.2) However, there were 45 patients (55.6%) with negative anti-dsDNA Abs in the active disease group. In addition, there were 9 patients (9.8%) with positive anti-dsDNA Abs found in inactive disease (Figure 4.1). The sensitivity and specificity of anti-dsDNA Ab testing were 61% and 76% respectively. The prevalence of SLE patients at King

Chulalongkorn Memorial Hospital in this study was 79%. The positive and negative value of anti-dsDNA Ab testing was 90% and 35% respectively (Table 4.3).

Table 4.2 Association between Anti-dsDNA Abs and Disease Activity in SLE using MEX-SLEDAI

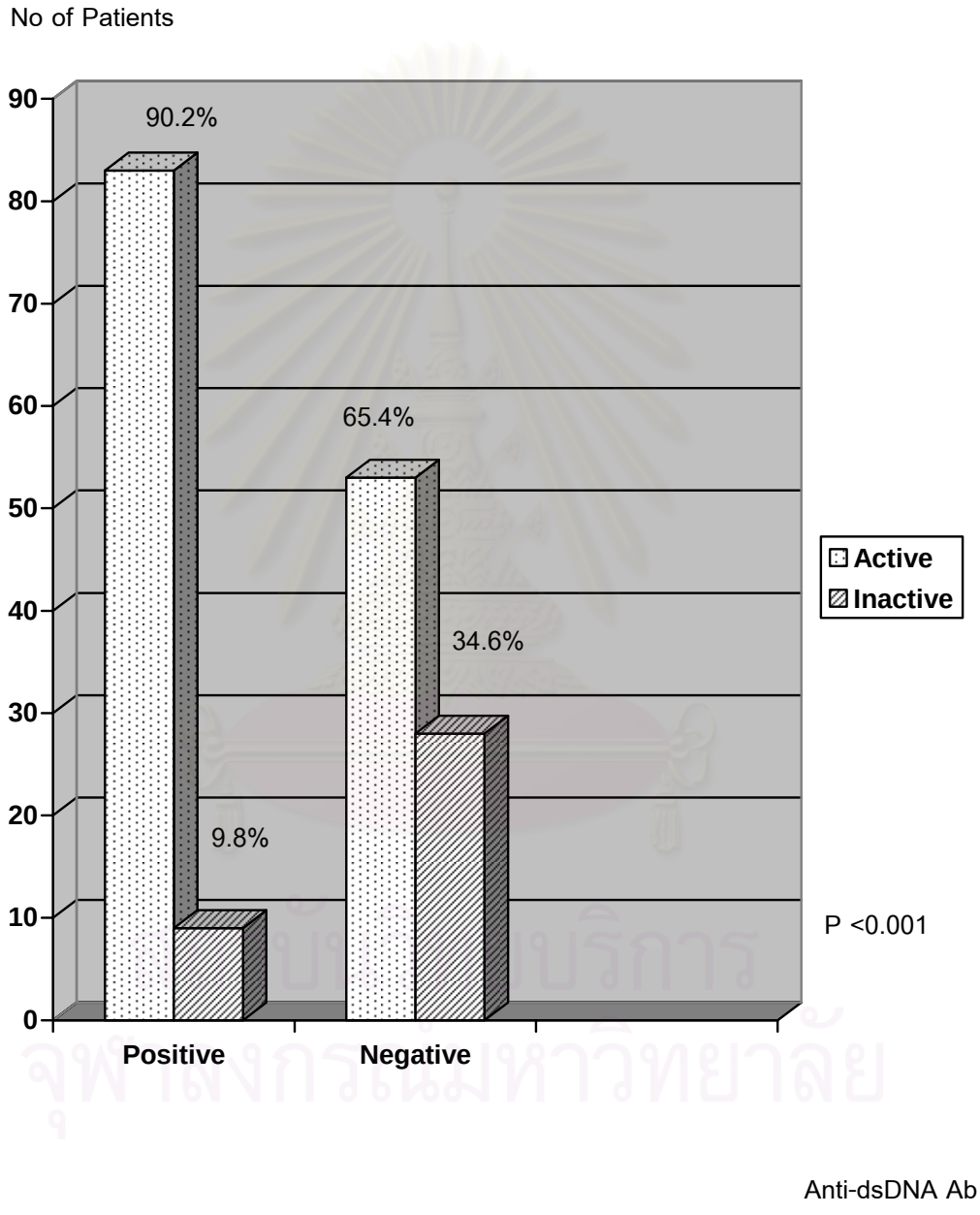
MEX-SLEDAI	Positive AntidsDNA Ab (n=92)	Negative AntidsDNA Ab (n=81)
Score < 2 (Inactive disease)	9 (9.8%)	28 (34.6%)
Score 2-5 (Probably active)	20 (21.7%)	8 (9.9%)
Score >5 (Clearly active)	63 (68.5%)	45 (55.6%)

Table 4.3 Sensitivity, Specificity, Positive and Negative Predicitve Value of Anti-dsDNA Ab

Anti-dsDNA Ab	Disease activity	
	Active	Inactive
Positive	83	9
Negative	53	28

- Sensitivity = 83/136 = 0.61
- Specificity = 28/37 = 0.76
- Positive predicitve value = 83/92 = 0.90
- Negative predicitve value = 28/81 = 0.35
- Prevalence = 136/173 = 0.79

Figure 4.1 Association between Anti-dsDNA Abs and Disease Activity in SLE using MEX-SLEDAI



4.3 Association between Anti-dsDNA Abs Titer and Disease Activity

Positive anti-dsDNA Abs in this study ranged from titer ≥ 1:10 (the lowest titer) to titer ≥ 1:1280 (the highest titer). The titer 1:10 mostly correlated with probably active disease group (MEX-SLEDAI score 2-5). The titer 1:20, 1:40, 1:80, 1:160, 1:320, ≥ 1:640 and ≥ 1:1280 were all correlated with clearly active disease group (MEX-SLEDAI score > 5). However, the titer <1:10 was also found in clearly active disease group (55.6%). In inactive disease group, there was no titer beyond 1:320 found (Table 4.4).

Table 4.4 Association between Anti-dsDNA Abs titer and Disease Activity in SLE Using MEX-SLEDAI

AntidsDNA Ab	MEX-SLEDAI		
Titer	<2	2-5	>5
< 1:10 (n=81)	28 (34.6%)	8 (9.9%)	45 (55.6%)
1:10 (n=16)	3 (18.8%)	7 (43.8%)	6 (37.5%)
1:20 (n=10)	3 (30.0%)	0	7 (70.0%)
1:40 (n=11)	2 (18.2%)	2 (18.2%)	7 (63.6%)
1:80 (n=14)	0	5 (35.7%)	9 (64.3%)
1:160 (n=8)	1 (12.5%)	0	7 (87.5%)
1:320 (n=9)	0	2 (22.2%)	7 (77.8%)
≥ 1:640 (n=17)	0	2 (11.8%)	15 (88.2%)
≥ 1:1280 (n=7)	0	2 (28.6%)	5 (71.4%)

4.4 The Relationship between Anti-dsDNA Abs and Organ Involvement in SLE

The relationship between anti-dsDNA Abs and organ involvement in SLE was demonstrated in table 4.5.

Table 4.5 The Relationship between Anti-dsDNA Abs and Organ Involvement in SLE

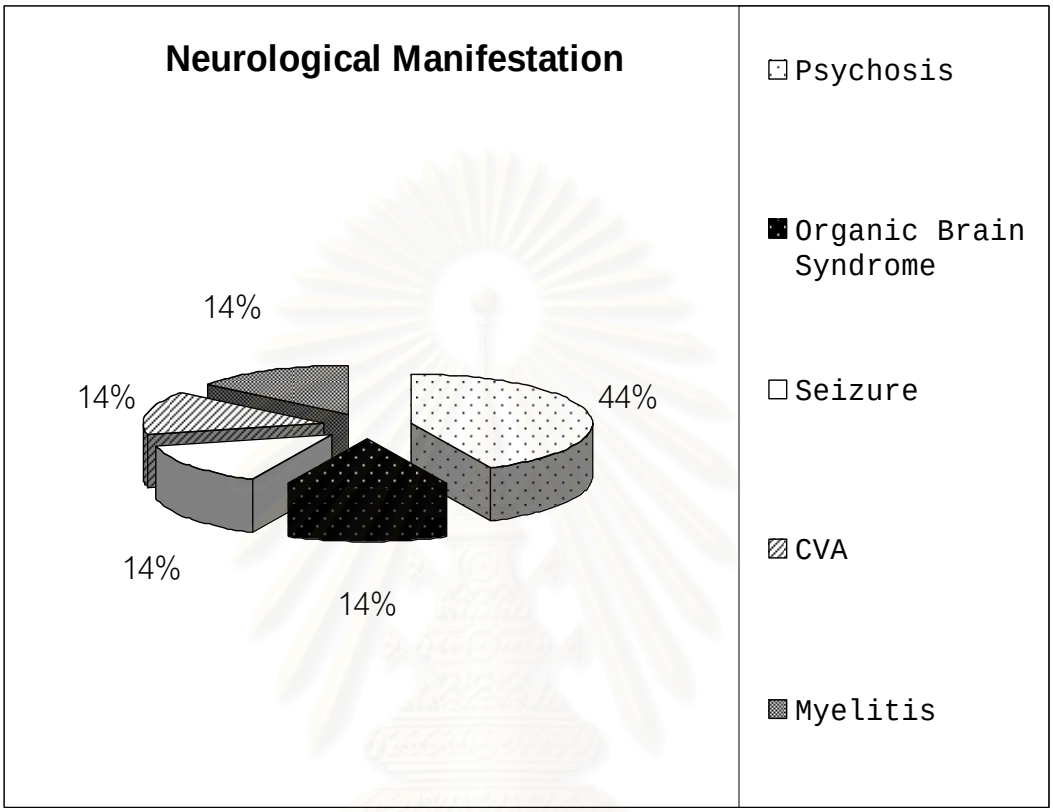
Organ Involvement	Positive AntidsDNA Ab (n=92)	Negative AntidsDNA Ab (n=81)	*P* value
Neurological	6 (6.5%)	3 (3.7%)	0.504
Renal	51 (55.4%)	40 (49.4%)	0.426
Hematological	69 (75.0%)	46 (56.8%)	0.011*
Musculoskeletal	11 (12.0%)	3 (3.7%)	0.047*
Mucocutaneous	37 (40.2%)	19 (23.5%)	0.019*
Cardiovascular	6 (6.5%)	2 (2.5%)	0.286
Respiratory	9 (9.8%)	6 (7.4%)	0.580
Gastrointestinal	1 (1.1%)	3 (3.7%)	0.341
Constitutional symptoms	33 (35.9%)	14 (17.3%)	0.015*

* *P* value < 0.05 was considered statistical significant.

Neurological Involvement

Six patients (6.5%) and 3 patients (3.7%) with neurological involvement had positive and negative anti-dsDNA Abs (p=0.504). The neurological manifestations in positive Abs group consisted of psychosis in 3 patients (50%), organic brain syndrome, seizure, cerebrovascular accident and myelitis 1 patient (16.6%) in each group (Figure 4.2). One patient in this group had both seizure and psychosis. The neurological manifestations in negative Abs group consisted of in seizure 1 patient (33.3%), cerebrovascular accident 1 patient (33.3%), and myelitis 1 patient (33.3%).

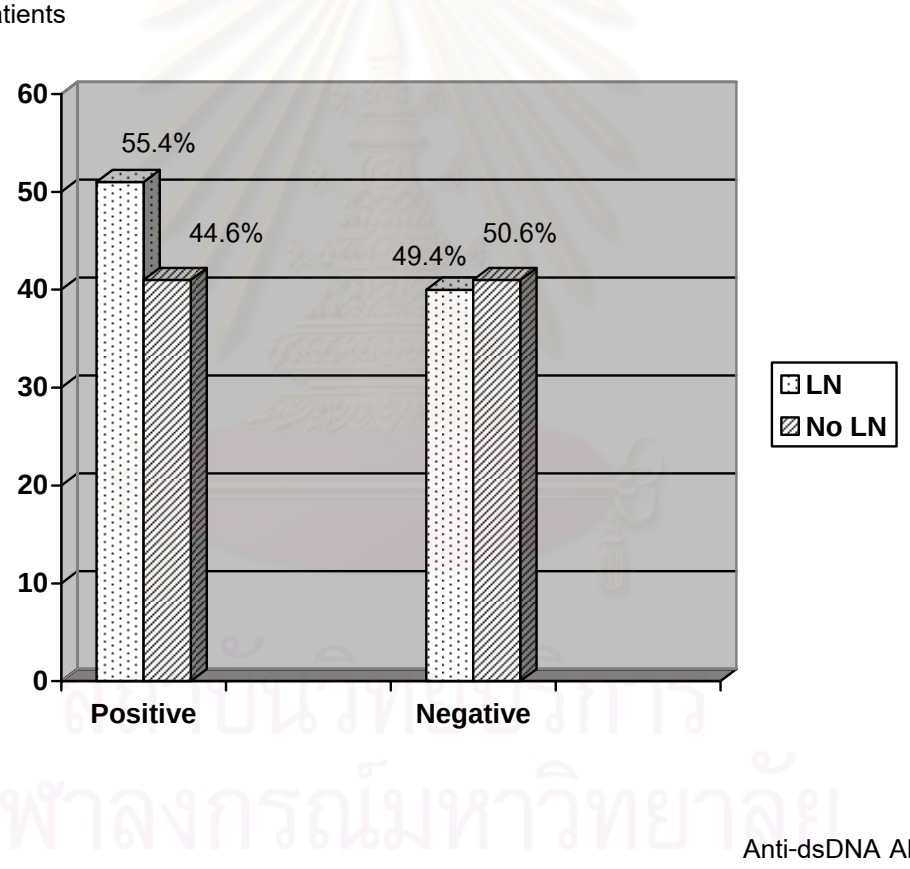
Figure 4.2 Neurological Manifestation with Positive Anti-dsDNA Abs



Renal Involvement

SLE patients with renal involvement had positive and negative anti-dsDNA Abs in 51 patients (55.4%) and 40 patients (49.4%) respectively (p=0.426). There was no significant correlation between lupus nephritis and anti-dsDNA Abs and negative Abs were also frequently found in patients with lupus nephritis (Figure 4.3).

Figure 4.3 The Relationship between Renal Involvement and Anti-dsDNA Abs



Ninety-one patients had lupus nephritis by clinical features and laboratory findings but renal biopsy was performed in 61 patients (67.0%). Biopsy results were classified according to WHO classification criteria as follows: 1 patient had class II, 3 had class III, 36 had class IV and 16 patients had mixed classes of lupus nephritis. Lupus nephritis class IV was the major renal involvement in SLE in this study (Table4.6). Negative Abs was found in 17 patients (54.8%) with lupus nephritis class IV. There was no renal biopsy in 21 patients (41.2%) with positive anti-dsDNA Abs group and 9 in the negative Abs group (22.5%). Positive anti-dsDNA Abs titer in lupus nephritis ranged from ≥ 1:10 to ≥ 1:1280 (Table 4.7). There was 1 patient (100%) with lupus nephritis class II had anti-dsDNA Abs titer ≥ 1:1280. The patient with lupus nephritis class III did not have positive anti-dsDNA Abs. In lupus nephritis class IV, the positive Abs titer were mainly above ≥ 1:20 whereas the positive Abs titer in lupus nephritis class V and mixed class of lupus nephritis were not specific at any levels.

Table 4.6 The Relationship of Lupus Nephritis Classified by WHO Classification and Anti-dsDNA Abs

Lupus Nephritis	Positive AntidsDNA Ab (n=30)	Negative AntidsDNA Ab (n=31)
Class II	1 (3.3%)	0
Class III	0	3 (9.7%)
Class IV	19 (63.3%)	17 (54.8%)
Class V	2 (6.7%)	3 (9.7%)
Class III+IV	1 (3.3%)	6 (19.4%)
Class III+V	3 (10%)	2 (6.5%)
Class IV+V	4 (13.3%)	0

Table 4.7 The Relationship of Anti-dsDNA Abs Titer and Class of Lupus Nephritis by WHO Classification

AntidsDNA	Lupus Nephritis Classification						
Ab Titer	II (n=1)	III (n=3)	IV (n=36)	V (n=5)	III+IV (n=1)	III+V (n=5)	IV+V (n=10)
< 1:10	0	3(100%)	17(47.2%)	3 (60%)	0	2 (40%)	6(60%)
1:10	0	0	1 (2.8%)	0	0	1 (20%)	0
1:20	0	0	3 (8.3%)	1 (20%)	0	0	0
1:40	0	0	2 (5.6%)	0	1(100%)	0	1 (10%)
1:80	0	0	4 (11.1%)	0	0	0	2
1:160	0	0	2 (5.6%)	1(20%)	0	1(20%)	0
1:320	0	0	2 (5.6%)	0	0	0	0
≥ 1:640	0	0	4 (11.1%)	0	0	1(20%)	1(10%)
≥ 1:1280	1(100%)	0	1 (2.8%)	0	0	0	0

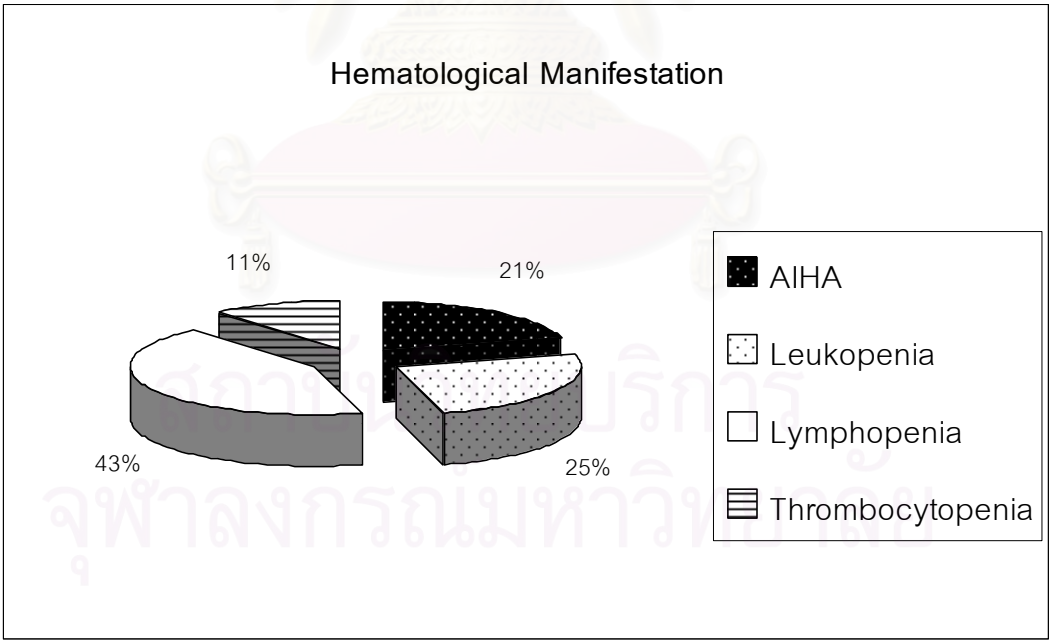
Hematologic Involvement

SLE patients with hematologic involvement had positive and negative anti-dsDNA Abs in 69 patients (75%) and 46 patients (56.8%), respectively (p=0.011). In positive Abs group, hematologic involvement included autoimmune hemolytic anemia (AIHA) in 31 patients (33.7%, p=0.002), leukopenia in 37 patients (40.2%, p <0.0001), lymphopenia in 63 patients (68.5%, p=0.112) and thrombocytopenia in 17 patients (18.5%, p = 0.383) (Figure4.8). Therefore, there was a significant difference between positive and negative Ab groups in AIHA and leukopenia.

Table 4.8 Hematological Manifestation with Positive Anti-dsDNA Abs

Hematological Manifestations	Positive AntidsDNA Ab (n=92)	Negative AntidsDNA Ab (n=81)	p value
AIHA	31 (33.7%)	11 (13.6%)	0.002*
Leukopenia	37 (40.2%)	8 (9.9%)	<0.0001*
Lymphopenia	63 (68.5%)	46 (56.8%)	0.112
Thrombocytopenia	17 (18.5%)	11 (13.6%)	0.383

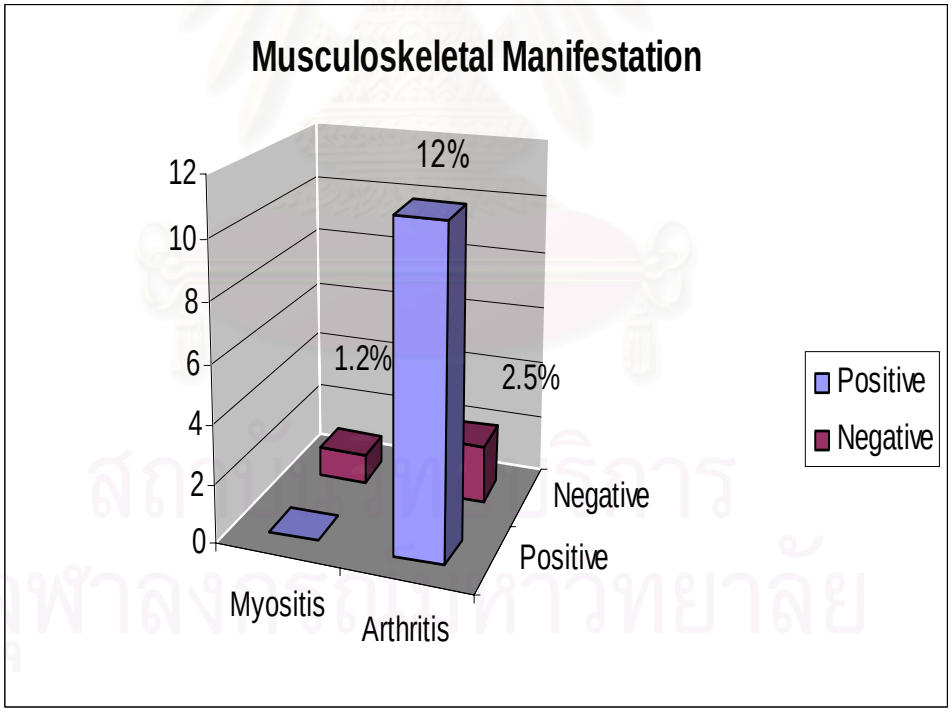
Figure 4.4 Hematological Manifestation with Positive Anti-dsDNA Abs



Musculoskeletal involvement

A significant difference in the number of SLE patients in both groups had musculoskeletal signs and symptoms. Eleven patients (12%) in positive Abs group and 3 (3.7%) in negative Abs group had musculoskeletal manifestations. (p=0.047). The musculoskeletal manifestation included polyarthritis and myositis. Arthritis was found in 11 patients (12%) with positive Abs group and 2 (2.5%) with negative Ab group (p=0.018). Myositis was found in 1 patient (1.2%) with negative Abs (p = 0.468). (Figure 4.5)

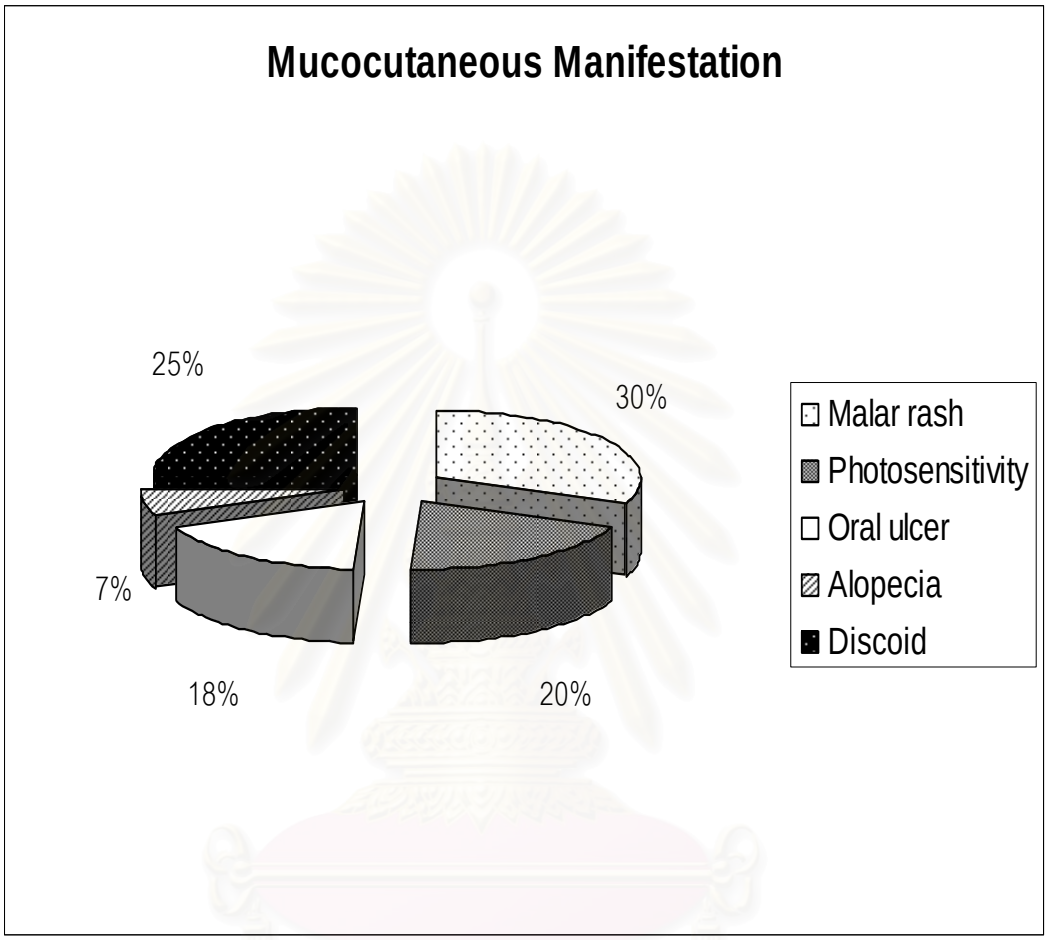
Figure 4.5 Musculoskeletal Manifestation with Anti-dsDNA Abs



Mucocutaneous Involvement

Positive anti-dsDNA Abs was found to correlate with mucocutaneous manifestation in SLE. The number of patients with mucocutaneous involvement in the positive and negative Ab groups was 37 patients (40.2%) and 19 patients (23.5%), respectively (p=0.019). In positive Abs group, the mucocutaneous manifestations consisted of malar rash in 19 patients (20.7%), photosensitivity rash in 12 (13.0%), oral ulcer in 11 (12.0%), alopecia in 4 patients (4.3%), and discoid lesions in 15 (16.3%). There was a statistical significance in the number of patients with photosensitivity rash in compared with negative Ab (p=0.029). (Figure 4.6) In negative Abs group, 19 patients (23.5%) had the mucocutaneous manifestations.

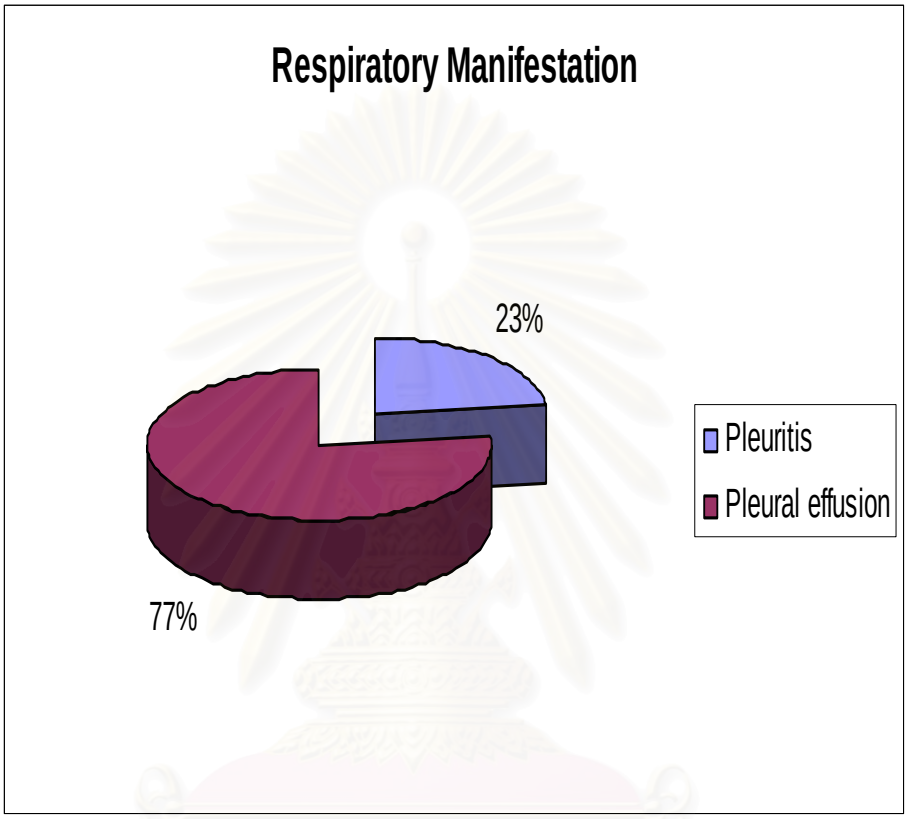
Figure 4.6 Mucocutaneous Manifestation with Positive Anti-dsDNA Abs



Respiratory Involvement

There was no correlation between of positive Abs and respiratory manifestations. SLE patients with pulmonary involvement had positive Abs in 9 patients (9.8%) and negative Abs in 6 patients (7.4%) (p=0.580). Pulmonary manifestations in positive Ab group included pleuritic symptoms in 3 patients (3.3%) and pleural effusion in 10 patients (10.9%). (Figure 4.7) Pulmonary manifestations in negative Ab group included pleuritic symptoms in 3 patients (3.7%) and pleural effusion 6 patients (7.4%).

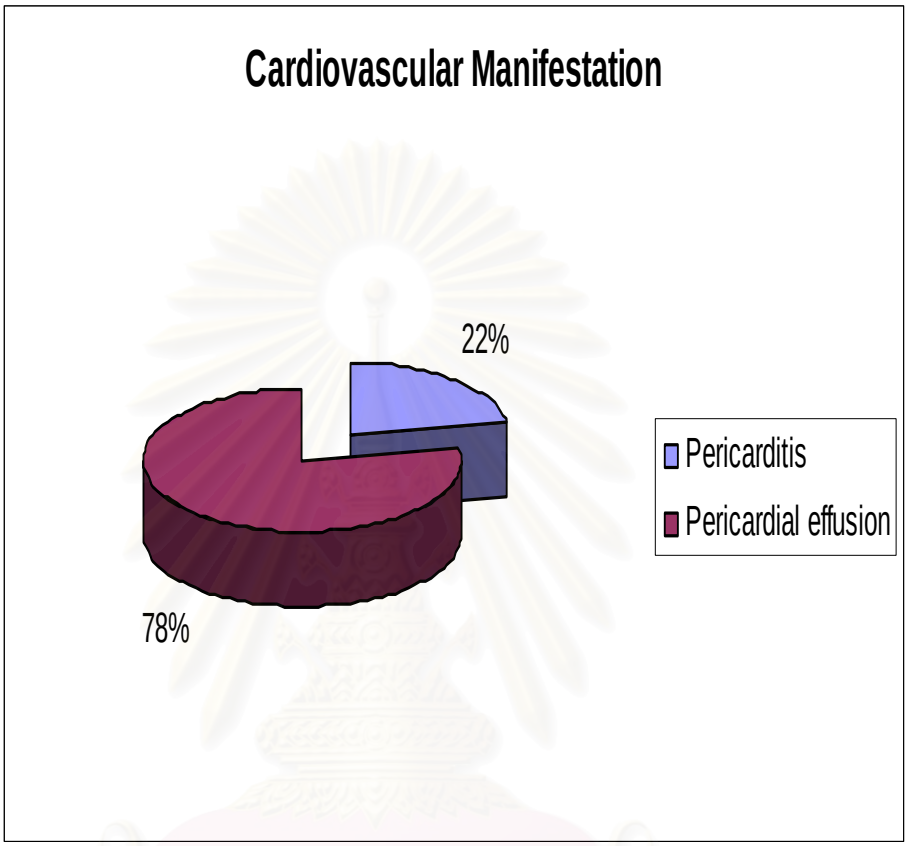
Figure 4.7 Respiratory Manifestation with Positive Anti-dsDNA Abs



Cardiovascular Involvement

There was no correlation between positive Anti-dsDNA Abs and cardiovascular manifestations. SLE patients with cardiac involvement had positive and negative anti-dsDNA Abs in 6 patients (6.5%) and 2 patients (2.5%), respectively (p=0.286). In positive Ab group, the cardiac manifestation included pericarditis in 2 patients (2.2%, p=0.637) and pericardial effusion in 7 patients (7.6%, p=0.176). Both clinical symptoms and signs had no statistical significance (Figure 4.8). Cardiovascular manifestations in negative Abs group included pericarditis in 1 patient (1.2%) and pericardial effusion in 2 patients (2.5%).

Figure 4.8 Cardiovascular Manifestation with Positive Anti-dsDNA Abs



Gastrointestinal Involvement

Gastrointestinal manifestation was found in 1 patient with positive anti-dsDNA Abs (1.1%) and 3 patients (3.7%) with negative anti-dsDNA Abs There was no statistical significance (p =0.341). One patient with positive anti-dsDNA Abs had ascites and peritonitis. The other 2 patients with negative anti-dsDNA Abs had gastrointestinal vasculitis and 1 patient had ascites and peritonitis.

Serositis

There was no correlation of positive Abs with serositis. SLE patients with serositis had positive and negative anti-dsDNA Abs 15 patients (16.3%) and 7 patients (8.6%) respectively (p=0.131). In positive Ab group, 10 patients had pleural effusion which were proved by chest radiography, 7 patients had pericardial effusion which were confirmed by echocardiography and 1 patient had ascites which was demonstrated by CT scan of abdomen. In negative Ab group, 6 patients had pleural effusion, 2 patients had pericardial effusion.

Constitutional symptoms

Constitutional symptoms comprised of fever and fatigue. There were 33 patients in positive Ab group (35.9%) and 14 patients in negative Ab group had constitutional symptoms (p=0.015), which represented a statistically significant difference between the two groups.

Laboratory investigation

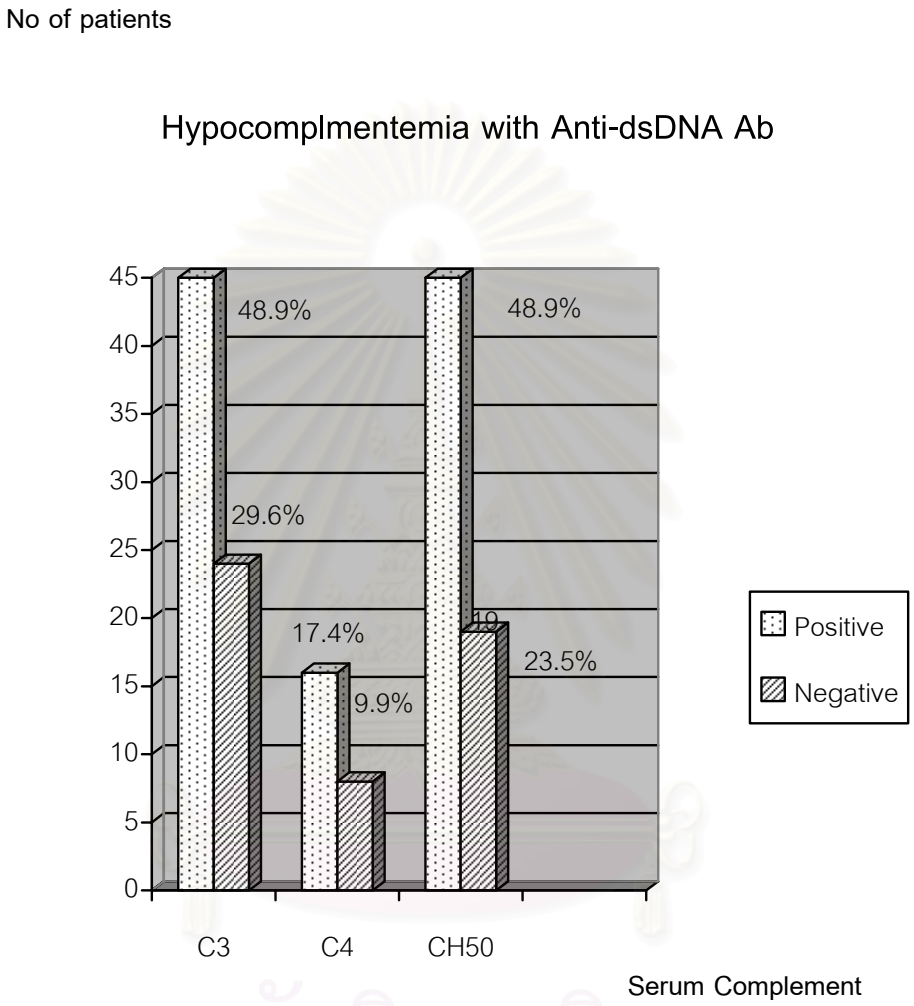
In positive anti-dsDNA Ab group, mean ± SD urine 24-hour protein was 3.1±2.7 gm/day and mean± SD serum creatinine was 2.6±3.6 mg/dL. In negative anti-dsDNA Abs group, mean ± SD urine 24-hour protein was 3.5±3.8 gm/day and mean ± SD serum creatinine was 3.1±4.4 mg/dL. Low level of C3 was detected in 45 patients (48.9%) from positive Abs group and 24 patients (29.6%) from negative Abs group (p=0.005). Low level of C4 was detected in 16 patients (17.4%) from positive Abs group and 8 patients (9.9%) from negative Abs group (p=0.046). Low level of CH50 was detected in 45 patients (48.9%) from positive Abs group and 19 patients (23.5%) from negative Abs group (p <0.0001). Thus, there was a significant difference in the complement levels between the 2 groups. (Table 4.9)

Table 4.9 Laboratory Investigation and Anti-dsDNA Abs

Laboratory investigation	Positive anti-dsDNA Ab	Negative anti-dsDNA Ab	*P* value
Proteinuria (g/day)	3.1±2.7 (0-11.0)	3.5±3.8 (0-20)	0.577
Cr (mg/dL)	2.6±3.6 (0.49-17.8)	3.1±4.4 (0.30-29.9)	0.197
Low C3	45 (48.9%)	24 (29.6%)	0.005*
Low C4	16 (17.4%)	8 (9.9%)	0.046*
Low CH50	45 (48.9%)	19 (23.5%)	<0.0001*

* *P* value < 0.05 was considered statistical significant.

Figure 4.9 Hypocomplementemia with Anti-dsDNA Abs



CHAPTER V

DISCUSSION, CONCLUSIONS AND SUGGESTION

5.1 Discussion

5.1.1 General Baseline Characteristics

During the 2005-2006 Study Year, consecutive cases of 173 patients were studied in the Rheumatology and Nephrology Outpatient Clinics and Inpatient Department of Medicine. The studied patients consisted of positive and negative anti-dsDNA Ab group 92 and 81 patients respectively. Most of the SLE patients were women and most of them were young and middle-aged. The mean age and mean age at onset between 2 groups was similar whereas the disease duration in negative Ab group was longer than positive Ab group (5.3 ± 5.7 and 6.7 ± 6.1 years) but there was no statistical difference.

Most of the patients were mainly from inpatient department (61.3%) and they were all collected as consecutive cases. This might result in the significantly higher percentage of IPD patients with positive anti-dsDNA Ab (61.3%) than those of negative Ab group (38.7%). In contrast, most OPD patients who mostly had milder symptoms or inactive disease activity had positive anti-dsDNA Ab (40.3%) and negative Ab (59.7%) (p=0.007).

The patients in negative anti-dsDNA Ab group were receiving prednisolone more than positive Ab group (81.5% and 66.3% respectively, p=0.024) with higher the average daily doses of the drug (17.8 ± 20.4 and 11.2±16.1 respectively) than positive Ab group, however, there was no statistical significance (p=0.275). It implied that the current medication may play an important role for the controlling the disease activity of SLE.

Overall baseline findings on age, gender distribution and medication apart from prednisolone were unremarkable between 2 groups.

5.1.2 Association between anti-dsDNA Abs and disease activity

Positive anti-dsDNA Ab was found 53.2% whereas negative anti-dsDNA Ab was found 46.8%. The patients who had positive Abs more often had active disease of SLE than those without active disease (90.2% in active disease group and 9.8% in inactive

group). However, the patients who had negative Abs could also have disease flare (55.6%).

Anti-dsDNA Abs have been shown in several studies to be correlated with SLE disease activity.[47,48,49] This Ab may be used as markers of disease activity in SLE.[79] However, these antibodies are also found in clinically inactive patients. In this study, it also supported the finding of former studies and the higher titer is also related with disease activity, although not all cases.

Changes in anti-dsDNA Ab levels need not be followed by disease flares, while flares may occur in the absence, as well as decreased, increased or stable presence of Ab.[62,80] It has become gradually clear that not all detectable anti-dsDNA Abs are clinically relevant. [24,81,82,83,84,85]

The sensitivity and specificity of anti-dsDNA Ab test vary according to the assay. When comparing among *Critidia luciliae* immunofluorescence tests, the results are quite similar for sensitivity and specificity of the tests but they have different results of PPV and NPV. In general, the sensitivity of the test is low and this study reported the same result while the specificity is high which helps confirm the benefit of using this Ab as a specific marker in diagnosis patients with SLE. However, this study demonstrated the finding of a very high PPV and low NPV. This based on the fact that most SLE patients in this study were mainly IPD cases and had active diseases. In other word, it is the characteristic of SLE patients in tertiary care hospital.

Table 5.1 Sensitivity, Specificity, PPV and NPV in SLE

	Study year 2005 [86]	Study year 2006 [87]	Present study
Sensitivity (%)	45.8	43	61
Specificity (%)	57.8	69	76
PPV (%)	27.8	43	90
NPV (%)	75	85	35

5.1.3 Association between anti-dsDNA Abs titer and disease activity

The titers of anti-dsDNA Ab in this study ranged from titer <1:10 to ≥ 1:1280. The titers in inactive disease group were mostly low (34.6%) and some patients who had higher titers did not exceed more than the titer of 1:320. the titers in probably active and clearly active disease group were more often to have the titers higher than 1:320. The highest titers (the titer of ≥ 1:1280 were also found in the patients with clearly active disease (71.4% with MEX-SLEDAI score >5) However, there were some patients who were classified in active disease group and had the titers of this Ab <1:10 (55.6%). It can be explained by that not all detectable anti-dsDNA Abs are clinically relevant. In clinical practice, the association between the Ab and disease activity needs to be addressed at the individual level, and it is unclear if following anti-dsDNA Ab is likely to be most helpful in whom disease activity previously concurred with anti-dsDNA Ab.[88,89]

5.1.4 The relationship between anti-dsDNA Abs and organ involvement in SLE

There were relationship of positive anti-dsDNA Abs and some organ involvements in this study: hematologic 75% (p 0.011), mucocutaneous 40.2% (p=0.019) musculoskeletal involvement 12.0% (p=0.047) and constitional symptoms 35.9% (p=0.015). However, this Ab was not related to renal manifestation (55.4%) in our study (p= 0.426). Among hematologic manifestation, they had association with AIHA (p=0.002) and leucopenia (<0.0001). Among mucocutaneous involvement, they had association with photosensitivity rashes (p=0.029).

Esdaile JM, et al.[19] revealed the relationship of positive anti-dsDNA Ab and SLE flare in renal 45%, serositis 35%, skin 29.8%, arthritis 31.6% and CNS 26%

whereas Isenberg DA, et al. [69] showed that anti-dsDNA Abs were correlated with renal disease activity, cardiopulmonary disease and global score but not with disease activity in the musculoskeletal system, the central nervous system or with hematological involvement.

Many literatures revealed that these anti-dsDNA antibodies are related with lupus nephritis with high percentage. However, there was no correlation between anti-dsDNA antibodies and renal involvement in this study. This might explain by the following theories:

1. Not all anti-dsDNA antibodies are pathogenic. There is a significant number of myeloma proteins may have antibody reactivity and yet in spite of having 10 g/L or more of igG anti-dsDNA antibody in the circulation, these patients did not develop features suggestive of lupus.[90]
2. Certain characteristics of some anti-dsDNA antibodies make them likely to be pathogenic.[91]
3. The pathogenic features include IgG1 and IgG3 isotypes, high avidity for dsDNA, cationic charge, crossreactivity with alpha actinin.[91]
4. There are evidences of pathogenic anti-dsDNA antibodies eluted from the kidneys of both patients with lupus and murine models of the disease.[92]
5. Some murine monoclonal antidsDNA antibodies and some affinity purified human serum anti-dsDNA antibodies could bind directly to renal glomeruli and significantly increase proteinuria.[93]

Therefore, the high percentage of negative anti-dsDNA antibodies in active disease group might be caused by detection of other IgG isotypes other than IgG1 and IgG3.

In addition to the pathogenic anti-dsDNA antibodies theories, assay problems are considered an important issue because some assays can detect anti-single stranded DNA antibodies. However, using the Crithidia luciliae assay already exclude this problem as it does not detect anti-single stranded DNA antibodies.

In this study, the hypocomplementemia was associated with positive anti-dsDNA Ab (low C3, $p=0.005$, low C4, $p=0.046$ and low CH50, $p<0.0001$).

The value of complement in assessment of SLE flares is considered to be useful parameter to monitor lupus activity (sensitivity 77%, specificity80%) [94] when correlating with good activity scoring system. However, it is difficult to make the interpretation in this study although there was a statistical significance due to small size of population.

5.2 Conclusions

1. There was no correlation between demographic data of the SLE patients and anti-dsDNA antibodies including age, gender, age at onset of the disease and the disease duration.

2. The prevalence of positive anti-dsDNA Ab in SLE in our study was 53.2% and this represented higher percentage of IPD patients who were recruited into the study. The studied patients were from OPD 67 patients and IPD 106 patients. Positive Ab group was mostly from IPD (61.3%) while negative Ab group was mostly from both OPD (59.7%).

3. There was a significant higher percentage of prednisolone use in negative Ab group (81.5%) than positive group (66.3%) however there was no statistical significance of average steroid dosage between 2 groups.

4. Positive anti-dsDNA Ab is mostly correlated with disease activity in SLE patients whereas negative titer cannot totally exclude disease flare.

5. Positive anti-dsDNA Ab was associated with hematological manifestation (AIHA and leukopenia), mucocutaneous (photosensitivity rash), musculoskeletal (arthritis) involvement and constitutional symptoms.

. 6. There was no correlation between anti-dsDNA antibodies and neurological, renal, pulmonary, cardiovascular and gastrointestinal involvement.

7. Hypocomplementemia (low C3, lowC4 and low CH50) was associated with positive anti-dsDNA antibodies.

5.3 SUGGESTION

Further studies should be performed with

1. Larger population size to clearly discriminate the similarity and difference of positive and negative anti-dsDNA antibodies.
2. Studying together with other specific antibodies such as anti-nucleosome or anti-C1q antibodies to measure reliability and specificity between groups.
3. Investigation serial anti-dsDNA antibodies as it might demonstrate the different clinical course of active and inactive disease and anti-dsDNA antibodies titer.
4. Using other types of immunofluorescence assay to compare reliability or find out technical error of laboratory investigation.

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APPENDIX

1997 American College of Rheumatology revised criteria for the classification of SLE

Criterion	Definition
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging: atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
5. Arthritis	Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling or effusion
6. Serositis	a) Pleuritis-convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion OR b) Pericarditis-documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	a) Persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantitation not performed OR b) Cellular casts-may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	a) Seizures-in the absence of offending drugs or known metabolic derangements (e.g.,uremia, ketoacidosis, or electrolyte imbalance) OR b) Psychosis-in the absence of offending drugs or known metabolic derangements (e.g.,uremia, ketoacidosis, or electrolyte imbalance)
9. Hematologic disorder	a) Hemolytic anemia-with reticulocytosis OR b) Leukopenia-less than 4000/mm^3 total on two or more occasions OR c) Lymphopenia- less than 1500/mm^3 total on two or more occasions OR d) Thrombocytopenia- less than 100000/mm^3 in the absence of offending drugs
10. Immunologic disorder	a) Positive LE cell preparation OR b) Anti-DNA: antibody to native DNA in abnormal titer OR c) Anti-Sm: presence of antibody to Sm nuclear antigen OR d) Faise positive serologic test for syphilis known to be positive for at least six months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with 'drug-induced lupus' syndrome

The proposed classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person shall be said to have SLE if any four or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.

หนังสือแสดงความยินยอมเข้ารับการศึกษาในโครงการวิจัย

(Informed Consent Form)

ชื่อโครงการวิจัย ความสัมพันธ์ระหว่างแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้และการวัดการกำเริบของโรคในผู้ป่วยโรคลูปุส

ชื่อผู้วิจัย แพทย์หญิงเรวดี เดชเทวพร

สาขาวิชาอายุรศาสตร์ (โรคข้อและรูมาติสซั่ม) คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

1. **คำชี้แจงเกี่ยวกับการตรวจหาแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เพื่อวัดการกำเริบของโรค**

โรคลูปุสเป็นโรคภูมิคุ้มกันผิดปกติที่เกิดขึ้นในผู้ป่วยที่ร่างกายมีการผลิตโปรตีนภูมิคุ้มกันในเลือดที่เรียกว่าแอนติบอดี้มากเกินปกติ เมื่อแอนติบอดี้เหล่านี้ไปปรากฏอยู่ในอวัยวะส่วนต่างๆของร่างกาย ทำให้เกิดการอักเสบของอวัยวะส่วนต่างๆทั่วร่างกาย เช่น ผิวหนัง ข้อ ไต สมอง ระบบโลหิต เป็นต้น

สาเหตุของโรคลูปุสยังไม่ทราบแน่ชัด แต่พบว่ามีความเกี่ยวข้องกับพันธุกรรมและฮอร์โมน ในปัจจุบันนี้ ความก้าวหน้าในการรักษาโรคลูปุสได้พัฒนามากขึ้น แต่อย่างไรก็ตาม บางครั้งการวินิจฉัยแยกโรคระหว่างภาวะติดเชื้อและการกำเริบของโรคลูปุสทำได้ยาก

แอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้เป็นโปรตีนภูมิคุ้มกันในเลือดชนิดหนึ่งที่มีข้อมูลอ้างอิงจากการศึกษาในต่างประเทศว่า มักจะมีระดับเพิ่มขึ้นสัมพันธ์กับการกำเริบของโรคลูปุส แต่ยังไม่มีข้อมูลดังกล่าวในประเทศไทย

การวิจัยดังกล่าวนี้มีวัตถุประสงค์เพื่อศึกษาความสัมพันธ์ระหว่างแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอ-แอนติบอดี้และการวัดการกำเริบของโรคในผู้ป่วยโรคลูปุส โดยพิจารณาทั้งระดับเลือดและการกำเริบของโรค

2. **คำชี้แจงเกี่ยวกับขั้นตอน วิธีการและการปฏิบัติตัวในการตรวจหาแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอ**

ท่านจะได้รับการซักประวัติ ตรวจร่างกายและตรวจเลือดเพื่อวัดระดับแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอ-แอนติบอดี้ การตรวจเลือดดังกล่าวนี้มีวิธีการเหมือนการตรวจเลือดตามปกติที่ท่าน

เคยได้รับบริการ ณ โรงพยาบาลจุฬาลงกรณ์ ไม่มีความจำเป็นต้องงดอาหารก่อนการตรวจเลือด

ข้อมูลที่ได้รับจากการวิจัยจะถูกวิเคราะห์และรายงานผล หากมีการเผยแพร่ก็จะเผยแพร่เป็นข้อมูลโดยรวมของผู้ป่วยโรคลูปุสทั้งหมดโดยไม่มีการเปิดเผยข้อมูลส่วนตัวของท่านต่สาธารณชนโดยเด็ดขาด

3. **ประโยชน์ที่จะได้รับจากการวิจัยนี้**

ทราบความสัมพันธ์ระหว่างแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้และการวัดการกำเริบของโรคลูปุส ท่านจะได้รับทราบผลการตรวจเลือดของท่านว่ามี การกำเริบของโรคลูปุสหรือไม่

คำชี้แจงเกี่ยวกับสิทธิของผู้เข้ารับการศึกษาในโครงการวิจัยนี้

การเข้าร่วมโครงการวิจัยนี้เป็นไปตามความสมัครใจของท่านโดยท่านสามารถปฏิเสธไม่เข้าร่วมโครงการวิจัยได้โดยไม่ส่งผลต่อการรักษาใดๆ

หากท่านมีข้อสงสัยประการใดในเรื่องของโครงการวิจัยดังกล่าวนี้ สามารถติดต่อสอบถามได้ที่

แพทย์หญิงเรวดี เดชเทวพร สาขาวิชาโรคข้อและรูมาติสซั่ม ตึกอายุรศาสตร์ ชั้น 2 โรงพยาบาลจุฬาลงกรณ์

เบอร์โทรศัพท์ติดต่อในเวลาราชการ (02)-2564526

เบอร์โทรศัพท์ติดต่อนอกเวลาราชการ (01)-7319221

คำยินยอมเข้ารับการศึกษาในโครงการวิจัย

ข้าพเจ้า นาย/ นาง/ นางสาว .....
ได้ทราบรายละเอียดของโครงการวิจัยโรคลูปุสและข้อความทั้งหมดของหนังสือแสดงความยินยอมเข้ารับการศึกษาในโครงการวิจัยฉบับนี้จาก แพทย์หญิงเรวดี เดชเทวพร โดยครบถ้วนแล้ว โดยแพทย์หญิงเรวดี เดชเทวพร ได้ตอบข้อซักถามของข้าพเจ้าจนเป็นที่เข้าใจแล้ว ข้าพเจ้ายินยอมที่จะเข้ารับการตรวจเลือดวัดระดับแอนตีดับเบิ้ลสะแตรนด์ดีเอ็นเอแอนติบอดี้นี้ด้วยความสมัครใจ

ลงชื่อ ..... (ผู้ยินยอมเข้ารับการศึกษา)

(.....)

ลงชื่อ ..... (ผู้ให้คำยินยอม
เข้ารับการศึกษา)

(.....)

ลงชื่อ ..... (พยาน)

(.....)

วันที่ .....

Data Collection Form

1. Identification Data

First name……. Family name…….

Sex ☐ Male ☐ Female

Age……..years Date of birth……..

Age at diagnosis……...years Date of diagnosis……

Disease duration……...years

Type of patient ☐ OPD ☐ IPD

2. Current Medication

Prednisolone	☐ Yes	☐ No
Dose ……(mg/d)		
Anti-malarial agents	☐ Yes	☐ No
Cyclophosphamide IV	☐ Yes	☐ No
Cyclophosphamide po	☐ Yes	☐ No
Azathiopine	☐ Yes	☐ No
Mycophenolate mofetil	☐ Yes	☐ No
Rituximab	☐ Yes	☐ No
Cyclosporin	☐ Yes	☐ No

3. Anti-dsDNA Antibodies Titer

……

4. MEX-SLEDAI Score

……

5. Classification Criteria for SLE

Criteria		
1. Malar rash	☐ Present	☐ Absent
2. Discoid rash	☐ Present	☐ Absent
3. Photosensitivity	☐ Present	☐ Absent
4. oral ulcer	☐ Present	☐ Absent
5. Arthritis	☐ Present	☐ Absent
6. Serositis	☐ Present	☐ Absent
• Pleuritis	☐ Present	☐ Absent
• Pericarditis	☐ Present	☐ Absent
7. Renal disorder	☐ Present	☐ Absent
• Proteinuria > 0.5 g/day	☐ Present	☐ Absent
• UA : Cellular cast, RBC	☐ Present	☐ Absent
8. Neurologic disorder	☐ Present	☐ Absent
• Seizure	☐ Present	☐ Absent
• Psychosis	☐ Present	☐ Absent
9. Hematologic disorder	☐ Present	☐ Absent
• Hemolytic anemia	☐ Present	☐ Absent
• Leukopenia < 4,000 cells/mm^3	☐ Present	☐ Absent
• Lyphopenia < 1,500 cells/mm^3	☐ Present	☐ Absent
• Thrombocytopenia < 100,000 cells/mm^3	☐ Present	☐ Absent
10. Immunologic disorder	☐ Present	☐ Absent
• Anti-dDNA	☐ Present	☐ Absent
• Anti-Sm	☐ Present	☐ Absent
11. ANA: Titer…… / Pattern……	☐ Present	☐ Absent

6. Laboratory Investigation

CBC

WBC..... cell/mm^3 Lymphocyte..... cell/mm^3

Hb..... g/dL Hct..... %

Platelet..... cell/mm^3

Urine analysis

Protein ☐ neg ☐ trace ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+

RBC..... cells/HPF RBC cast/LPF ☐ yes ☐ no

24-hour urine protein ..... g/day

Blood chemistry

BUN..... mg/dL Cr..... mg/dL

Complement

C3..... mg/dL

C4..... mg/dL

CH50..... U/mL

7. Renal Pathology

Renal biopsy

Class I	☐ Yes	☐ No
Class II	☐ Yes	☐ No
Class III	☐ Yes	☐ No
Class IV	☐ Yes	☐ No
Class V	☐ Yes	☐ No
Class VI	☐ Yes	☐ No
Others	.....	

8. Organ Involvement

Organ Involvement		
1. Neurological involvement	☐ Present	☐ Absent
2. Renal involvement	☐ Present	☐ Absent
3. Hematological involvement	☐ Present	☐ Absent
4. Musculoskeletal involvement	☐ Present	☐ Absent
5. Mucocutaneous involvement	☐ Present	☐ Absent
6. Respiratory involvement	☐ Present	☐ Absent
8. Cardiovascular involvement	☐ Present	☐ Absent
9. Gastointestinal involvement	☐ Present	☐ Absent
10. Constitutional involvement	☐ Present	☐ Absent

MEX-SLEDAI

Enter weight in MEX-SLEDAI if descriptor present at the time of the visit or in the preceding 10 days.

Weight	Descriptor	Definition
8	Neurological disorder	Psychosis. Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include: hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized or catatonic behavior. Exclude presence of uremia and offending drugs. CVA, New syndrome. Exclude arteriosclerosis. Seizure. Recent onset. Exclude metabolic, infectious or drug causes. Organic Brain Syndrome. Altered mental function with impaired orientation, memory or other intellectual function with rapid onset, fluctuating clinical features. Such as any of the following: a) Clouding of consciousness with reduce capacity of focus and inability to sustain attention to environment. Plus at least 2 of : b) Perceptual disturbance; incoherent speech; insomnia or daytime drowsiness; increased or decreased psychomotor activity. Exclude metabolic, infectious and drug causes. Mononeuritis. Recent onset of sensorial or motor deficit in one or several cranial or peripheral nerves. Myelitis.
6	Renal disorder	Recent onset of paraplegia and/or bladder/bowel control disorder. Exclude other causes. Casts. Heme granular or RBC. Hematuria. > 5 rbc/hpf. Excluding other causes (stone, infection).
4	Vasculitis	Proteinuria. New onset.>0.5 g/l in random specimen. Creatinine increase (>5 mg/dl).
3	Hemolysis Thrombocytopenia	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter haemorrhages. Biopsy or angiogram data of vasculitis.
3	Myositis	Hb<12.0g/dl and corrected reticulocytes>3%.
2	Arthritis	<100.000 platelets. Not due to drugs.
2	Mucocutaneous disorder	Proximal muscle aching and weakness, associated with elevated CPK. More than 2 tender joints with swelling or effusion. Malar rash. New onset or recurrence of raised malar erythema.
2	Serositis	Mucous ulcers. New onset or recurrence of oral or nasopharyngeal ulcerations. Alopecia. Abnormal patch of diffuse loss of hair or easily falling hair. Pleurisy. Convincing history of pleuritic pain or pleural rub or pleural effusion on physical exam. Pericarditis. Convincing history of pericardial pain or audible rub.
1	Fever Fatigue	Peritonitis. Diffuse abdominal pain with rebound tenderness (exclude intra-abdominal disease).
1	Leukopenia Lymphopenia	>38^{0}C after exclusion of infection. Unexplained fatigue. WBC < 4,000/ mm^{3}, not due to drugs. Lymphocytes < 1,200/mm^{3}, not due to drugs.

BIOGRAPHY

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ACADEMIC QUALIFICATIONS:

2004	Diploma, Thai Board of Internal Medicine Royal College of Physicians of Thailand
2002	Diploma in Clinical Science (Internal Medicine) Mahidol University, Bangkok, Thailand
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