การตรวจเครื่องหมายชีวภาพในเซลล์จากปัสสาวะ เพื่อการวินิจฉัยและรักษาการกำเริบของโรคไตอักเสบลูปุสเปรียบเทียบกับการวินิจฉัยและรักษาโดยการเจาะชิ้นเนื้อไต

นางสาวธนรัตน์ ศุภศิริ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอายุรศาสตร์ ภาควิชาอายุรศาสตร์
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

Urinary Cellular Biomarkers as an Early Diagnostic Test for Treatment of Active Lupus Nephritis compare with kidney biopsy guided therapy

Miss Thanarat Supasiri

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Medicine
Department of Medicine
Faculty of Medicine
Chulalongkorn University
Academic Year 2015

Thesis Title	Urinary Cellular Biomarkers as an Early Diagnostic Test for Treatment of Active Lupus Nephritis compare with kidney biopsy guided therapy
By	Miss Thanarat Supasiri
Field of Study	Medicine
Thesis Advisor	Professor Yingyos Avihingsanon

Accepted by the Faculty of Medicine, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Medicine

(Professor Suttipong Wacharasindhu)

THESIS COMMITTEE

.....Chairman

(Professor Kammant Phanthumchinda)

.....Thesis Advisor

(Professor Yingyos Avihingsanon)

.....Examiner

(Naricha Chirakalwasan)

.....External Examiner

(Prapaporn Pisitkun)

ธนรัตน์ ศุภศิริ : การตรวจเครื่องหมายชีวภาพในเซลล์จากปัสสาวะ เพื่อการวินิจฉัยและรักษาการกำเริบของโรคไตอักเสบลูปุสเปรียบเทียบกับการวินิจฉัยและรักษาโดยการเจาะชิ้นเนื้อไต (Urinary Cellular Biomarkers as an Early Diagnostic Test for Treatment of Active Lupus Nephritis compare with kidney biopsy guided therapy) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. นพ. ยิ่งยศ อวิหิงสานนท์, 69 หน้า.

ที่มา: โรคไตอักเสบลูปัสมีความรุนแรง นำไปสู่ภาวะไตวายระยะสุดท้ายได้ การพยากรณ์โรคขึ้นกับผลลัพธ์การรักษา การเริ่มการรักษาที่เหมาะสมโดยรวดเร็ว ทำให้ผลลัพธ์การรักษาดีขึ้น

วัตถุประสงค์: เพื่อศึกษาว่าการใช้วิธีการตรวจเครื่องหมายชีวภาพในเซลล์จากปัสสาวะสามารถเริ่มให้การรักษาการกำเริบโรคไตอักเสบลูปัสได้อย่างรวดเร็ว อันนำไปสู่ผลลัพธ์การรักษาที่ดีขึ้น

วิธีการศึกษา: แบ่งการศึกษาเป็นสองกลุ่ม กลุ่มแรกเป็นการศึกษาแบบไปข้างหน้า ในผู้ป่วยโรคไตอักเสบลูปัส (N=14) โดยส่งตรวจสารชีวภาพจากเซลล์ในปัสสาวะ (Interferon Inducible Protein 10; IP-10) หากผลเป็นบวกจะเริ่มให้การรักษาด้วยยากดภูมิทันที เปรียบเทียบกับผู้ป่วยอีกกลุ่มหนึ่ง ซึ่งเป็นข้อมูลจากแฟ้มประวัติผู้ป่วย (N=26) โดยการรักษาด้วยยากดภูมิมักจะเริ่มในภายหลังจากที่เห็นผลพยาธิวิทยาชิ้นเนื้อไตแล้ว ผลการศึกษาหลักคือการเปรียบเทียบอัตราการหายของโรคระหว่างสองกลุ่ม ผลการศึกษารองคือการเปรียบเทียบปริมาณยากดภูมิคุ้มกันที่ใช้ อัตราการติดเชื้อ ผลข้างเคียงจากยา

ผลการศึกษา: ผู้ป่วยในกลุ่มที่ใช้การตรวจสารชีวภาพในปัสสาวะมีอัตราการหายของโรคสูงเป็น 1.23 เท่า (ค่าความเชื่อมั่น 95% 0.57-2.67; P=0.595) มีการใช้ยาเพรดนิโซโลนในขนาดที่น้อยกว่า (13.1±6.8 vs. 20.7±9.1 มิลลิกรัมต่อวัน P=0.010) และระยะเวลาตั้งแต่โรคกำเริบจนหายจากโรคในกลุ่มที่ใช้การตรวจสารชีวภาพในปัสสาวะ เร็วกว่าอีกกลุ่ม (ค่ามัธยฐานเวลา 16 สัปดาห์ [ค่าความเชื่อมั่น 95% 9.89-22.1 สัปดาห์] เปรียบเทียบกับ 25 สัปดาห์ [ค่าความเชื่อมั่น 95% 13.8-36.2 สัปดาห์])

สรุปผล: การตรวจสารชีวภาพ (IP-10) ในปัสสาวะ ยังไม่สามารถแสดงประสิทธิภาพในการเพิ่มอัตราการหายของโรคไตอักเสบลูปัส แต่สามารถลดระยะเวลาที่มีไตอักเสบและลดขนาดยาสเตียรอยด์ลงได้ ควรมีงานวิจัยขนาดใหญ่เพื่อยืนยันความแม่นยำต่อไป

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา อายุรศาสตร์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2558

5774031730 : MAJOR MEDICINE
KEYWORDS: LUPUS NEPHRITIS / URINE BIOMARKER / COHORT / CLINICAL VALIDATION

THANARAT SUPASIRI: Urinary Cellular Biomarkers as an Early Diagnostic Test for Treatment of Active Lupus Nephritis compare with kidney biopsy guided therapy. ADVISOR: PROF. YINGYOS AVIHINGSANON, 69 pp.

Background: Both patient and renal survival in lupus nephritis depend mainly on treatment response. Early management increase remission rate and improve renal survival.

Objective: To demonstrate the better renal remission by the early non-invasive biomarkers testing compared to the conventional approach.

Material and method: patients who met proteinuric or nephritic flare criteria were recruited. Biomarker group (N=14) used urine IP-10 to guide treatment. Induction immunosuppression was initiated without waiting for renal pathology. Whereas in the historical cohort (conventional group, N=26) induction therapy was initiated based on renal biopsy results. Primary outcome was overall renal remission. Secondary outcomes were time to remission, adverse event and immunosuppressive dosage

Results: The HR of overall renal remission in biomarker group compared to conventional group was 1.23 (95% 0.57-2.67; P=0.595). The mean steroid dosage was 13.1±6.8 vs. 20.7±9.1 P=0.015 and the median time from renal flare to renal remission was 16 weeks (95%CI 9.89-22.1 weeks) vs. 25 weeks (95% CI 13.8-36.2 weeks) in biomarker arm vs. conventional arm respectively.

Conclusion: Using urinary IP-10 as LN biomarker couldn’t show better overall renal remission, but can shorten time from renal flare to renal response and decrease net prednisolone dosage. Further larger study is needed to confirm this results.

Department: Medicine
Field of Study: Medicine
Academic Year: 2015

Student's Signature .....

Advisor's Signature .....

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor Professor Yingyos Avihingsanon, MD. He was always open whenever I had a trouble and gave me support in every aspect.

I would like to thank Miss Jariya Pongsaisophon for all the help she did for me all her best. I also would like to thank Miss Chutipha Phromjeen and all technician at the Nephrology Laboratory Unit for all the collaboration. I would also like to thank Ajarn Vasant Panyasang for all the statistic counselling sessions.

I am gratefully indebted to Ratchadapisek Sompoch Funding for the budget to run this project.

I would also like to thank all the patients for participating in this study.

I would like to thank all the thesis examination committee for sacrificing time reading my thesis paper and joining my research presentation.

Finally, I must express my very profound gratitude to my parents for providing me all the support and encouragement throughout my years of study.

CONTENTS

Page

Page

Page

Figure index

Table index

CHAPTER I

INTRODUCTION

1.1 Background and Rationale

Lupus nephritis (LN) is one of the major organ involvement in systemic lupus erythematosus (SLE)[1]. LN has high morbidity and mortality with a 26-fold excess in the risk of death than in general population[2]. Clinical manifestations include edema, foamy urine, hypertension, or oliguria[3]. The severity of disease varies from asymptomatic hematuria to rapid progressive deterioration in kidney function and end stage kidney disease. The pathology of LN is classified in to 6 classes by the International Society of Nephrology/ Renal Pathology Society (ISN/RPS). The class I pathology is minimal change disease and class II pathology is mesangial hypercellularity which need no definite immunosuppressive course and the main treatment depends on other major organ involvement. The class V pathology is membranous LN which manifests as heavy proteinuria without a significant decline in GFR. The class VI pathology consists of > 90% fibrosis. The class III pathology is focal proliferative pattern and class IV pathology is diffuse proliferative pattern which are considered as severe pathology and need aggressive immunosuppressive course[4].

Both patient survival and renal survival in proliferative lupus, the class III and class IV pathology, depend mainly on treatment response[5]. Achieving complete remission results in the best prognosis with 10 year patient survival of 95%, 10 year renal survival of 94%, and survival without end-stage renal disease of 92%. While, achieving partial remission results in 10 year patient survival of 76%, 10 year renal survival of 45%, and survival without end-stage renal disease of 43%. Eventually, without remission result in the worst prognosis with 10 year patient survival of 46%, 10 year renal survival of 19%, and survival without end-stage renal disease of 13%.

Early detection and prompt treatment can improve rate of renal remission and improve renal survival[6]. Kidney biopsy is the gold standard for diagnosis of LN flare. The risk of this procedure includes hemorrhage (either hematuria, or sub-capsular hematoma), perinephric infection, or arterio-venous fistula, which could lead to morbidity and mortality. Physician usually perform this invasive procedure when overt clinical flare-up appears, thus delay in proper management might occur. Furthermore, the intrarenal inflammation is proof to proceed the clinical flare which can be observed by the visualizing of electron dense deposit material by electron microscopy[7]. This leads to the encouragement to discover the laboratory marker with the capability to predict the pathological flare in order to early detect pathological flare and to guide appropriate aggressive management[6]. This study is aimed to prove the hypothesis that biomarker guiding lupus nephritis management will early detect lupus nephritis flare, and ensure physician to early perform kidney biopsy or early initiate immunosuppressive medication. This strategy will promptly terminate the ongoing renal inflammation and result in better renal outcome.

1.2 Research Question

1.2.1 Primary question

Whether the use of urinary biomarkers could initiate early immunosuppressive treatment and provide the better overall renal remission compared to the conventional kidney biopsy guided approach in LN patient.

1.2.2 Secondary question

Whether urine biomarkers approach could decrease overall immunosuppressive dosage and decrease incidence rate of adverse infection events, could prevent disease progression to rapidly progressive glomerulonephritis (RPGN), and shortening time from renal flare to renal response compared to the conventional kidney biopsy approach in LN patient.

1.3 Research Objective

1.3.1 To demonstrate the better overall renal remission of treatment approach by the early non-invasive biomarkers testing compared to the conventional approach.

1.3.2 To demonstrate that the non-invasive biomarkers guided therapy, compared to the kidney biopsy guided treatment can decrease overall immunosuppressive dosage, decrease incidence of adverse event from immunosuppression, decrease progressive disease to RPGN, and shortening time from clinical flare to renal response.

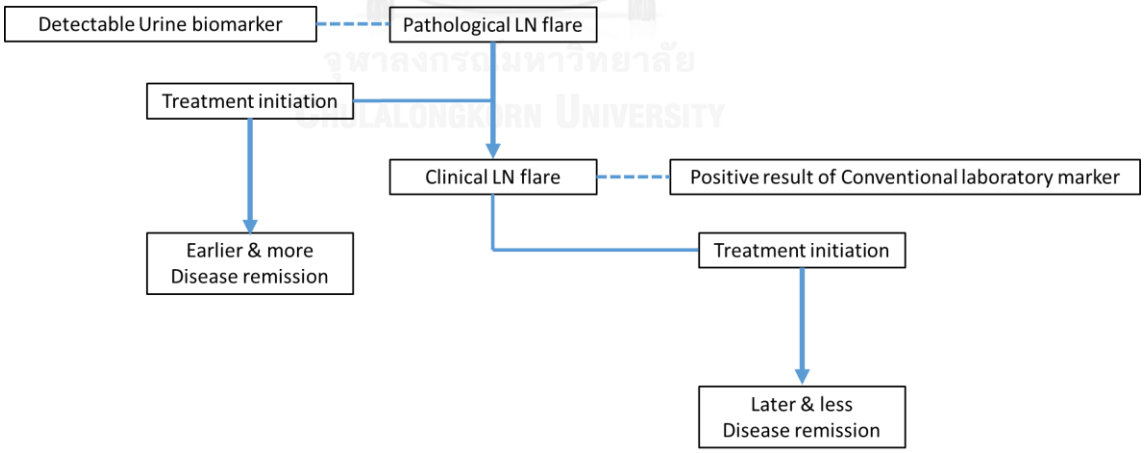
1.4 Research Hypothesis

1.4.1 The urine biomarkers can lead to an initiation of early treatment. This approach should improve overall renal remission, reduce the severely active events, and shortening the time from clinical flare to treatment response.

1.4.2 An early treatment may not require high dosages of immunosuppression as compared to the conventional approach. Therefore, this may lead to lesser infections and other related adverse events.

1.5 Conceptual Framework

Figure 1 Shows conceptual framework



1.6 Assumption

The participating patients must be fulfilled with American College of Rheumatology (ACR) criteria for SLE diagnosis with laboratory evidence of renal involvement and must not have rapidly progressive glomerulonephritis or must not match resistant criteria.

1.7 Operational Definition

Lupus nephritis is a renal involvement in SLE patient.

Urine biomarker is a substance detected in urine which correlated with disease activity.

Kidney biopsy is a procedure attempt to obtain kidney tissue in order to inspection the histopathology of the tissue.

Induction immunosuppression is the treatment given to active LN patient in order to cease inflammation process and induce renal remission.

Complete remission define as return of serum creatinine to previous baseline plus decline in the UPCI to < 500 mg/g (< 50 mg/mmol).

Partial remission define as Stabilization, or Improvement of serum creatinine, but not to normal plus a ≥ 50% decrease in UPCI. If there was nephrotic-range proteinuria Improvement requires a ≥ 50% reduction in UPCI, and a UPCI < 3000 mg/g (<300 mg/mmol)

Deterioration defined as a sustained 25% increase in serum creatinine. This definition is widely used but has not been validated.

Overall renal remission is either complete remission or partial remission is achieved.

1.8 Study Design

Biomarker group is a prospective cohort study. Conventional kidney biopsy group is a historical cohort data.

1.9 Concise Method

In biomarker group, after screening with inclusion and exclusion criteria, patients will be measured for urine biomarker, Interferon inducible protein 10 (IP-10). With positive result patients will be promptly initiated with induction immunosuppression prior to kidney pathology result, then routine standard of care with kidney biopsy will be schedule. Treatment will be adjusted according to kidney pathology. The other arm is historical data from lupus clinic of nephrology unit, the King Chulalongkorn Memorial Hospital. In his arm patients with active LN were usually initiated induction

immunosuppression after the pathology result obtained. This process usually took several weeks to months. Overall renal remission, either complete or partial remission, will be recorded and analyzed as primary outcome. Secondary outcome is adverse event rate, time from renal flare to response, and overall immunosuppressive dosage.

1.10 Ethical Consideration

1.10.1 Respect for person

Protocol detail will be reviewed and approved by the IRB and/or EC of the faculty of medicine, the King Chulalongkorn Memorial hospital.

Information about the purpose, the risk and benefit of this study will be informed to every patient. Patient can decide whether or not to join this study. Informed consent will be obtained prior to the initiation of study and a copy will be given to the patient. All questions from the patient will be answered. All patient data will be concealed, except for the investigator team.

1.10.2. Beneficence

This study will utilize urine biomarker to early diagnosis active lupus nephritis and early management. Patients who have contraindication or unwilling to perform kidney biopsy will benefit from this diagnostic tool, without under-standardised management, since all patients will be informed risk and benefit of kidney biopsy procedure. Patient could be withdrawn from study participation any time with any reason without affecting their treatment. In the other way, investigator or IRB/EC could terminate study anytime if safety issues is concerned.

1.10.3 Justice

Investigators in this study have license from Good Clinical Practice guidelines (GCP). The principles of the Declaration of Helsinki, October 2000 and the ICH Harmonized Tripartite Guidelines for Good clinical Practice, May 1997 will be followed. Patients matched with inclusion criteria have right to choose joining this trial.

1.11 Limitation

1.11.1 This study is not a randomized controlled trial (RCT). There might be an unequal baseline patient characteristic between groups.

1.11.2 Relatively short duration of study period, only small number of patients will be recruited, thus resulting in limit study power.

1.12 Expected Benefit and Application

Kidney biopsy is an invasive procedure that could lead to for example, hemorrhage (either intra-urinary tract or extra-capsule) that could lead to nephrectomy or death, peri-nephric collection with or without infection, intra-renal arteriovenous fistula. Risk increased with person with bleeding diathesis, obese or hypertension. In clinical practice, physician usually decides to do this procedure when overt flare occurred, that the biopsy result could change the management. With this reason, prolonged duration from onset of clinical flare to definite immunosuppressive management is usually occurred. In this study, the inclusion criteria with proteinuria of > 1 g/d in the patient with baseline urine protein of < 0.5 g/d can detect early cases with LN flare. Together with positive urine cytokine, indicating proliferative kidney pathology, wound ensure the physician decision to perform the early kidney biopsy. Furthermore, in patient with contraindication to undergone kidney biopsy or patients who deny the procedure could also have benefit from this biomarker testing. Since this test has good sensitivity and specificity to diagnose LN class IV pathology. Therefore, this non-invasive urine biomarker testing could be a good alternative way guiding early treatment.

1.13 Obstacles and Strategies to Solve Problem

1.13.1 In case there is discordance between the kidney biopsy result and the positive cytokine which indicating active proliferation LN, the management will be adjusted according to the gold standard kidney biopsy result. And this false positive of the cytokine will be reported.

1.13.2 If the clinical course of the patient get worse, rescue therapy will be given, and the patient will be strongly encouraged to undergone kidney biopsy if he or she previously denied the procedure.

CHAPTER II

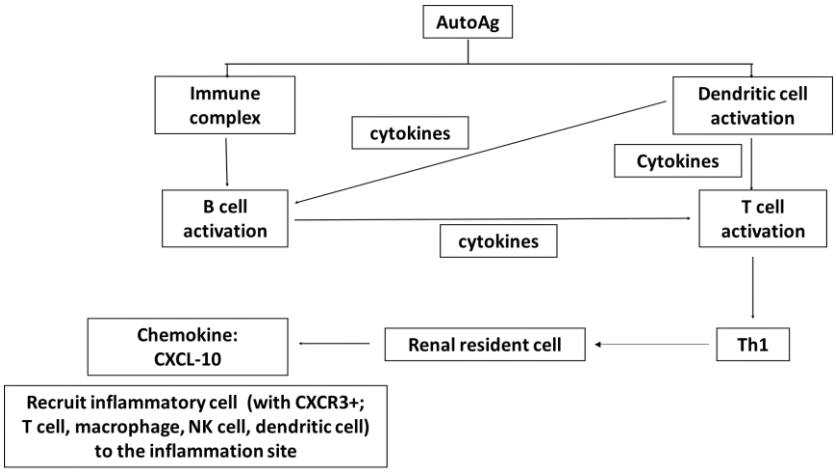
LITRATURE REVIEW

2.1 Pathogenesis of Lupus Nephritis

The pathogenesis of lupus nephritis starts from the abnormal apoptosis which leading to the production of auto-antibodies, for example anti ds DNA, anti-histone, anti C1q, anti-nucleosome antibodies etc. form an immune complex with self-antigen within renal tissue or form a circulating immune complex and deposit within renal tissue. These immune complex is not only activate the complement system, but also lead to the activation of dendritic cell, myeloid cell, renal cells including mesangial cells, endothelial cells, and podocytes. The activated dendritic cell and monocyte produce cytokines, for example IL12, IL4, IL23, TGF-B, to activate T cell to differentiate into Th1, Th2, Th17 and Treg, respectivel[8]. The predominant Th1 is associated with WHO LN class IV[9]. The activated dendritic cell and monocyte also produce cytokines Blys or BAFF to activate B cell into plasma cell and further enhance the production of auto-antibodies[10]. The activated dendritic cell also produces cytokines for example TWEAK to activate renal cell such as mesangial cell, epithelial cell to produce chemokine (chemokine is a chemotactic-cytokine, to recruit inflammatory cell to the inflammatory site) for example, IP-10 MCP1, RANTES etc. to enhance further inflammatory process in renal tissue[11]. The schematic diagram of LN pathogenesis is shown in figure 2

.

Figure 2 Shows schematic diagram of LN pathogenesis



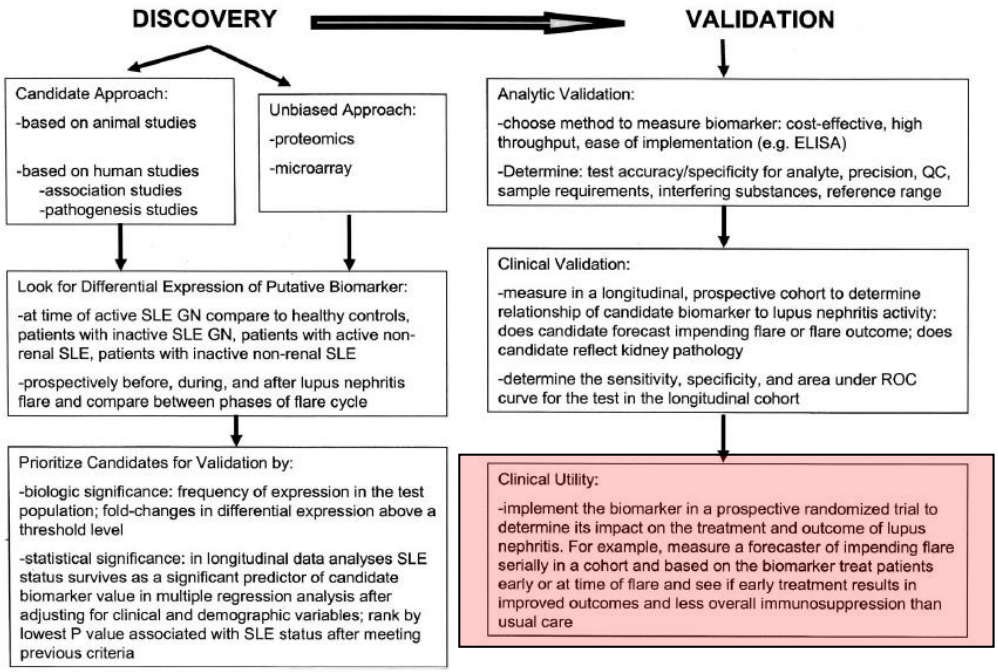
2.2 Novel Biomarker Developmental Process

The biomarker science is developed in order to early detect the ongoing intra-renal inflammation before clinically flare-up appears. As previously reported, urine biomarkers are capable to predict active LN before laboratory flare up, with some markers found to be novel in predicting LN class pathology[12].

The developmental process of biomarker starts from the discovery of the protein or RNA transcript which found to be associated, either up-regulation or down-regulation, with disease activity, as shown in figure3. After prioritized the candidate for validation by either biologic significant or statistical significant, next process is to do the clinical validation.

Figure 3 Shows an algorithm to discovery biomarker and clinical validation in lupus nephritis,

[(Figure reproduced from Brad H. Rovin 2009[13]]



Conventional biomarker for lupus nephritis, for example anti dsDNA, has the capability to distinguish lupus nephritis from other glomerulonephritis but without the capability to distinguish lupus nephritis histopathological classes[14, 15].

Since treatment strategies is emphasized mainly on proliferative pathological pattern[16], biomarker to differentiate LN class pathology are the most benefit in guiding management. While serum cytokine/chemokine level reflects systemic lupus activity rather than intra-renal inflammation, urine cytokine/chemokine level directly reflects intra-renal pathology. There are many techniques to detect urine cytokine level, for example urine protein detection with ELISA technique, urine cellular mRNA transcripts detection with real time PCR. Urine protein detection such as by ELISA technique, is interfered by filtrated plasma protein, and protein level could rapidly decline after collection without immediate freezing the sample[17]. On the other hand, cytokine RNA transcripts extracted from the shedding renal cells directly reflect an ongoing intra-renal inflammatory process without being interfered with those factors[18]. So in this

study, in order to detect early proliferative renal pathology, we choose to measure urinary cellular RNA biomarker to early predict active class IV lupus nephritis.

2.3 Upcoming Novel Biomarker for Lupus nephritis

The current knowledge of LN pathogenesis could be applied to categorize biomarker into those with cytokine, chemokine, cellular adhesion properties and other biomarker that reflect and ongoing intra-renal injury[19-21]. To choose the most appropriate biomarker for this study, we review the articles of lupus nephritis biomarkers according to the pathogenesis, including these followings.

2.3.1 Cellular adhesion molecule; Vascular cell adhesion molecule 1 (VCAM1) and Intercellular adhesion molecule 1 (ICAM1)

VCAM1 is a ligand for integrin act as an adhesion molecule of leukocyte to endothelial cell.

Ikeda et al. measured plasma level of soluble VCAM1 and ICAM1 and found that the serum level of VCAM1 were significantly higher in patients with active LN WHO class III and IV than in patients with inactive LN class I and II[22]. Another finding from this study was that serum ICAM1 was higher in patients with active lupus nephritis WHO class III and IV but there was no correlation with SLEDAI score. Wu et al. studied SLE patient vs normal control and found that urine VCAM1 level was significantly higher in SLE compare to normal controls. And they also found a correlation between urine VCAM1 active renal lupus and found a high AUC of ROC curve (0.91-0.93) of urine VCAM1 to predict active renal disease[23]. A further study with longitudinal data and class pathology correlation is needed for clinical application.

2.3.2 Neutrophil gelatinase-associated lipocalin (NGAL) is a glycosylated protein produced by many tissues including renal tubular cell. It is upregulated during renal injury, ischemia or inflammation. Pitashny et al. conducted a cross sectional study and found that urine NGAL was significantly higher in lupus nephritis compare with SLE without LN or healthy control[24, 25]. Torres et al. found that fractional excretion of urine NGAL/urine protein correlated with active lupus nephritis and can predict treatment

response but quite comparable sensitivity and specificity to the conventional C3 and anti dsDNA[25].

2.3.3 Cytokine and chemokine biomarkers

2.3.3.1 Forkhead box P3 (FOXP3) is a transcription factor involving in the developmental process of regulatory T cell (Treg). Treg is act like an inhibitor of the inflammatory process[26]. The suppressed level of Treg is associated with active SLE[27]. Wang et al. found that FOXP3 mRNA in urinary sediment in 25 subjects with active LN correlated with histological activity index (r=0.541; P=0.009). This could be explained by the hypothesis that in parallel with inflammation, the counter balance system of the inhibition pathway is also activated, but the inhibitory function of Treg might be defective. So, a further study to confirm this hypothesis and a further study with sensitivity, specificity and a postulated cutoff value is required before the clinical practice could be applied.

2.3.3.2 B lymphocyte stimulator protein (Blys) or B cell activating factor (BAFF) is a cytokine of TNF family produced by myeloid or dendritic cells to differentiate B cell into plasma cell. Stohl et al. studied 68 SLE patients follow up for 369 days, they failed to find the correlation between serum Blys level and SLE disease activity, only significant finding was that serum Blys correlated with anti dsDNA level[28]. This could be explained by the lack of pro-inflmmatory property of Blys. So there is no data support the use of Blys as renal class pathology prediction.

2.3.3.3 A proliferation inducing ligand (APRIL) is a chemokine with the function to proliferate B cell. Treamtrakanpon conducted a study in 47 SLE patients and found that blood APRIL level correlated with renal histopathological activity with Rs = 0.34 and intra-renal mRNA expression of APRIL was correlated with resistant LN. But there are some conflicting data, for example, a study of Vincent et al. studied 98 SLE patients on longitudinal 245 samples and found that serum APRIL was decreased in patients with renal lupus. And there was no cross-sectional correlation between APRIL and SLE

disease activity (30). This data couldn't support the role of APRIL to apply to clinical practice of LN class pathology prediction.

2.3.3.4 Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is cytokine produced by myeloid or dendritic cells in order to activate the renal mesenchymall cell to express the inflammatory mediators such as MCP1, RANTES, IP10, ICAM, and VCAM. Schwartz et al found that urine TWEAK levels rose up during renal flare and associated with renal disease activity score[29], but no data found uTWEAK to be correlated with renal histopathology.

2.3.3.5 A Regulated upon activation, normal T cell expressed (RANTES) is a chemokine attract leukocyte especially monocyte to the inflammation site. Chan studied 128 chinese SLE patients and found that urine RANTES mRNA expression had the AUC of ROC curve was 0.4 to predict renal flare, and found poor correlation between urine RANTES and renal pathological activity score[30]. So, this biomarker does not suit our goal in differentiating LN class pathology.

2.3.3.6 Monocyte chemoattractant protein 1 (MCP-1) belongs to C-C chemokine family. Its function is a potent chemotactic factor for monocyte[31]. Chan et al. conducted a study in 106 Chinese SLE patients and found that urinary mRNA MCP1 expression correlated with SLE clinical activity and also correlated with renal histologic activity index [32]. Study from Rovin et al. found that urine MCP1 level measured by ELISA technique rose up 4 months before renal flare and was associated with proliferative LN WHO class III and IV. Alharazy et al postulated the AUC of ROC curve of urine MCP1 for lupus activity, defined by clinical parameters, for example, serum albumin, serum creatinine, complement levels etc. was 0.87, compare to 0.89 of proteinuria[32]. The cutoff value of 3,594 pg/mg Cr gave the sensitivity of 0.09 and specificity of 0.79. The limitation of this study is that they couldn't find the correlation between urine MCP1 level and histo-pathological class due to time lag between kidney biopsy and urine sample collection. So, another biomarker with consistent data on LN class pathology prediction is required for clinical application.

2.2.3.7 Interferon inducible protein-10 (IP-10) is a chemokine in a family of C-X-C motif, function as a Th1 chemo-attractant, expressing by many cell

types, i.e. endothelial cell, fibroblast, or monocyte, in response to interferon gamma. This protein is found in many Th1 mediated inflammatory process, including LN[33]. Bauer conducted a study in 267 SLE patients follow up for 1 year with a total visit of 1166. Serum level of interferon inducible chemokine was measured by chemiluminescent sanchwich-based immunoassay. A sum score of chemokine were calculated and found the association between serum IP-10 and SLE disease activity, lower complement and higher anti dsDNA. And in longitudinal analysis, serum chemokine rose up when clinically flare-up appears and decreased when response to treatment obtained.

Previous study of Avihingsanon et al. showed that urine IP-10 can accurately predict LN class IV pathology and also predict response to treatment(14) with AUC of ROC 0.89, sensitivity of 73, and specificity of 94 [12]. Also, urine sediment mRNA of IP-10 was found to be higher 2 weeks before renal flare and declined after response to treatment. From these knowledge, measuring urine cellular mRNA could help predicting active LN class IV and lead to early appropriate management including kidney biopsy and induction with immunosuppressive medication.

In this study we choose urine mRNA Interferon inducible protein-10 (IP-10) as a biomarker to detect class IV lupus nephritis. From figure1, the process of novel biomarker development, starting from the discovery, IP-10 has biological significant to differentiate proliferative lupus class IV from other active lupus class pathology with great sensitivity and specificity. In the clinical validation phase, we choose urine cellular mRNA level because there are abundant of data supporting that with this technique will be less interfered with filtered serum protein and best reflect intra-renal pathology. And there are longitudinal data finding the good correlation between treatment response and IP-10 level, with the provision of sensitivity, specificity and also the cutoff value to apply to the clinical practice. The next step, as we highlight the box in the figure 1, is to provide clinical utility. So, this study is designed to implement the biomarker in a prospective trial to prove the hypothesis that with biomarker guided therapy will early diagnose active proliferative LN class IV which lead to early induction

treatment and result in better response rate and decrease the immunosuppressive dosage.

CHAPTER III

METHODOLOGY

3.1 Study design

There are two arms in this study. The biomarker arm is a prospective study and the conventional arm (kidney biopsy guided treatment group) is a historical cohort. Each arm method will be described separately.

3.2 Population and samples

3.2.1 Target population: lupus nephritis patients in Thailand

Sample population: Both arms in this study are LN patients who following up at the lupus clinic of nephrology unit, The King Chulalongkorn memorial hospital

3.2.2 Biomarker group (prospective cohort)

3.2.2.1 Inclusion criteria

3.2.2.1.1 Age 18-60 year

3.2.2.1.2 Willing to provide informed consent and to comply with the schedule of protocol requirement

3.2.2.1.3 Diagnosis of SLE by 4/11 of SLICC or ACR criteria

3.2.2.1.4 Active lupus nephritis defined by either nephritic flare or proteinuric flare

3.2.2.1.4.1 Proteinuric flare:

3.2.2.1.4.1.1 24h-urine protein > 1 g/d or UPCI > 1 if baseline 24h-urine protein < 0.5 g/d or UPICI < 0.5 OR

3.2.2.1.4.1.2 Doubling urine protein if 24h-urine protein > 1 g/d or UPCI > 1

3.2.2.1.4.2 Nephritic flare:

3.2.2.1.4.2.1 present of red blood cell cast OR

3.2.2.1.4.2.2 urine RBC > 5 cells/HPF or urine WBC > 5 cells/HPF

3.2.2.2 _Exclusion criteria

3.2.2.2.1 Relates to SLE

3.2.2.2.1.1 RPGN

3.2.2.2.1.2 Baseline serum Cr > 2 mg/Dl

3.2.2.2.1.3 Resistant case; defined by high dose/induction immunosuppressive drug currently being prescribed

3.2.2.2.1.3.1 Previous treatment with IVCY in the previous 3 months

3.2.2.2.1.3.2 Previous treatment with oral CY > 50 mg/d in the previous 3 months

3.2.2.2.1.3.3 Previous treatment with MMF > 1 g/d in the previous 3 months

3.2.2.2.1.3.4 Previous treatment with steroid equivalent to prednisolone > 20 mg/d in the previous 3 months

3.2.2.2.2 Relates to general health

3.2.2.2.2.1 pregnancy or breast feeding mothers

3.2.2.2.2.2 evidence of significant uncontrolled concomitant disease in any organ system not related to SLE

3.2.2.2.2.3 active HIV infection

3.2.2.2.2.4 active malignancy

3.2.2.2.2.5 active uncontrolled serious infection

3.2.2.2.2.6 evidence of current abuse of drugs or alcohol

3.2.2.2.3 Relates to laboratory findings

3.2.2.2.3.1 neutrophil < 1,000 /mm3, Hb < 7 g/L, platelet < 20,000 /mm3

3.2.3 Conventional group (historical cohort)

3.2.3.1 Inclusion criteria

3.2.3.1.1 Age 18-60 year

3.2.3.1.2 Willing to provide informed consent and to comply with the schedule of protocol requirement

3.2.3.1.3 Diagnosis of SLE by 4/11 of SLICC or ACR criteria

3.2.3.1.4 Active lupus nephritis defined by either nephritic flare or proteinuric flare

3.2.3.1.4.1 Proteinuric flare:

3.2.3.1.4.1.1 24h-urine protein > 1 g/d or UPCI > 1 if baseline 24h-urine protein < 0.5 g/d or UPICI < 0.5 OR

3.2.3.1.4.1.2 Doubling urine protein if 24h-urine protein > 1 g/d or UPCI > 1

3.2.3.1.4.2 Nephritic flare:

3.2.3.1.4.2.1 present of red blood cell cast OR

3.2.3.1.4.2.2 urine RBC > 5 cells/HPF or urine WBC > 5 cells/HPF

3.2.3.2 _Exclusion criteria

3.2.3.2.1 Relates to SLE

3.2.3.2.1.1 RPGN

3.2.3.2.1.2 Baseline serum Cr > 2 mg/Dl

3.2.3.2.1.3 Resistant case; defined by high dose/induction immunosuppressive drug currently being prescribed

3.2.3.2.1.3.1 Previous treatment with IVCY in the previous 3 months

3.2.3.2.1.3.2 Previous treatment with oral CY > 50 mg/d in the previous 3 months

3.2.3.2.1.3.3 Previous treatment with MMF > 1 g/d in the previous 3 months

3.2.3.2.1.3.4 Previous treatment with steroid equivalent to prednisolone > 20 mg/d in the previous 3 months

3.2.3.2.2 Relates to general health

3.2.3.2.2.1 pregnancy or breast feeding mothers

3.2.3.2.2.2 evidence of significant uncontrolled concomitant disease in any organ system not related to SLE

3.2.3.2.2.3 active HIV infection

3.2.3.2.2.4 active malignancy

3.2.3.2.2.5 active uncontrolled serious infection

3.2.3.2.2.6 evidence of current abuse of drugs or alcohol

3.2.3.2.3 Relates to laboratory findings

3.2.3.2.3.1 neutrophil < 1,000 /mm3, Hb < 7 g/L, platelet < 20,000 /mm3

3.2.3.2.4 According to kidney pathology

3.2.3.2.4.1 No kidney biopsy results or kidney biopsy other than class 3 or class 4 or class 3+5 or class 4+5; active or active/chronic pattern

3.3 Sample Size

Define at alpha 5%, beta 80%, success rate in control 50%, and success rate in experimental group 95%

$$N= [Z_{\alpha}\sqrt{2P/(1-P)}+Z_{\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}]^2/ (P_1-P_2)^2$$

N= 14 per arm

Z_{α} = alpha 0.05

Z_{β} = beta 0.2

P_1 = success rate in control group (0.5)

P_2 = success rate in experimental group (0.95)

P = mean of success rate between group

3.4 Methods

3.4.1 Biomarker arm

3.4.1.1 SLE patients following up at the lupus clinic of nephrology unit, the King Chulalongkorn Memorial Hospital, during January 2015 to June 2015 with clinically active LN will be asked to join At “screening visit” Inclusion criteria will be reviewed. Compatible cases will be asked for informed consent. Detail process are as this following

3.4.1.1.1 Screen patient according to inclusion criteria

3.4.1.1.2 Ask for informed consent

3.4.1.1.3 Send investigation according to “screening visit” CRF as shown in appendix 1.

3.4.1.1.4 Medication part of this “screening visit” form is the baseline current treatment of patient

3.4.1.1.5 Urine cytokine (IP-10) will be sent to the Lupus laboratory center for measurement

3.4.1.1.5.1 Fifty milliliters of urine sample will be collected for cytokine analysis using mid-stream clean catch technique.

Method of running cytokine analysis is as this followings Urine sample collection was performed with mid-stream clean catch technique. Urine was immediately centrifuged after collection at 1000g for 30 min at 4 C. Total RNA was isolated from the cell pellets using an RNA blood mini kit (Qiagen, Chatworth, CA), measured for concentration and reverse-transcribed into complementary DNA, then real time PCR technique will be performed in order to measure the mRNA copies. The mRNA levels of IP-10 and the housekeeping gene, 18s rRNA, were measured by a Light Cycler machine (Roche Molecular Biochemicals, Indianapolis, IN).

3.4.1.1.5.2 Schedule for induction visit, should be within 1 week

3.4.1.1.5.3 Steroid dosage can be titrated up according to physician preferences and the baseline dosage must be documented

3.4.1.2 One week after screening visit is “Induction visit”, all laboratory investigation results will be obtained. The CRF of “induction visit” is shown in appendix 2. Details of the process are this followings

3.4.1.2.1 Check exclusion criteria

3.4.1.2.1.1 If all reveal negative, then start induction treatment

3.4.1.2.1.2 If HBV or HCV infection reveal positive

3.4.1.2.1.2.1 Active infection; exclude from study

3.4.1.2.1.2.2 Not active status; schedule GI clinic and induction treatment can be initiated

3.4.1.2.1.3 Active pulmonary infection, parasitic infection, patient is excluded from study

3.4.1.3 Patients who pass the exclusion criteria will be recruited in this study and treated according to study protocol

3.4.1.3.1 Patient with positive urine IP-10 with level of > 2.09 copies/ug RNA will be initiated induction immunosuppression in this visit. This followings are the protocol

3.4.1.3.1.1 Start induction treatment with one of this following two regimen

3.4.1.3.1.1.1 Mycophenolate mofetil:

3.4.1.3.1.1.1.1 BW < 50 kg; 1.5 g/d

3.4.1.3.1.1.1.2 BW > 50 kg; 2 g/d

In case the therapeutic trough level couldn't be achieved by the dosage of 3 g/d, and without improving of clinical, switching to intravenous cyclophosphamide is allowed. Follow up plan for biomarker arm are shown in table 1 (eurolupus regimen) and table 2 (MMF regimen)

3.4.1.3.1.1.2 Eurolupus: IVCY 500 mg IV q 2 week

3.4.1.3.1.2 Increase prednisolone dosage to 0.5 mg/kg/d and reduce by 5 mg/day every 2 weeks for two times then every 4 weeks until dosage of 5 mg/day was reached. More rapid decline of prednisolone dosage can be performed if the patient has adverse effect from this medication, for example: infection, avascular necrosis, osteoporosis/osteopenia, cushingoid appearance, according to physician's judge.

3.4.1.3.1.3 Start hydroxychloroquine (200) 1 tab OD and schedule for retina examination.

3.4.1.3.1.4 Start infection prophylaxis with

3.4.1.3.1.4.1 Bactrim (80/400) 2 tab OD

3.4.1.3.1.4.2 Acyclovir (200) 1 tab bid

3.4.1.3.1.4.3 Ivermectin (6) 2 tab OD for 2 days and repeat dose next 2 week

3.4.1.3.1.5 If patient has previous history of peptic ulcer, then prescribe omeprazole (20) 1 tab OD

3.4.1.3.1.6 Start ACEI/ARB will be prescribed and adjusted to the maximal tolerated dosage, determined by

3.4.1.3.1.6.1 keeping within the upper normal limit of serum potassium level (5.0 mEq/L)

3.4.1.3.1.6.2 Keeping GFR not lower than 30% from baseline

3.4.1.3.1.6.3 Keeping blood pressure > 90/60 mmHg and without symptom of hypotension

3.4.1.3.1.7 Start vitamin D2 (20,000u) 1 tab OD

3.4.1.3.1.8 Schedule for kidney biopsy

3.4.1.3.1.8.1 If the patients willing to be performed kidney biopsy, the procedure will be scheduled.

3.4.1.3.1.8.2 If the patient has contraindication for kidney biopsy or deny the procedure, induction treatment will be prescribed according to clinical flare and positive urine cytokine.

3.4.1.3.2 Patients with negative urine IP-10 with level IP-10 ≤ 2.09 copies/ug RNA will be managed according to standard clinical practice guideline.

3.4.1.4 Following up every 2 weeks for 1 times and then every 4 weeks will be scheduled, Clinical record forms of follow up visit are shown in appendix 3 and 4. Details of study protocol are shown in appendix 5.

3.4.1.5 Response rate will be evaluated every month, and be classified as complete or partial response or resistance at month 6 (definition of responses are presented in operational definition).

3.4.1.6 Adverse event will be recorded, either from kidney biopsy complication: bleeding, from immunosuppression; infection, or from disease progression; ESRD.

3.4.1.7 Regarding the risk of infection after an initial immunosuppression, in this study we use the less potent and steroid-minimization protocol. In our database, the incidence of opportunistic infection was less than 10 percent. Furthermore, we will provide antimicrobial prophylaxis including Peumocystis Jirovecii prophylaxis (cotrimoxazole), anti-herpes virus prophylaxis (acyclovir) and anti-parasitic prophylaxis (ivermectin). Incident rate of adverse immunosuppression reaction and infection will be collected and report.

3.4.2 Conventional biomarker guided treatment group (historical cohort)

3.4.2.1 The historical cohort data will be collected from the lupus clinic, King Chulalongkorn Memorial Hospital database. Medical records of LN patients who followed up during June 2014 to December 2014 will be chosen.

Table 1 follow up plan for eurolupus (intravenous cyclophosphamide) group

Eurolupus	screening	Induction IVCY							Induction MMF		
		week							week		
		0	2	4	6	8	10	12	16	20	24
Date											
Inclusion/exclusion	x										
Consent for kidney bx	x										
Consent for this study	x										
Hx/demography	x	x	x	X		x		x	x	x	x
PE/BP/Wt&Ht	x	x	x	x		x		x	x	x	x
SLEDAI/SLICC	x	x	x	X		x		x	x	x	x
CBC	x	x	x	X		x		x	x	x	x
PT/PTT/INR	x										
BUN/Cr/Elyte	x	x	x	x		x		x	x	x	x
LFT/FPG/lipid	x										
Complement /anti dsDNA	X			x		x		x	x	x	x
ANA	x										
Urine cytokines	X	x		x		x		x	x	x	x
UA/UPCI	x	x	x	x		x		x	x	x	x
24 h urine	x										
Stool exam/agar plate culture	x										
UPT	x										
MPA level									x		
CXR	X										
IVCY eurolupus		x	x	x	x	x	x				
Start pred 0.5 MKD		x									
Oral prednisolone will be initiated at 0.5 mg/kg/day and reduced by 5 mg/day every 2 weeks for two times then every 4 weeks until dosage of 5 mg/day was reached											
MMF 1.5 g/d BW < 50 (keep C0 > 2.5 mg/mL)								x	x	x	x
MMF 2 g/d BW > 50 (keep C0 > 2.5 mg/mL)								x	x	x	x

Table 2 follow up plan for mycophenolate group

Mycophenolate	**screening**	**Induction mycophenolate**							
		week							
		0	2	4	8	12	16	20	24
Date									
Inclusion/exclusion	x								
Consent for kidney biopsy	x								
Consent for this study	x								
Hx/demography	x	x	x	X	x	x	x	x	x
PE/BP/Wt&Ht	x	x	x	x	x	x	x	x	x
SLEDAI/SLICC	x	x	x	X	x	x	x	x	x
CBC	x	x	x	X	x	x	x	x	x
PT/PTT/INR	x								
BUN/Cr/Elyte	x	x	x	x	x	x	x	x	x
LFT/FPG/lipid	x								
Complement /anti dsDNA	X			x	x	x	x	x	x
ANA	x								
Urine cytokines	X	x		x	x	x	x	x	x
UA/UPCI	x	x	x	x	x	x	x	x	x
24 h urine	x								
Stool exam/agar plate culture	x								
UPT	x								
MPA level			x						
CXR	X								
IVCY eurolupus									
Start pred 0.5MKD		x							
Oral prednisolone will be initiated at 0.5 mg/kg/day and reduced by 5 mg/day every 2 weeks for two times then every 4 weeks until dosage of 5 mg/day was reached									
MMF 1.5 g/d BW < 50 (keep C0 > 2.5 mg/mL)		x	x	x	X	x	x	x	x
MMF 2 g/d BW > 50 (keep C0 > 2.5 mg/mL)		x	x	x	x	x	x	x	x

3.4.2.2 The latest episode of flare which matched the inclusion and exclusion criteria will be chosen. There will be 26 cases in this arm to match the 1:2 ratio of sample size between both arms. Random selection technique will be used to select these 26 cases from the compatible cases.

3.4.2.3 The patient demography, laboratory data and medication will be collected in the same fashion as in biomarker group.

3.5 Statistical Analysis

3.4.3 Statistical analysis was performed using SPSS version 17.0.

3.4.4 Patient demography data was presented in mean±SD if it was continuous data and median (IQR) if it was categorical data.

3.4.5 Overall renal remission was analyzed with multivariate cox proportional hazard ratio.

3.4.6 Median time from renal flare to renal response and to immunosuppressive initiation were shown in Kaplan Meier curve.

3.4.7 Overall adverse event rates were compared between groups using chi-square test.

3.4.8 Average immunosuppressive dosages were compared between groups using paired t-test.

3.4.9 Statistical significant was considered if P value < 0.05.

CHAPTER IV

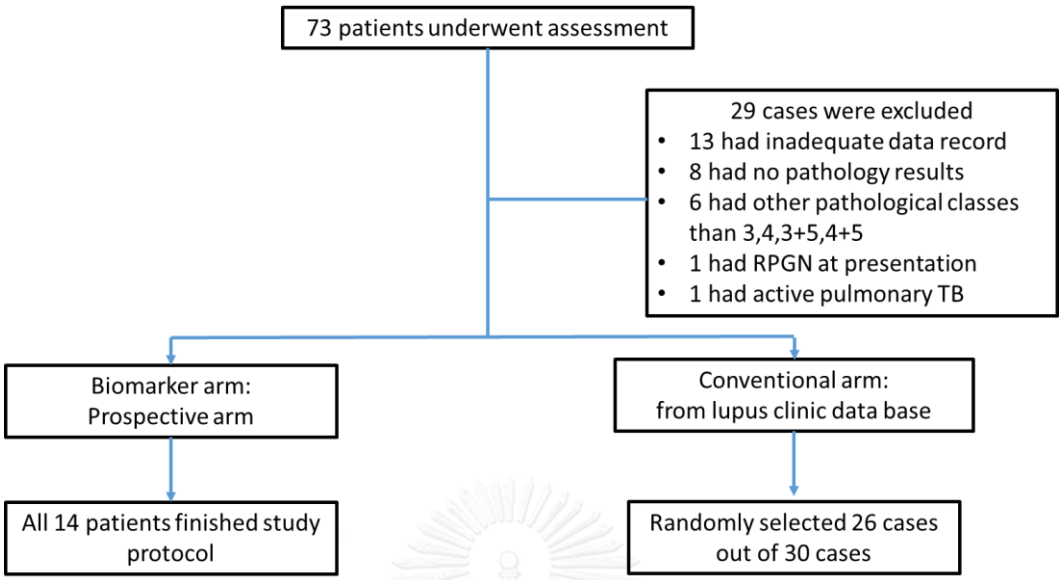
RESULTS

4.1 Enrollment Process

This study had two arms. The first arm was “the biomarker group” which the patients were prospectively followed up from the onset of LN flare. There were 14 patients who matched the inclusion criteria. None of them matched the exclusion criteria so all 14 patients were participated in this study.

The other arm was called “the conventional group” which was the historical cohort. Data in this arm was collected from the database of lupus clinic. There were 59 medical records of patients who followed up at the lupus clinic during June 2014-december 2014 that matched with inclusion criteria. The latest episode of LN flare were reviewed. Twenty-nine cases were excluded and details of exclusion are as these followings; 13 cases had in adequate data in the medical records, 8 cases without kidney biopsy and 6 cases with kidney pathology revealed other pathological classes other than class 3, 4, 3+5 or 4+5, one case had RPGN at presentation and one case had active pulmonary tuberculosis (TB). There were 30 cases left for analysis. Twenty-six cases were randomly selected by random selection technique. Overall there were 14 cases in biomarker group and 26 cases in conventional group. Flow diagram of enrollment process is shown in figure 4.

Figure 4 Shows Enrollment process



4.2 Patient Demography

Patient demography are shown in table 3. The mean age of patients in both groups were 35 and 36 year old. Thirteen out of 14 cases in biomarker group and all 26 cases in conventional group were female. Every cases were Thai race. Baseline clinical severity of LN were as these followings. There were 36% and 58% in biomarker group and conventional group with hypertension at diagnosis of clinical flare which corresponding with clinical nephritic flare. There were 42.9% vs. 88.5% of cases in biomarker group and conventional group respectively with clinical nephritic flare and this difference was statistically significant. Mean level of serum creatinine was 0.87 mg/dL and 1.02 mg/dL in biomarker and conventional group respectively. Urine protein in biomarker group was higher than in conventional group with mean value of 3.86 g/d compare to 3.44 g/d. Mean urine RBC was 12 cells/HPF in biomarker group and 28 cells/HPF in conventional group and mean urine WBC was 16 cells/HPF in biomarker group and 15 cells/HPF in conventional group and these parameters were not statistically difference. Serological parameter include complement factor 3 (C3). C3 level in biomarker group was 63.4 mg/dL vs 28.3 mg/dL in conventional group and this parameter was statistically significant. Baseline immunosuppression in biomarker group were MMF, AZA and prednisolone and baseline immunosuppression in conventional

group were MMF, AZA, prednisolone and oral cyclophosphamide. All patients in conventional group were performed kidney biopsy while 64.3% of patient in biomarker group had kidney pathology results. Majority of both group had class 4 pathology (55.6% in biomarker group and 73.1% in conventional group). Forty-four percent of cases with biopsy result in biomarker group and 65.4% of cases in conventional group had crescentic lesion.

4.3 Primary Outcome

We performed three model of analysis. The first model was univariate analysis to analyze the effect of biomarker on overall renal remission. The second model was multivariate analysis adjusted by baseline renal severity which composed of serum creatinine, urine protein and urine sediments (RBC and WBC). The third model was multivariate analysis adjusted by different induction regimen which were IVCY or MMF or tacrolimus. The hazard ratio (HR) of overall renal remission in the first model was 1.23 (95%CI 0.57-2.67; P=0.595) comparing biomarker group to conventional group. For the second model, the HR of overall renal remission was 1.75 (95%CI 0.74-4.15; P=0.204) comparing biomarker group to conventional group. For the third model, the HR of overall renal remission was 1.70 (95%CI 0.73-3.94; P=0.218) comparing biomarker group to conventional group

Table 3 Patient demography

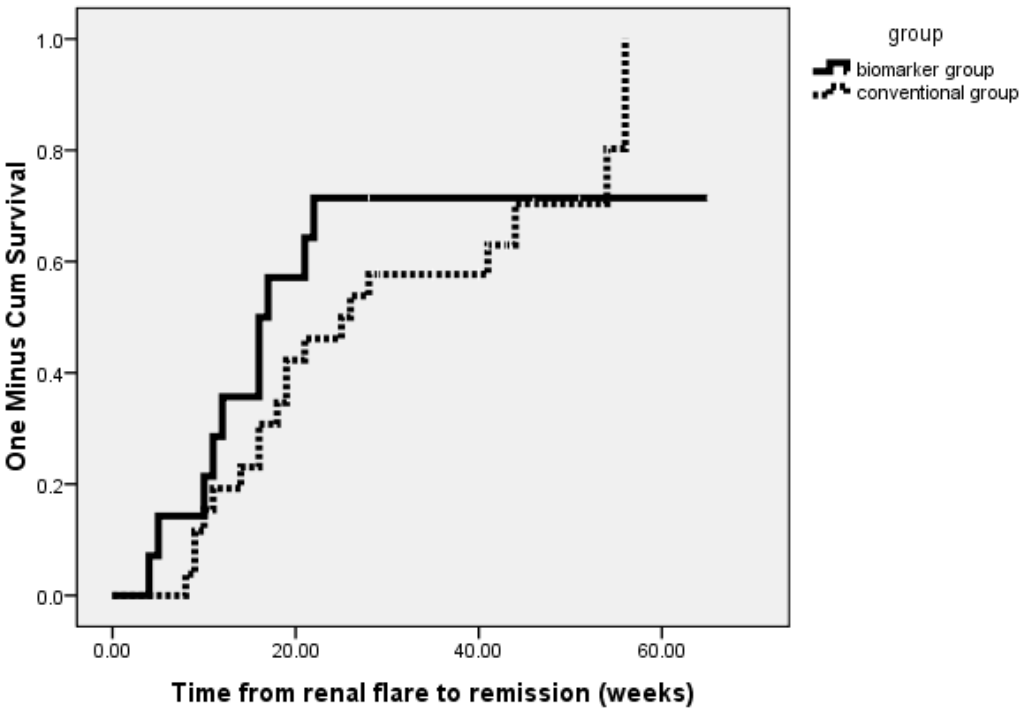
Demography	Biomarker group	Conventional group	P value
Age (years)	35±10	36±8	0.654
Sex, N (F/M)	13/1	26/0	0.350
Race (%)			
Thai	100	100	NA
others	0	0	NA
Clinical severity			
Nephritic flare	42.9	88.5	0.007
Proteinuric flare	85.7	100	0.117
Hypertension (%)	36	58	0.320
Laboratory parameters			
Serum creatinine (mg/dL)	0.8±0.39	1.02±0.27	0.240
24 h-urine protein	3.86±3.38	3.44±2.52	0.657
Serum albumin (g/dL)	3.2±0.4	3.0±0.5	0.156
C3 (mg/dL)	63.4±27.7	28.3±30.5	0.003
Urine RBC (cells/HPF)	12±15	28±41	0.172
Urine WBC (cels/HPF)	16±26	15±21	0.899
Baseline medication (%)			
MMF	21	4	0.123
AZA	29	8	0.163
pred	79	42	0.049
HCQ	43	12	0.047
POCY	0	4	1.000
Kidney pathology			
Without pathology	35.7	0	
With pathology	64.3	100	
Biopsy class (%)			
3	22.2	3.8	
4	55.6	73.1	
3+5	11.1	7.7	
4+5	11.1	15.4	
With crescent (%)	44.4	65.4	0.432

4.4 Secondary Outcomes

4.4.1 Time from renal flare to overall renal remission

Median time from renal flare to overall renal remission (either complete remission or partial remission) was 16 weeks (95%CI 9.89-22.1 weeks) in biomarker group compare to 25 weeks (95%CI 13.8-36.2 weeks). The Kaplan Meier curve of time from renal flare to overall renal remission is shown in figure 5. with P=0.589 by log rank test.

Figure 5 Shows Kaplan Meier curve of time from renal flare to overall renal remission.

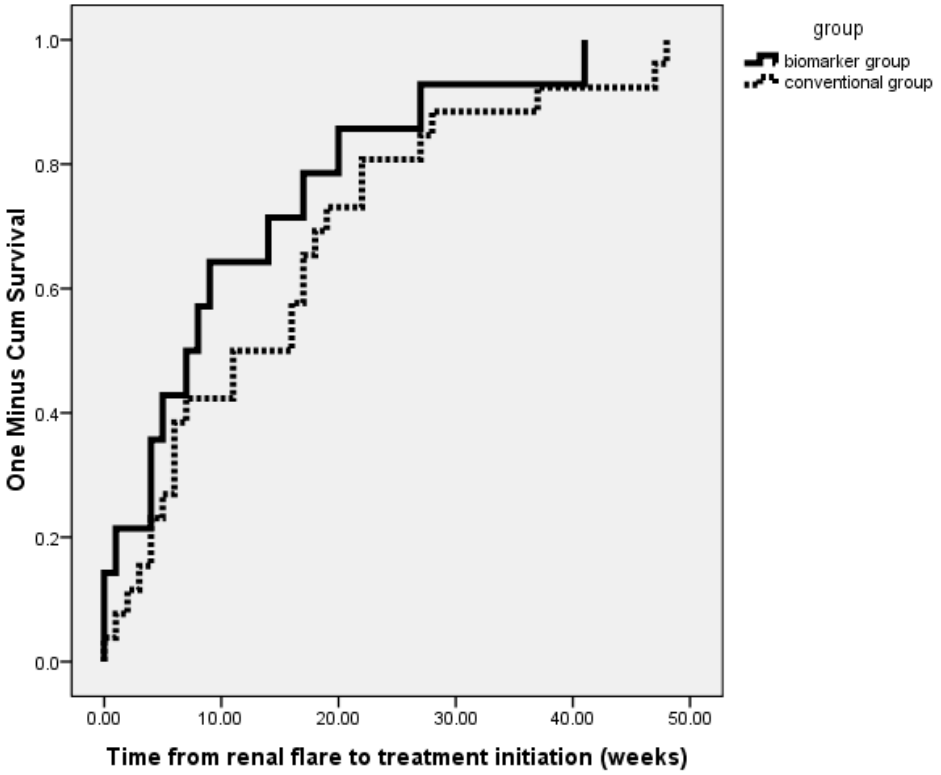


4.4.2 Time from renal flare to treatment initiation

Median time from renal flare to induction treatment was 7 weeks (95%CI 1.5-12.5 weeks) in biomarker group compare to 11 weeks (95%CI 0-22.2 weeks). The Kaplan

Meier curve of time from renal flare to induction treatment is shown in figure 6. with P=0.288 by log rank test.

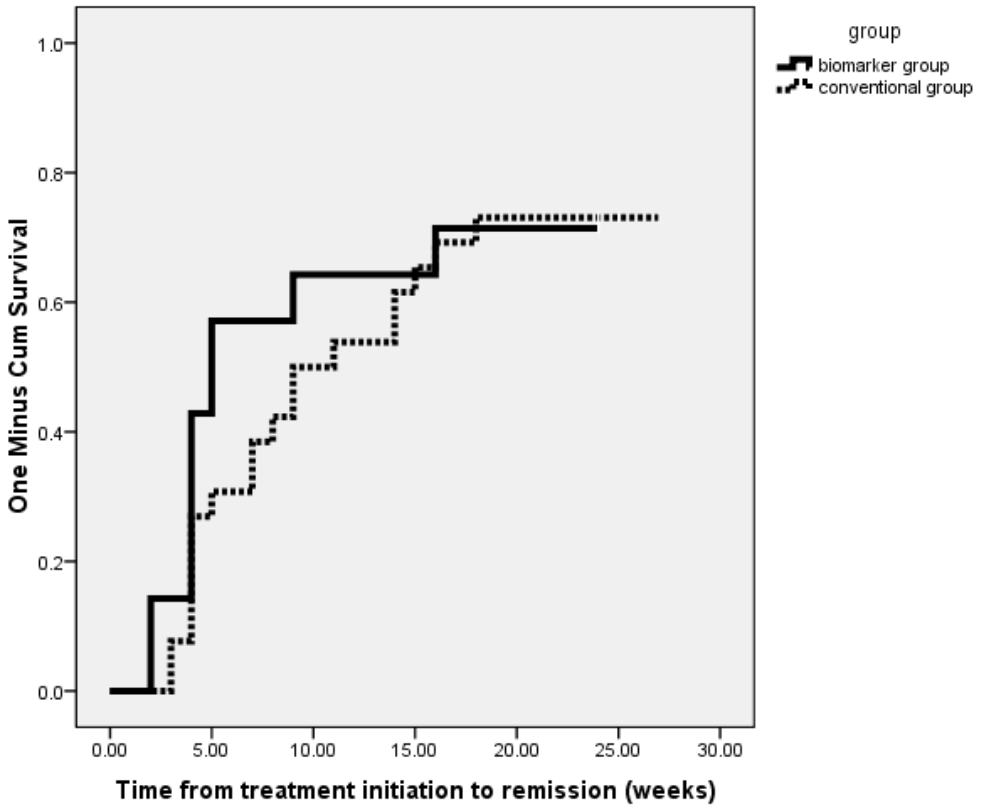
Figure 6 Shows Kaplan Meier curve of time from renal flare to induction treatment



4.4.3 Time from treatment initiation to overall renal response

Median time from induction treatment to overall renal remission (either complete remission or partial remission) was 5 weeks (95%CI 3.2-6.8 weeks) in biomarker group compare with 9 weeks (95%CI 3.0-15.0 weeks) in conventional group. The Kaplan Meier curve of time from induction treatment to overall renal remission shown in figure 7 with P=0.702 by log rank test.

Figure 7 Shows Kaplan Meier curve of time from induction treatment to overall renal remission



4.4.4 Post hoc analysis, subgroup patient with nephritis flare

In post hoc analysis, subgroup patient with nephritis flare was analyzed. Median time from induction treatment to overall renal remission (either complete remission or partial remission) was 16 weeks (95%CI 10-22 weeks) in biomarker group compare with 21 weeks (95%CI 12.8-29.2 weeks) in conventional group. The hazard ratio (HR) of overall renal remission was 1.73 (95%CI 0.62-4.85; P=0.299) comparing biomarker group to conventional group. The Kaplan Meier curve of time from renal flare to overall renal remission is shown in figure 8.

Figure 8 Shows Kaplan Meier curve of time from renal flare to overall renal remission in subgroup patient with nephritis flare

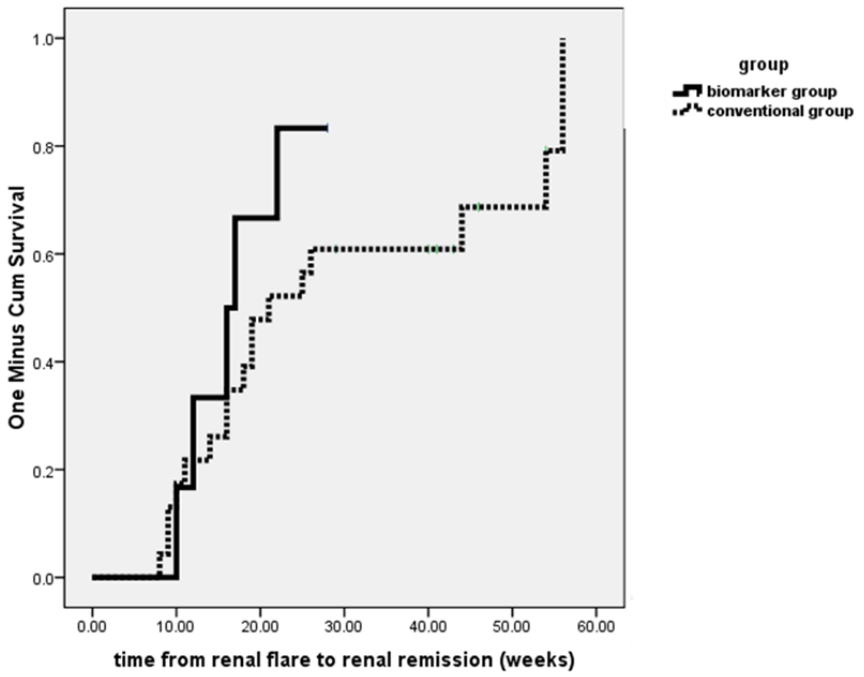
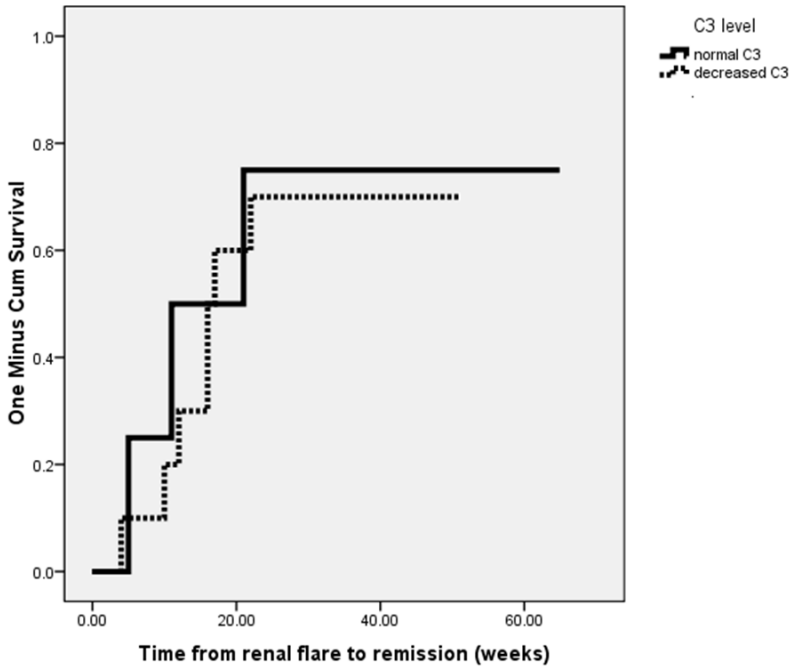


Figure 9 Shows Kaplan Meier curve of time from renal flare to overall renal remission in subgroup patient categorized by C3 level



4.4.5 Post hoc analysis, subgroup patient categorized with C3 level

In post hoc analysis, patients in biomarker group, all with positive urine IP-10, were categorized into 2 groups, subgroup with decreased C3 and normal C3 level. Median time from induction treatment to overall renal remission was 11 weeks (95%CI 0-26.7 weeks) in biomarker group compare with 16 weeks (95%CI 10.8-21.2 weeks) in conventional group. The hazard ratio (HR) of overall renal remission was 0.81 (95%CI 0.21-3.15; P=0.760) comparing biomarker group to conventional group. The Kaplan Meier curve of time from renal flare to overall renal remission is shown in figure 9.

4.4.6 Remission rate

Renal remission rate is shown in table 4. Complete remission rate in biomarker group was higher than in conventional group with the rate of 69.2% vs. 50% respectively. Non-responder rate was comparable between groups (23.1% vs, 26.9%).

Table 4 Shows renal remission rate

	Biomarker group	Conventional group	P value
Complete remission	64.3	50	0.510
Partial remission	7.1	23.1	0.387
Non responder	28.6	26.9	1.000

4.4.7 Disease progression

Some patients had disease progression despite immunosuppressive initiation as shown in table 5. There was 7.7% of patient in biomarker group and 11.5% of patient in conventional group that progressed to RPGN.

Table 5 Shows percent of patient progressed to RPGN

	Biomarker group	Conventional group	P value
Disease progression to RPGN (%)	7.1	11.5	1.000

4.4.8 Adverse events

Adverse events either from immunosuppressive side effect or infection complication following immunosuppression are shown in table 6. There was 7.7% of patients in biomarker group and 15.4% of patients in conventional group experienced side effect from immunosuppression. The adverse drug events in biomarker group was lymphopenia while in conventional group were leukopenia, bicytopenia and diarrhea. There were 15.4% of patients in both biomarker group and conventional group had infection complication from immunosuppressive state. The infection in biomarker group was herpes simplex while in conventional group were herpes simplex, herpes zoster, cellulitis and infectious diarrhea.

Table 6 Shows adverse events from treatment

Adverse events (%)	Biomarker group	Conventional group	P value
Adverse drug events	7.1	15.4	0.640
Infection complication	14.3	15.4	1.000

4.4.9 Average immunosuppressive dosage

Percent of immunosuppressive regimens are shown in table 7. Majority of cases in biomarker group received MMF as induction regimen (84.6%) while cases in conventional group received comparable percent of IVCY (42.3%) either NIH regimen (26.9%) or eurolupus regimen (15.4%) and MMF (46.2%) as induction medication.

Table 7 Shows percent of immunosuppressive regimen for induction treatment

Induction medication (%)	Biomarker group	Conventional group	P value
NIH	0	26.9	0.075
Eurolupus	21.4	15.4	0.679
MMF	78.6	46.2	0.092
TAC	0	11.5	0.539

4.4.10 Average immunosuppressive dosages

Average immunosuppressive dosages are shown in table 8. Average dosage of IVCY was 3000±0 mg/d in biomarker group which was lower than 4208±2060 mg/d in conventional group (P=0.067). average dosage of MMF was 1944±242 mg/d in biomarker group and 1692±405 mg/d in conventional group (P=0.091). Average dosage of tacrolimus in conventional group was 3.5±0.7 mg/d but none of the case in biomarker group received tacrolimus as induction medication. Average dosage of prednisolone was 13.3±7.0 mg/d in biomarker group and 20.7±9.1 mg/d in conventional group (P=0.015).

Table 8 Shows average dosage of immunosuppression

Average dosage (mean ±SD)	Biomarker group	Conventional group	P value
IVCY (mg/d)	3000±0	4208±2060	0.067
MMF (mg/d)	1944±242	1692±405	0.091
Tacrolimus (mg/d)	0	3.5±0.7	NA
Prednisolone (mg/d)	13.0±6.8	20.7±9.1	0.010

CHAPTER V

DISCUSSION CONCLUSION AND SUGGESTION

5.1 Discussion

Patient survival and renal survival in LN patient depends mainly on whether patient achieves or not achieves renal remission. Strategies to achieve this goal include early detection of renal flare and promptly initiate immunosuppression. Theoretically patients who meet the criteria of renal flare for example urine protein more than 1 g/d or urine RBC > 5 cells/HPF could be definitely diagnosed as LN flare, but in practice some patients who meet this criteria are considered to have mild clinical severity. Physicians might considered these clinical picture as having a less severe pathology for example LN class II or other classes than proliferative pathology. They might manage these cases conservatively. Therefore, depending on conventional laboratory marker alone could result in delaying appropriate management in some patients. Previous data from Ioannidis et al. showed that delaying time from renal flare to immunosuppression initiation decrease renal remission[34]. This data encourages us to find a strategy to improve early detection and management strategy. Conventional serologic marker for example anti dsDNA was studied as a marker for pathology prediction but data turned out disappointedly that there was no pathological correlation with this marker(15).

Many investigators around the world attempt to find a novel LN biomarker in many specific proposes for example biomarker to predict active renal disease, biomarker to predict renal prognosis etc. The most benefit of biomarker that we were interested in was the pathology predicting performance. The proliferative pathology has the most aggressive clinical course and need promptly aggressive immunosuppression. Of all biomarkers that we reviewed, urine IP-10 had a promising result in predicting LN class IV pathology with available data of cut-off value,

sensitivity and specificity. Then we chose to further investigate this biomarker in this prospective cohort study.

Benefit and limitation in study design and patient selection.

Every patients in this study were from the same clinic, the lupus clinic, which gave less confounder in the aspect of treatment protocol. Even though this could decrease generalizability of the result, in the manner of study design which compare prospective arm to historical cohort, data from the same clinic might decrease intervention bias.

Primary outcome

From the study results, the patient demography (age, sex, and race) between both groups were comparable. Most of them were young to middle age with female predominance and all of them were Thai. For baseline renal severity, biomarker group had milder disease severity, as observed by lower serum creatinine, lower urine protein, higher serum albumin, and less urine sediments. These unequal baseline characteristic was resulted from a non-randomized effect of study design, but could be explained by the concept of early LN flare detection in biomarker group. These finding could emphasize the benefit of using biomarker to detect case with LN flare. Concerning of this unequal clinical severity, we performed the multivariate analysis adjusted by these factors (serum creatinine, urine protein and urine sediments). The result showing that with univariate analysis overall renal remission was higher in biomarker group but not statistically significant. In multivariate analysis adjusted by baseline clinical severity, biomarker group also showing higher overall renal remission but also without statistically significant. This trend toward better outcome could be explained by the early initiation of immunosuppression, as the median time from renal flare to immunosuppression initiation is shorter in biomarker group. We also performed multivariate analysis adjusted by treatment regimen (MMF, cyclophosphamide, or tacrolimus). The majority of immunosuppressive regimen in conventional group was NIH regimen, which known to be the most potent regimen (refer to table 7), so this confounder is less likely to be the reason of worse outcome in conventional group. However, overall renal remission adjusted by these different in treatment regimen came out to be comparable to result adjusted by baseline clinical severity. There was data from secondary outcome showing that there was no different of time from

treatment initiation to renal remission, implying that the treatment regimen didn't affect the outcome.

Trend toward higher overall renal remission in biomarker group couldn't be explained by lead time bias. Since the inclusion criteria between both groups were the same, so we recorded the date of renal flare with the same definition. Prolongation of time from renal flare to renal remission truly reflected the delay in management.

There are limitation in this analysis since the power of this study at this time is only 80% and with sample size of 14 cases, the amount of factors for adjustment should be 1 to 2 factors, not as many as 4 factors that we chose for this analysis. The reason we chose these 4 factors for adjustment was that they were all the laboratory parameters of renal severity defined in the definition of nephritic and proteinuric flare.

Renal pathology and IP-10

Every cases in biomarker group who had been performed kidney biopsy showing proliferative pathological pattern and half of the cases had crescentic lesion. This finding confirm the performance of IP-10 in predicting intrarenal proliferative pathology. However, there was limitation in pathology interpretation since not every cases in biomarker group was performed kidney biopsy. The main reason was patient unwillingness, but there was one case with severe scoliosis and the interventionist failed to perform the procedure. This case with scoliosis, after immunosuppression initiation according to urine IP-10 result, the clinical of renal flare improved and had been achieved complete remission. This showed the benefit of biomarker in case with contraindication or inappropriate to be performed the invasive procedure. There was limitation, in those who didn't have pathology report, they might had less severe pathology, so the specificity of IP-10 for proliferative pathology couldn't entirely be analyzed. Since there were only few cases in biomarker group with pathology report, we didn't perform correlation analysis between IP-10 level at time of flare and renal pathology because this might not give the accurate information.

Secondary outcome

In secondary outcomes, there were a trend toward less adverse drug event rate and less infection complication in biomarker group, however not statistically significant.

These could be explained by the less prednisolone dosage in biomarker group. In conventional group, after renal flare was diagnosed, majority of cases were titrated prednisolone dosage up in order to awaiting for kidney pathology result, then specific immunosuppression was later initiated. This strategy resulted in higher accumulative steroid dosage. Higher steroid exposure related to infection, diabetes mellitus, osteoporosis or avascular necrosis of bone, glaucoma etc. In contrast to biomarker group, steroid sparing strategy could be achieved by shortening time from renal flare to specific immunosuppression initiation. The average MMF dosage in biomarker group was higher than in conventional group, this was explained by the fact that we used mycophenolic acid trough level (MPA C0) to guide adjust MMF dosage in order to achieve the best treatment base on the current available data(39). But the accumulative IVCY dosage was higher in conventional group, this could be explained by the fact that more percent of patient in conventional group received NIH regimen than eurolupus regimen. This could resulted from the more severe LN flare in conventional group than biomarker group. These results emphasize the concept that early detection of LN flare will benefit in less clinical severity, which result in overall immunosuppression reduction, decrease adverse drug event and infection complication. Another benefit of biomarker guided therapy is to achieve more complete renal remission rate. As previously mentioned, achieving renal remission especially complete remission significantly improve renal survival and patient survival.

Post hoc analysis in subgroup with nephritis flare

In post hoc analysis, IP-10 showed its performance in improving renal remission in subgroup patient with nephritis flare. The explanation could be that IP-10 is a Th1 specific biomarker. The major function of Th1 is to activate cellular inflammation cascade which results in proliferative pathology, thus resulting in nephritis clinical picture. This showed benefit of urine IP-10 in patients with nephritis flare, implying proliferative pathological pattern, in alerting physician to either perform kidney biopsy or initiate immunosuppression. Unlike clinical nephritic flare, proteinuric flare could be a result from multiple pathway of pathogenesis. IP-10 is not specific to this pathology, and clinical proteinuric flare might not get the best response with immunosuppression.

Post hoc analysis categorized patient with C3 level

In post hoc analysis, patients in biomarker group were categorized in to normal C3 level or decreased C3 level in order to see the add-on performance of C3 to IP-10 biomarkers. There was no different in median time from renal flare to renal remission shown by log rank test. Meaning that either normal or decreased C3 level couldn't predict renal outcome.

Subgroup of LN population who will get the most benefit from urine IP-10 measurement

Patients with overt clinical LN flare or who present with RPGN are usually performed kidney biopsy and initiate immunosuppression without delay. This subgroup of LN patient might not gain the most benefit of biomarker measurement. The population that could gain the most benefit of urine IP-10 biomarker might be the patient with mild clinical severity, since urine IP-10 gives the information of an ongoing intra-renal inflammation. Positive urine biomarker alerts physicians to appropriately early manage these cases. Furthermore, patients with contraindication or unwilling to be performed kidney biopsy might get some benefit of measuring urine IP-10 to guide immunosuppressive management.

Test interpretation

With positive test result patient will gain the benefit of shortening time to induction, thus shortening time on renal flare. From the previous data, specificity of urine IP-10 is 94% so the false positive rate is low. In this study the false negative result was zero. For test negative, patient will not get the add-on benefit of urine biomarker measurement, but management will be according to the standard of care. From the previous data sensitivity of this test is 73%, result from larger study might give further information with false negative test. Repeated urine IP-10 measurement might give better sensitivity.

5.2 Strength of This Study

This study is the first prospective study to utilize urine biomarker in early detect case with LN flare and guiding immunosuppression initiation. We showed the performance of IP-10 in early detection of active LN flare with shortening time from renal flare to

renal remission. There was a trend toward higher complete renal remission rate with significant lower prednisolone dosages in biomarker guided therapy.

5.3 Limitation

Relatively shortening time of this study, small sample size was obtained with small power of 70%. Further larger study is anticipated to accurately show the benefit of urine biomarker in guiding LN management.

5.4 Conclusion

Urinary IP-10 is a promising and simple biomarker for early diagnosis and treatment of active lupus nephritis. This approach may improve LN outcomes and could avoid invasive kidney biopsy procedure.

5.5 Suggestion

A larger prospective study or RCT should be conducted to confirm the benefit of biomarker guided strategy in active LN management.

REFERENCES

1. Avihingsanon Y, Hirankarn N. Major lupus organ involvement: severe lupus nephritis. Lupus 2010;19:1391-8.
2. Yap DY, Tang CS, Ma MK, Lam MF, Chan TM. Survival analysis and causes of mortality in patients with lupus nephritis. Nephrol Dial Transplant 2012;27:3248-54.
3. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD, et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care Res (Hoboken) 2012;64:797-808.
4. Beck L, Bomback AS, Choi MJ, Holzman LB, Langford C, Mariani LH, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis. Am J Kidney Dis 2013;62:403-41.
5. Chen YE, Korbet SM, Katz RS, Schwartz MM, Lewis EJ. Value of a complete or partial remission in severe lupus nephritis. Clin J Am Soc Nephrol 2008;3:46-53.
6. Bootsma H, Spronk P, Derksen R, de Boer G, Wolters-Dicke H, Hermans J, et al. Prevention of relapses in systemic lupus erythematosus. Lancet 1995;345:1595-9.
7. Wagrowska-Danilewicz M, Danilewicz M. Current position of electron microscopy in the diagnosis of glomerular diseases. Pol J Pathol 2007;58:87-92.
8. Tanaka H, Demeure CE, Rubio M, Delespesse G, Sarfati M. Human monocyte-derived dendritic cells induce naive T cell differentiation into T helper cell type 2 (Th2) or Th1/Th2 effectors. Role of stimulator/responder ratio. J Exp Med 2000;192:405-12.
9. Akahoshi M, Nakashima H, Tanaka Y, Kohsaka T, Nagano S, Ohgami E, et al. Th1/Th2 balance of peripheral T helper cells in systemic lupus erythematosus. Arthritis Rheum 1999;42:1644-8.
10. Schneider P, MacKay F, Steiner V, Hofmann K, Bodmer JL, Holler N, et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. J Exp Med 1999;189:1747-56.
11. Nowling TK, Gilkeson GS. Mechanisms of tissue injury in lupus nephritis. Arthritis Res Ther 2011;13:250.
12. Avihingsanon Y, Phumesin P, Benjachat T, Akkasilpa S, Kittikowit V, Praditpornsilpa K, et al. Measurement of urinary chemokine and growth factor messenger RNAs: a noninvasive monitoring in lupus nephritis. Kidney Int 2006;69:747-53.
13. Rovin BH, Zhang X. Biomarkers for lupus nephritis: the quest continues. Clin J Am Soc Nephrol 2009;4:1858-65.
14. Heidenreich U, Mayer G, Herold M, Klotz W, Stempfl Al-Jazrawi K, Lhotta K. Sensitivity and specificity of autoantibody tests in the differential diagnosis of lupus nephritis. Lupus 2009;18:1276-80.
15. Nossent JC, Henzen-Logmans SC, Vroom TM, Huysen V, Berden JH, Swaak AJ. Relation between serological data at the time of biopsy and renal histology in lupus nephritis. Rheumatol Int 1991;11:77-82.
16. Cybulsky AV, Walsh M, Knoll G, Hladunewich M, Bargman J, Reich H, et al. Canadian Society of Nephrology Commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis: management of glomerulonephritis in adults. Am J Kidney Dis 2014;63:363-77.
17. Wu J, Chen YD, Gu W. Urinary proteomics as a novel tool for biomarker discovery in kidney diseases. J Zhejiang Univ Sci B 2010;11:227-37.

18. Kopetschke K, Klocke J, Griessbach AS, Humrich JY, Biesen R, Dragun D, et al. The cellular signature of urinary immune cells in Lupus nephritis: new insights into potential biomarkers. Arthritis Res Ther 2015;17:94.
19. Adhya Z, Borozdenkova S, Karim MY. The role of cytokines as biomarkers in systemic lupus erythematosus and lupus nephritis. Nephrol Dial Transplant 2011;26:3273-80.
20. Liu CC, Kao AH, Manzi S, Ahearn JM. Biomarkers in systemic lupus erythematosus: challenges and prospects for the future. Ther Adv Musculoskelet Dis 2013;5:210-33.
21. Reyes-Thomas J, Blanco I, Putterman C. Urinary biomarkers in lupus nephritis. Clin Rev Allergy Immunol 2011;40:138-50.
22. Ikeda Y, Fujimoto T, Ameno M, Shiiki H, Dohi K. Relationship between lupus nephritis activity and the serum level of soluble VCAM-1. Lupus 1998;7:347-54.
23. Wu T, Xie C, Wang HW, Zhou XJ, Schwartz N, Calixto S, et al. Elevated urinary VCAM-1, P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. J Immunol 2007;179:7166-75.
24. Pitashny M, Schwartz N, Qing X, Hojaili B, Aranow C, Mackay M, et al. Urinary lipocalin-2 is associated with renal disease activity in human lupus nephritis. Arthritis Rheum 2007;56:1894-903.
25. Torres-Salido MT, Cortes-Hernandez J, Vidal X, Pedrosa A, Vilardell-Tarres M, Ordi-Ros J. Neutrophil gelatinase-associated lipocalin as a biomarker for lupus nephritis. Nephrol Dial Transplant 2014;29:1740-9.
26. Wang G, Lai FM, Tam LS, Li EK, Kwan BC, Chow KM, et al. Urinary FOXP3 mRNA in patients with lupus nephritis--relation with disease activity and treatment response. Rheumatology (Oxford) 2009;48:755-60.
27. Bonelli M, von Dalwigk K, Savitskaya A, Smolen JS, Scheinecker C. Foxp3 expression in CD4+ T cells of patients with systemic lupus erythematosus: a comparative phenotypic analysis. Ann Rheum Dis 2008;67:664-71.
28. Stohl W, Metyas S, Tan SM, Cheema GS, Oamar B, Xu D, et al. B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. Arthritis Rheum 2003;48:3475-86.
29. Schwartz N, Rubinstein T, Burkly LC, Collins CE, Blanco I, Su L, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther 2009;11:R143.
30. Chan RW, Lai FM, Li EK, Tam LS, Chow KM, Li PK, et al. Messenger RNA expression of RANTES in the urinary sediment of patients with lupus nephritis. Nephrology (Carlton) 2006;11:219-25.
31. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009;29:313-26.
32. Chan RW, Lai FM, Li EK, Tam LS, Wong TY, Szeto CY, et al. Expression of chemokine and fibrosing factor messenger RNA in the urinary sediment of patients with lupus nephritis. Arthritis Rheum 2004;50:2882-90.
33. Vincent FB, Northcott M, Hoi A, Mackay F, Morand EF. Association of serum B cell activating factor from the tumour necrosis factor family (BAFF) and a proliferation-inducing ligand (APRIL) with central nervous system and renal disease in systemic lupus erythematosus. Lupus 2013;22:873-84.
34. Ioannidis JP, Boki KA, Katsorida ME, Drosos AA, Skopouli FN, Boletis JN, et al. Remission, relapse, and re-remission of proliferative lupus nephritis treated with cyclophosphamide. Kidney Int 2000;57:258-64.

ภาคผนวก

Appendix 1.

Clinical Record Form of Screening visit

BIG STEP Clinical Record Form

Sticker

Demography
•DOB
•RACE

Week: screening

Date

Clinical note

Physical exmination: BW.....kg, Ht.....cm

BW Ht		BP PR	
	Normal	Ab normal	Detail
HEENT/LN			
H & L			
Abdomen			
Ext			
Neuro			
Ext			

SLEDAI

Weight	Score	Descriptor
8		Seizure
8		Psychosis
8		Organic brain syndrome
8		Visual disturbance
8		Cranial nerve disorder
8		Lupus headache
8		CVA
8		Vasculitis
4		Arthritis
4		Myositis
4		Urinary casts
4		Hematuria
4		Proteinuria
4		Pyuria
2		Rash
2		Alopecia
2		Mucosal ulcers
2		Pleurisy
2		Pericarditis
Total		

Laboratory: date.....

CBC		Chem				UA		Spot U		C3		HBsAg	
Hb		BUN		AST		Spec		Ptn		C4		Anti HBs	
Hct		Cr		ALT		pH		Cr		ANA		Anti HBc	
WBC		GFR		ALP		Ptn		UPCI		dsDNA		Anti HCV	
N		Na		TB		RBC						Anti HIV	
L		K		DB		WBC		24 h U					
M		Cl		Alb				Cr					
E		HCO3		Glb		U VEGF		Ptn				Stool agar	
Plt		FPG		TC		U IP10		vol				Stool conc	
PTT				TG				CrCl				CXR	
PT		ESR		LDL		UPT							
INR				HDL									

Assessment

Treatment

☐ MMF.....	Ivermectin (6).....	ACEI/ARB .....
☐ AZA.....	Acyclovir (200)	HCQ (200) .....
Prednisolone (5)	Bactrim (80/400).....	CCB.....
omeprazole (20)	Furosemide	BB.....
onsia (8)	.....	hydralazine.....
		doxazosin.....

Next visit Date
.....

Sign
.....
.....

Appendix 2

Clinical Record Form of Induction visit

BIG STEP Clinical Record Form

Sticker

Week 0: Induction

Date

Clinical note

Physical exmination: BW.....kg, Ht.....cm

BW Ht		BP PR	
	Normal	Ab normal	Detail
HEENT/LN			
H & L			
Abdomen			
Ext			
Neuro			
Ext			

SLEDAI

Weight	Score	Descriptor
8		Seizure
8		Psychosis
8		Organic brain syndrome
8		Visual disburtance
8		Crenial nerve disorder
8		Lupus headache
8		CVA
8		Vasculitis
4		Arthritis
4		Myositis
4		Urinary casts
4		Hematuria
4		Proteinuria
4		Pyuria
2		Rash
2		Alopecia
2		Mucosal ulcers
2		Pleurisy
2		Pericarditis
Total		

Laboratory: date.....

CBC		Chem				UA		Spot U					
Hb		BUN				Spec		Ptn					
Hct		Cr				pH		Cr					
WBC		GFR				Ptn		UPCI					
N						RBC							
L		K				WBC							
M													
E													
Plt													

Assessment

Treatment

☐ IVCY.....mg #	Ivermectin (6).....	ACEI/ARB .....
☐ MMF..... mg	Acyclovir (200)	HCQ (200) .....
Prednisolone (5)	Bactrim (80/400).....	CCB.....
omeprazole (20)	Furosemide	BB.....
onsia (8)	.....	hydralazine.....
		doxazosin.....

Next visit Date .....

Sign .....

.....

Version 1 date 26 May 2015

Page 2

Appendix 3

Clinical Record Form follow up visit week 2

BIG STEP Clinical Record Form

Sticker

Week 2,6,10,14,18,22

Date

Clinical note

Physical exmination: BW.....kg, Ht.....cm

BW Ht		BP PR	
	Normal	Ab normal	Detail
HEENT/LN			
H & L			
Abdomen			
Ext			
Neuro			
Ext			

SLEDAI

Weight	Score	Descriptor
8		Seizure
8		Psychosis
8		Organic brain syndrome
8		Visual disturbance
8		Cranial nerve disorder
8		Lupus headache
8		CVA
8		Vasculitis
4		Arthritis
4		Myositis
4		Urinary casts
4		Hematuria
4		Proteinuria
4		Pyuria
2		Rash
2		Alopecia
2		Mucosal ulcers
2		Pleurisy
2		Pericarditis
Total		

Laboratory: date.....

CBC		Chem				UA		Spot U					
Hb		BUN				Spec		Ptn					
Hct		Cr				pH		Cr					
WBC		GFR				Ptn		UPCI					
N						RBC							
L		K				WBC							
M													
E													
Plt													

Assessment

Treatment

ADR

Inf	Med

☐ IVCY.....mg # ..... Ivermectin (6)..... ACEI/ARB .....

☐ MMF..... mg Acyclovir (200) ..... HCQ (200) .....

Prednisolone (5) ..... Bactrim (80/400)..... CCB.....

omeprazole (20) ..... Furosemide ..... BB.....

onsia (8) .... hydralazine.....

doxazosin.....

Next visit Date

.....

Sign

.....

.....

Appendix 4

Clinical Record Form of follow up visit week 4,8,12,16,20,24

BIG STEP Clinical Record Form

Sticker

Week: 4,8,12,16,20,24

Date

Clinical note

Physical exmination: BW.....kg, Ht.....cm

BW Ht		BP PR	
	Normal	Ab normal	Detail
HEENT/LN			
H & L			
Abdomen			
Ext			
Neuro			
Ext			

SLEDAI

Weight	Score	Descriptor
8		Seizure
8		Psychosis
8		Organic brain syndrome
8		Visual disturbance
8		Cranial nerve disorder
8		Lupus headache
8		CVA
8		Vasculitis
4		Arthritis
4		Myositis
4		Urinary casts
4		Hematuria
4		Proteinuria
4		Pyuria
2		Rash
2		Alopecia
2		Mucosal ulcers
2		Pleurisy
2		Pericarditis
Total		

Laboratory: date.....

CBC		Chem				UA		Spot U		C3			
Hb		BUN				Spec		Ptn					
Hct		Cr				pH		Cr					
WBC		GFR				Ptn		UPCI		dsDNA			
N						RBC							
L		K				WBC							
M				Alb									
E						U VEGF							
Plt						U IP10							
		ESR											

Assessment

Treatment

☐ IVCY.....mg #	Ivermectin (6).....	ACEI/ARB .....
☐ MMF..... mg	Acyclovir (200)	HCQ (200) .....
Prednisolone (5)	Bactrim (80/400).....	CCB.....
omeprazole (20)	Furosemide	BB.....
onsia (8)	.....	hydralazine.....
		doxazosin.....

ADR

Inf	Med

Next visit Date .....

Sign .....

.....

Appendix 5

Study protocol

Screening visit

Screen patient according to inclusion criteria

Send investigation according to “screening visit” CRF

Medication part of this “screening visit” form is the baseline current treatment of patient

Schedule for induction visit, should be within 1 week

Steroid dosage can be titrated up according to physician preferences and the baseline dosage must be documented

Induction visit, this visit could be the same visit as screening visit if all inclusion and exclusion criteria is matched (Hx, PE and lab as CRF will be marked as same as screening visit)

Check exclusion criteria

Check infectious marker status and complete inclusion/exclusion criteria sheet

If all reveal negative, then start induction treatment

If HBV or HCV infection reveal positive

Active infection; exclude from study

Not active status; schedule GI clinic and induction treatment can be initiated

Active pulmonary infection, parasitic infection, patient is excluded from study

Sign inform consent if compatible with inclusion and exclusion criteria

Start induction treatment with one of this following two regimen

Mycophenolate mofetil:

BW < 50 kg; 1.5 g/d

BW > 50 kg; 2 g/d

Eurolupus: IVCY 500 mg IV q 2 week

Increase prednisolone dosage to 0.5 mg/kg/d

Start hydroxychloroquine (200) 1 tab OD and schedule for retina examination

Start infection prophylaxis with

Bactrim (80/400) 2 tab OD

Acyclovia (200) 1 tab bid

Ivermectin (6) 2 tab OD for 2 days and repeat dose next 2 week

If patient has previous history of peptic ulcer, then prescribe omeprazole (20) 1 tab OD

Start ACEI/ARB according to physician preference

Start vitamin D2 (20,000u) 1 tab OD

Schedule for kidney biopsy

Week 2

Record sign of ADR from medication, any infection, RPGN.

Check kidney biopsy result

Adjust regimen according to biopsy result, NIH regiment is allowed

Mycophenolate mofetil; check C0 MPA, Cr and K after start ACEI, order C0 MPA in next visit CRF if adjust dosage

Eurolupus; CBC for nadir WBC, Cr and K after start ACEI, prescribe 2nd IVCY

Week 4

Record sign of ADR from medication, any infection, RPGN

Check kidney biopsy result

Adjust regimen according to clinical and biopsy result, NIH regiment is allowed

Mycophenolate mofetil; check C0MPA if dosage was adjusted in last visit, order C0 MPA in next visit CRF if adjust dosage

Eurolupus; prescribe 3rd and 4th IVCY

NIH; prescribe according to clinical and kidney result

Week 8

Record sign of ADR from medication, any infection, RPGN

Check kidney biopsy result

Adjust regimen according to clinical and biopsy result, NIH regiment is allowed

Mycophenolate mofetil; check C0 MPA if dosage was adjusted inlast visit, order C0 MPA in next visit CRF if adjust dosage

Eurolupus; prescribe 5th and 6th IVCY

NIH; prescribe according to clinical and kidney result

Week 12

Record sign of ADR from medication, any infection, RPGN

Check kidney biopsy result

Adjust regimen according to clinical and biopsy result, NIH regiment is allowed

Mycophenolate mofetil; check C0 MPA if adjusted dose last visit, order C0 MPA in next visit CRF if adjust dosage

Eurolupus; prescribe 5th and 6th IVCY

NIH; prescribe according to clinical and kidney result

Week 16

Record sign of ADR from medication, any infection, RPGN

Check kidney biopsy result

Adjust regimen according to clinical and biopsy result, NIH regiment is allowed

Mycophenolate mofetil; check C0 MPA if dosage was adjusted inlast visit, order C0 MPA in next visit CRF if adjust dosage

Eurolupus; prescribe 5th and 6th IVCY

NIH; prescribe according to clinical and kidney result

Week 20

Record sign of ADR from medication, any infection, RPGN

Check kidney biopsy result

Adjust regimen according to clinical and biopsy result, NIH regiment is allowed

Mycophenolate mofetil; check C0 MPA if dosage was adjusted inlast visit, order C0 MPA in next visit CRF if adjust dosage

Eurolupus; prescribe 5th and 6th IVCY

NIH; prescribe according to clinical and kidney result

Week 24

Record sign of ADR from medication, any infection, RPGN

Check kidney biopsy result

Adjust regimen according to clinical and biopsy result, NIH regiment is allowed

Mycophenolate mofetil; check C0 MPA if dosage was adjusted inlast visit, order C0 MPA in next visit CRF if adjust dosage

Eurolupus; prescribe 5th and 6th IVCY

NIH; prescribe according to clinical and kidney result

This visit is the end of study protocol. Response to treatment will be diagnosed according to these following criteria

Complete remission: improvement of serum creatinine to baseline value, urine protein < 0.5 g/d, resolution of urine RBC

Partial remission: a reduction of urine protein for more than 50% or a reduction of urine protein for more than 50% and < 3 g/d in the baseline nephritic range proteinuria

Progressive disease: increase in serum creatinine or no urine protein reduction to < 50% of baseline value

VITA

1. Name Thanarat Supasiri, MD
2. Date of birth May, 14th 1984
3. Place of birth Bangkok, Thailand
4. Educations

- 2002 – 2008 medical student at Faculty of Medicine, Chulalongkorn University

(first class honors)

- 2011 - 2014 Internal medicine residency at Department of Medicine, Faculty of

Medicine, Chulalongkorn Univeristy

- 2014 – present Renal fellow at Nephrology division, Department of Medicine,

Faculty of Medicine, Chulalongkorn University

5. Working experiences

- 2008 – 2009 Internship at Samutprakarn hospital
- 2009 – 2011 Internship at Department of Biochemistry, Faculty of Medicine,

Chulalongkorn University

6. Research presentation

- 2010 Poster presentation in the topic of "Urinary Interferon Inducible

protein 10 is associated with lupus nephritis class III&IV" at The 9th international congress on systemic lupus erythematosus, held in Vancouver, Canada.