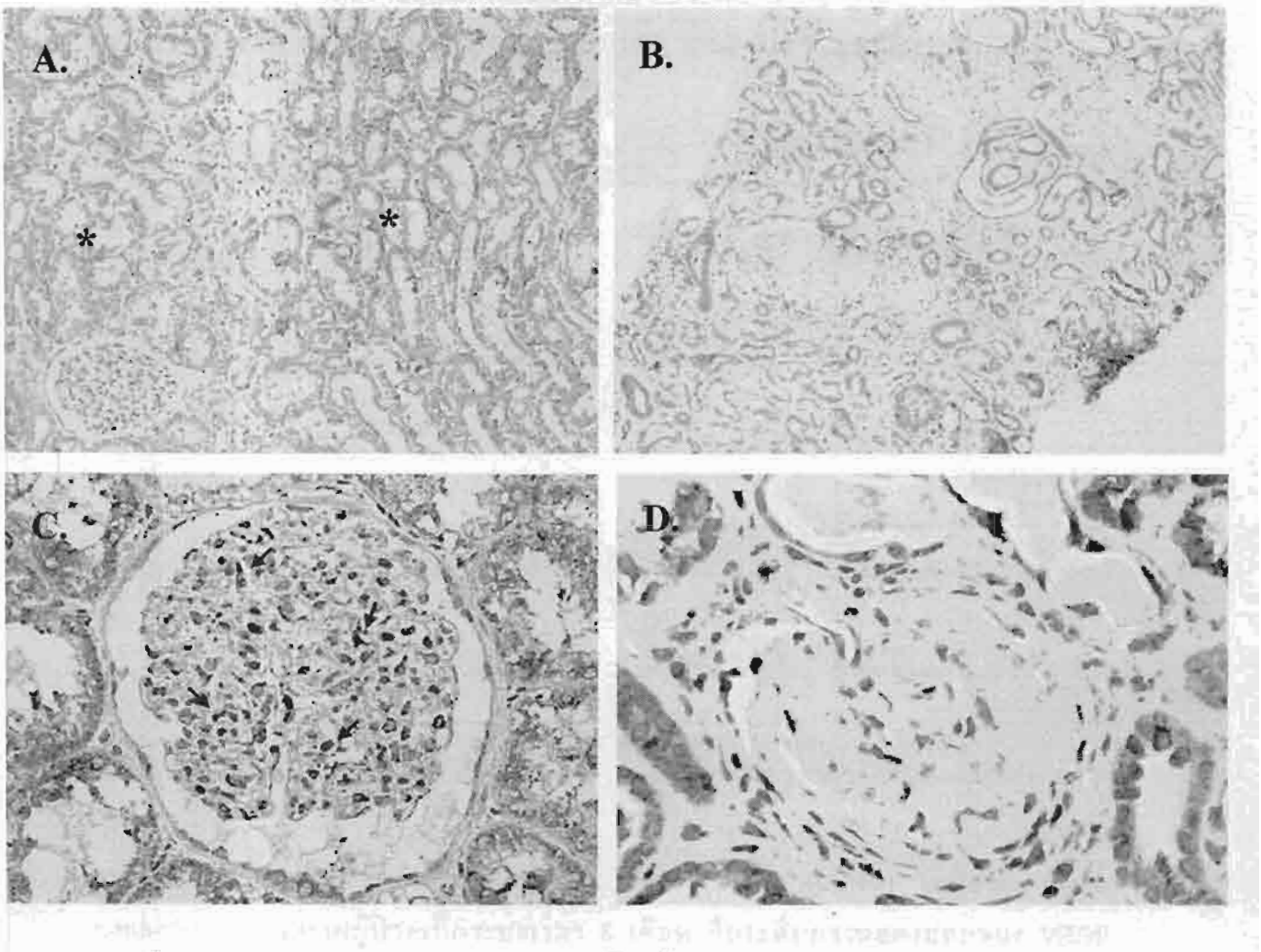
4. การศึกษาความสัมพันธ์ระหว่างระดับโปรตีน VEGF และการแสดงออกของยีนในเนื้อเยื่อไต รวมถึงความรุนแรงของพยาธิสภาพของไต

จากผลการศึกษาการแสดงออกของยีน VEGF ซึ่งลดลงในชิ้นเนื้อไตของผู้ป่วย lupus nephritis จึงสนใจศึกษาการแสดงออกของโปรตีนในชิ้นเนื้อเยื่อไตของผู้ป่วยและกลุ่มควบคุมด้วยวิธี Immunohistochemistry โดยใช้ Anti-VEGF antibody เพื่อศึกษาความสัมพันธ์ระหว่างระดับโปรตีนและการแสดงออกของยีน VEGF พบว่าผู้ป่วย lupus nephritis มีระดับการแสดงออกของโปรตีน VEGF ในชิ้นเนื้อไตลดลงเมื่อเปรียบเทียบกับกลุ่มควบคุมดังแสดงในรูปที่ 6 ซึ่งผลการทดลองแสดงให้เห็นว่าการแสดงออกของ VEGF โปรตีนสอดคล้องกับการแสดงออกของ VEGF mRNA โดยเฉพาะอย่างยิ่งในบริเวณ glomerulus ซึ่งเป็นที่อยู่ของเซลล์ที่สามารถสร้าง VEGF ได้ในไต (glomerular visceral epithelial) และจะยิ่งลดน้อยลงในผู้ป่วยที่มีพยาธิสภาพแบบ endocapillary proliferation และ crescent formation



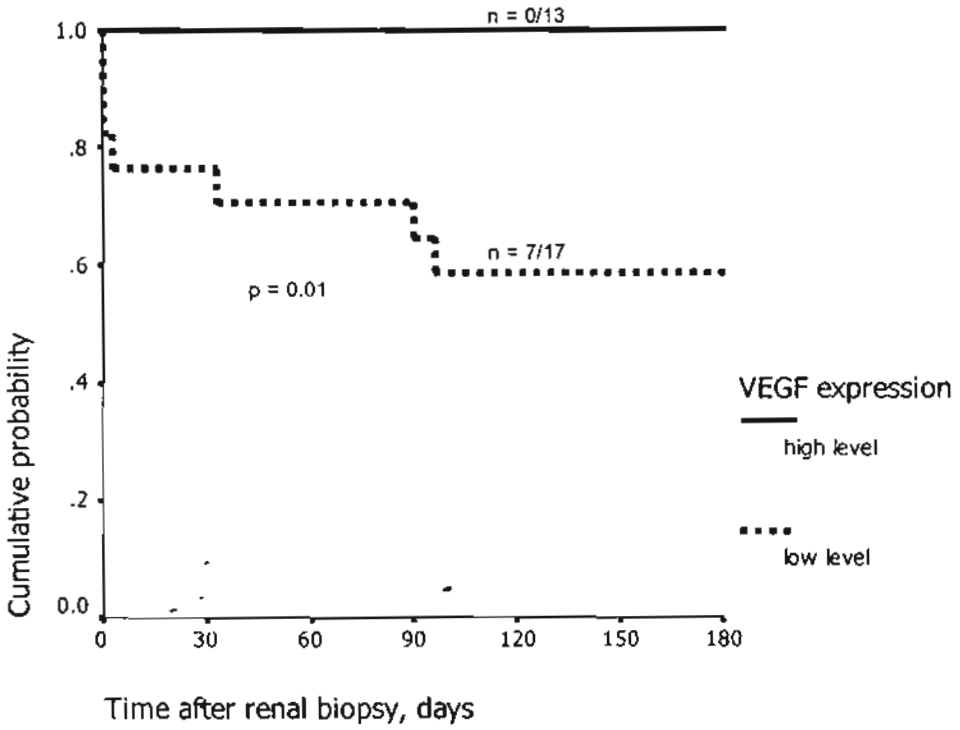
รูปที่ 6 การแสดงออกของโปรตีน VEGF ในชิ้นเนื้อไตด้วยวิธี Immunohistochemistry

หมายเหตุ : A และ C คือ ชิ้นเนื้อไต control (kidney transplant donor)

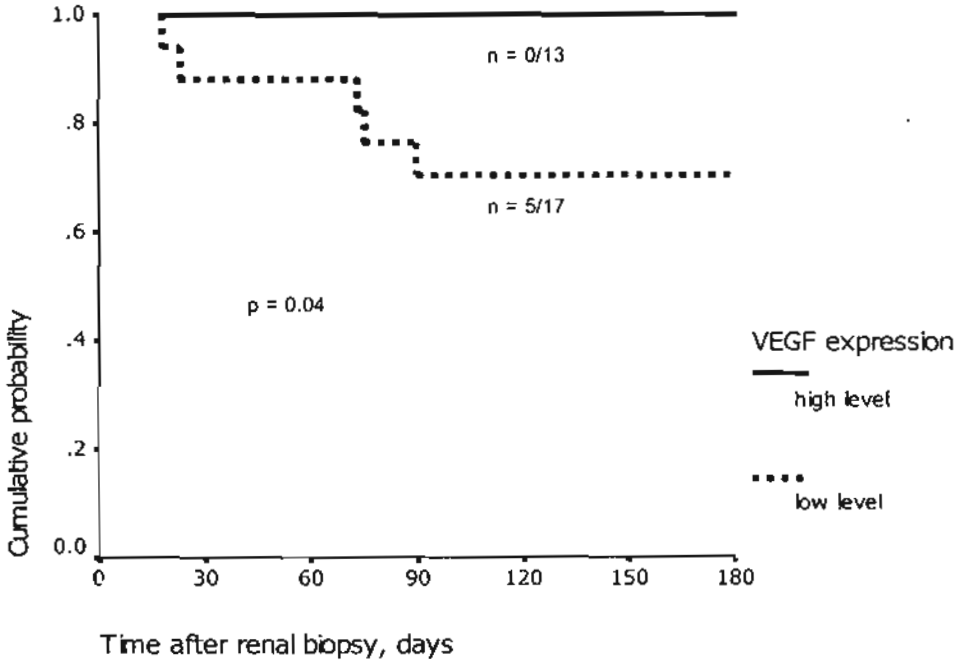
B และ D คือ ชิ้นเนื้อไตของผู้ป่วย lupus nephritis

5. การศึกษาความสัมพันธ์ของการแสดงออกของยีน VEGF กับการเกิดไตวายเรื้อรังระยะสุดท้าย

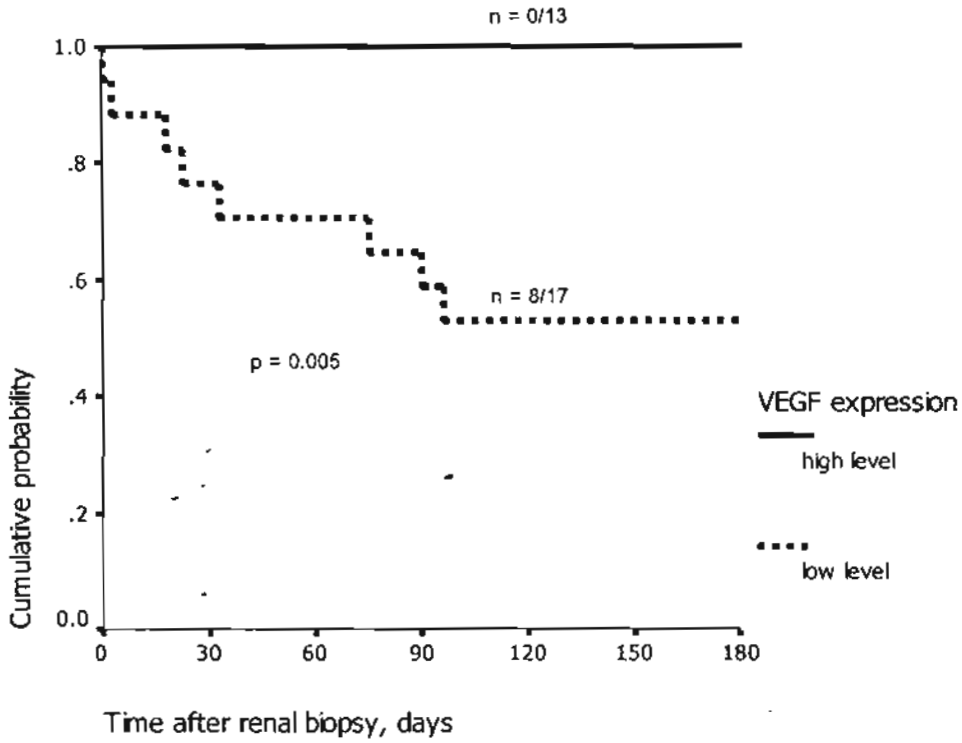
ในการศึกษานี้ได้ติดตามการรักษาและเก็บรวบรวมข้อมูลของผู้ป่วยทุกรายเป็นระยะเวลา 6 เดือนแล้วนำมาวิเคราะห์ร่วมกับผลการแสดงออกของยีน VEGF ซึ่งผู้ป่วยจะถูกแบ่งออกเป็น 2 กลุ่ม คือ กลุ่มที่มีการแสดงออกของ VEGF mRNA สูง (high level VEGF) และต่ำ (low level VEGF) โดยหลังจากติดตามผู้ป่วยเป็นระยะเวลา 6 เดือน พบว่ามีผู้ป่วยจำนวนหนึ่งมีการเพิ่มขึ้นของค่า serum creatinine เป็นสองเท่าจากค่าตั้งต้นในเดือนแรก (doubling serum creatinine) นอกจากนั้นยังพบว่ามีผู้ป่วยบางรายเกิดภาวะไตวายเรื้อรังระยะสุดท้าย (end-stage renal disease; ESRD) ซึ่งเมื่อนำมาหาความสัมพันธ์ระหว่างการแสดงออกของยีน VEGF และ outcome ดังกล่าวที่เกิดขึ้นกับผู้ป่วย จะพบว่าการแสดงออกของ VEFG mRNA ที่ลดต่ำลงยังสามารถใช้ในการทำนายการเกิด doubling serum creatinine หรือ ESRD หรือทั้งสองอย่างร่วมกัน เมื่อติดตามผู้ป่วยที่ได้รับการรักษาเป็นระยะเวลา 6 เดือนได้อีกด้วย ดังแสดงใน survival curve (p-value = 0.04, 0.01 และ 0.005, ตามลำดับ) ดังแสดงในรูปที่ 7-9



รูปที่ 7 กราฟแสดงการเกิดภาวะไตวายเรื้อรังระยะสุดท้าย (ESRD) ในผู้ป่วย lupus nephritis เมื่อติดตามผู้ป่วยเป็นระยะเวลา 6 เดือน กับระดับการแสดงออกของ VEGF mRNA



รูปที่ 8 กราฟแสดงการเกิด Doubling serum creatinine ในผู้ป่วย lupus nephritis เมื่อติดตามผู้ป่วยเป็นระยะเวลา 6 เดือน กับระดับการแสดงออกของ VEGF mRNA



รูปที่ 9 กราฟแสดงการเกิดภาวะไตวายเรื้อรังระยะสุดท้าย (ESRD) หรือ Doubling serum creatinine ในผู้ป่วย lupus nephritis เมื่อติดตามผู้ป่วยเป็นระยะเวลา 6 เดือน กับระดับการแสดงออกของ VEGF mRNA

6. การหารูปแบบของยีน VEGF โดยวิธี PCR-Restriction Fragment Length Polymorphism (RFLP) (1)

จากการศึกษารูปแบบของยีน VEGF ในผู้ป่วย SLE พบว่ารูปแบบของยีนที่ตำแหน่ง -460C/T และ +405C/G ในผู้ป่วยและกลุ่มควบคุมไม่มีสัมพันธ์กันอย่างมีนัยสำคัญ ดังแสดงในตารางที่ 5-8

ตารางที่ 5 แสดง Genotype และ allele frequencies ของยีน VEGF ที่ตำแหน่ง –460 C/T ในผู้ป่วย SLE และกลุ่มควบคุม

	SLE patients n = 193	Healthy controls n = 234
Genotype frequencies		
C/C	18 (9.33%)	20 (8.55%)
C/T	74 (38.34%)	97 (41.45%)
T/T	101 (52.33%)	117 (50.00%)
Allele frequencies		
C	110 (28.50%)	137 (29.27%)
T	276 (71.50%)	331 (70.73%)

No significant association

ตารางที่ 6 Risk of SLE associated with VEGF (-460C/T) genotype according to different models of inheritance.

	SLE patients n =193	Healthy controls n =234
C dominance, T wild type		
C/C or C/T	92 (47.67%)	117 (50.00%)
T/T	101 (52.33%)	117 (50.00%)
C recessive, T wild type		
C/C	18 (9.33%)	20 (8.55%)
T/T or C/T	175 (90.67%)	214 (91.45%)

No significant association

ตารางที่ 7 แสดง Genotype และ allele frequencies ของยีน VEGF ที่ตำแหน่ง +405 C/G ในผู้ป่วย SLE และกลุ่มควบคุม

	SLE patients n = 193	Healthy controls n = 234
Genotype frequencies		
C/C	26 (13.47%)	29 (12.39%)
C/G	97 (50.26%)	118 (50.43%)
G/G	70 (36.27%)	87 (37.18%)
Allele frequencies		
C	149 (38.60%)	176 (37.61%)
G	237 (61.40%)	292 (62.39%)

No significant association

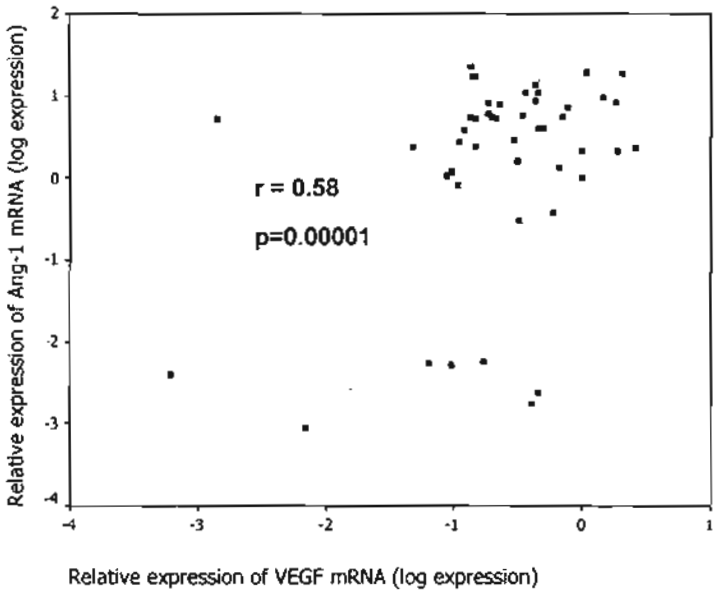
ตารางที่ 8 Risk of SLE associated with VEGF (+405C/G) genotype according to different models of inheritance.

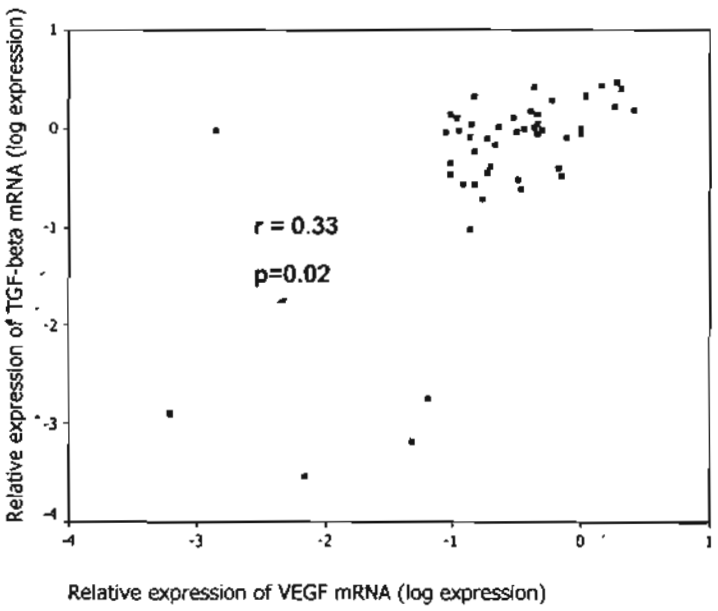
	SLE patients n = 193	Healthy controls n = 234
C dominance, G wild type		
C/C or C/G	123 (63.73%)	147 (62.82%)
G/G	70 (36.27%)	87 (37.18%)
C recessive, G wild type		
C/C	26 (13.47%)	29 (12.39%)
G/G or C/G	167 (86.53%)	205 (87.61%)

No significant association

7. ผลการศึกษาเบื้องต้นเกี่ยวกับยีน angiopoeitin-1 และ TGF-beta

จากการทบทวนวรรณกรรมเพิ่มเติมพบว่าการแสดงออกของยีน angiopoetin-1 และยีน TGF-beta มีส่วนเกียวข้องที่สำคัญต่อการแสดงออกของยีน VEGF ที่เราสนใจศึกษาข้างต้น ดังนั้นงานวิจัยนี้จึงได้ทำการศึกษาการแสดงออกของยีนทั้งสองเพิ่มเติม ผลการศึกษาพบว่าระดับการแสดงออกของยีน TGF-beta และ Angiopoetin-1 ไม่มีความแตกต่างกันระหว่างผู้ป่วยและกลุ่มคบคุม อีกทั้งยังไม่พบความสัมพันธ์กับความรุนแรงของโรคด้วย แต่อย่างไรก็ตามพบว่าระดับการแสดงออกของยีนทั้งสองมีความสัมพันธ์กับระดับการแสดงออกของยีน VEGF (TGF-beta : r=0.58, p=0.00001 และ Angiopoetin-1 : r=0.33, p=0.02)





รูปที่ 10 แสดงความสัมพันธ์ระหว่างการแสดงออกของยีน TGF-beta และ Angiopoetin-1 กับ กับการแสดงออกของยีน VEGF

สรุปและวิจารณ์ผลการทดลอง

Lupus nephritis เป็นภาวะไตอักเสบในผู้ป่วย SLE ซึ่งเป็นอาการแสดงที่พบได้บ่อย และสัมพันธ์กับการเพิ่มขึ้นของอัตราการเจ็บป่วยและเสียชีวิต (morbidity and mortality) ปัจจุบันยังไม่ทราบสาเหตุและกลไกการเกิดโรค lupus nephritis ที่ชัดเจน เชื่อว่ามีความผิดปกติของระบบภูมิคุ้มกัน นำไปสู่การสร้าง autoantibody และมี immune complex ไปจับเกาะที่เซลล์ต่างๆภายในไต Immune complex เหนี่ยวนำให้มีการชุมนุมกันของเซลล์อักเสบโดยเฉพาะที่ glomerulus ส่วนอาการของโรค Lupus nephritis นั้นมีความหลากหลายตั้งแต่ไม่มีอาการทางคลินิกเลยแต่พบการตรวจปัสสาวะผิดปกติ ไปจนถึงกลุ่มอาการ nephrotic หรือ ไตวายอย่างรวดเร็ว (Rapidly progress glomerulonephritis) ยังไม่ทราบปัจจัยที่กำหนดการเกิดโรคและความรุนแรงของโรค lupus nephritis เชื่อว่าบทบาทของพันธุกรรมและปัจจัยแวดล้อมน่าจะมีความสำคัญ การศึกษานี้มีสมมุติฐานว่าปัจจัยที่ควบคุมการเกิดโรครวมทั้งความรุนแรงของโรคส่วนหนึ่งถูกกำหนดโดยผ่านการแสดงออกของยีนบางกลุ่มภายในเนื้อไต (intra-renal gene expression) ยีน Vascular Endothelial Growth Factor (VEGF) เป็นยีนที่มีหน้าที่หลากหลาย ได้แก่ 1) ควบคุมการแพร่ผ่านของสารผ่านหลอดเลือด (vascular permeability) 2) ชักนำให้เกิดการสร้างหลอดเลือดใหม่ (angiogenesis) 3) ชักนำให้เกิดการเคลื่อนที่ของเซลล์เม็ดเลือดขาว (leukocytes chemotaxis) สำหรับภายในไตยีนนี้เป็นยีนที่มีบทบาทสำคัญในการเกิด proteinuria และ renal failure เห็นได้ชัดจากการศึกษาการให้ VEGF ป้องกันการเกิดพยาธิสภาพของไตวายเรื้อรังได้ในหนูทดลอง VEGF เป็นยีนที่มีหน้าที่ดูแลรักษา (cytoprotection) glomerular endothelial cell และ epithelial cell การแสดงออกของยีนมีความสัมพันธ์กับการเกิดโรคไตหลายชนิด ยิ่งกว่านั้นมีรายงานการให้ anti-VEGF antibody กระตุ้นการเกิดโปรตีนในปัสสาวะ (proteinuria) ในผู้ป่วยโรคมะเร็ง เชื่อว่ารูปแบบของยีน (genotype) และการแสดงออกของยีน (gene expression) ที่มีความแตกต่างกันในผู้ป่วยแต่ละรายมีส่วนกำหนดการเกิดโรคและพยากรณ์โรคได้ในผู้ป่วย lupus nephritis ดังนั้นผู้วิจัยจึงสนใจศึกษาบทบาทของยีน Vascular Endothelial Growth Factor (VEGF) กับการเกิดโรคและพยากรณ์โรคในผู้ป่วยไตอักเสบลูปัส

จากผลการศึกษาโดยวิธี real-time PCR พบว่าระดับการแสดงออกของยีน VEGF ในชิ้นเนื้อไต (intra-renal VEGF mRNA) ของผู้ป่วย lupus nephritis ลดต่ำลงกว่ากลุ่มควบคุม (kidney transplant donor) (-0.83 ± 0.7 และ -0.001± 0.39 log copies ตามลำดับ, p-value = 0.002) ซึ่งสอดคล้องกับการลดลงของปริมาณ VEGF โปรตีนในชิ้นเนื้อไตของผู้ป่วยโดยเฉพาะอย่างยิ่งในเซลล์ podocytes และเซลล์ tubular epithelial ซึ่งเป็นเซลล์สำคัญในการสร้าง VEGF ภายในไต นอกจากนั้นการลดลงของยีน VEGF ยังสอดคล้องกับพยาธิสภาพที่รุนแรงภายในไตของผู้ป่วย lupus nephritis อันได้แก่ การเกิด glomerular endocapillary proliferation,

crescent formation และ renal activity score จากการศึกษา animal model ของ Kung และคณะ (37, 38) แสดงให้เห็นว่าการลดลงของ intra-renal VEGF มีความสัมพันธ์กับการเข้ามาชุมนุมกันของ macrophage cells ภายในไต โดยมีความสัมพันธ์กับการเสียหายของหลอดเลือดภายในไตและการเกิด glomerulosclerosis หลังจากนั้นเมื่อให้ VEGF protein กับ RK mice สามารถลดการเกิด renal fibrosis และช่วยทำให้หนู mice มีการทำงานของไตดีขึ้นได้ด้วย ซึ่งสนับสนุนบทบาทของ VEGF ในการทำหน้าที่เป็น cytoprotective gene ภายในไต

การมีการแสดงออกของยีน VEGF ที่ต่ำลงในผู้ป่วยและเกิดพยาธิสภาพที่รุนแรงภายในไตนั้นอาจเกิดจากสองสาเหตุหลักคือ

1) มีการเสียหายและหลุดของเซลล์ podocytes ออกมาในปัสสาวะ ซึ่งเซลล์ดังกล่าวเป็นเซลล์ที่ทำหน้าที่เกี่ยวกับการกรองภายในไต มีการศึกษาพบว่าปัสสาวะของผู้ป่วย lupus nephritis มีปริมาณเซลล์ podocytes หลุดปนออกมามากกว่าในปัสสาวะของคนปกติ โดยเพาะอย่างยิ่งในผู้ป่วยที่มีอาการของโรครุนแรง (34, 33) ด้วยเหตุนี้น่าจะเป็นผลให้มีปริมาณ VEGF ลดลงและไตทำงานผิดปกติด้วย

2) เซลล์ภายในไตสามารถสร้าง proinflammatory cytokine และ chemotattractant cytokine เช่น MCP-1, MIP-1alpha และ MIP-1beta ซึ่งโมเลกุลเหล่านี้จะชักนำให้มีการเข้ามาชุมนุมกันของ monocytes/macrophage cells และหลั่งสารต่างๆรวมทั้ง IL-1beta, IL-6 และ TNF-alpha ซึ่งสามารถยับยั้งการสร้าง VEGF โปรตีนได้ (37)

ในการศึกษานี้ผู้วิจัยได้ทำการศึกษาเพิ่มเติมในส่วนของ Short-term outcome โดยติดตามผู้ป่วยเป็นระยะเวลา 6 เดือน พบว่าผู้ป่วยบางรายเกิดการเสื่อมสภาพการทำงานของไต ได้แก่ เกิดการเพิ่มขึ้นของระดับ serum creatinine เป็น 2 เท่า (doubling serum creatinine) และบางรายเกิดไตวายเรื้อรังระยะสุดท้าย โดยผู้ป่วยกลุ่มดังกล่าวนี้เป็นผู้ป่วยที่มีการแสดงออกของยีน VEGF อยู่ในระดับต่ำกว่าผู้ป่วยที่ไม่เกิดเหตุการณ์ดังกล่าวทั้งสิ้น นอกจากนั้นเมื่อผู้วิจัยทำการทบทวนวรรณกรรมเพิ่มเติมพบว่ายีน TGF-beta และ Angiopoetin-1 เป็นยีนกลุ่มที่มีส่วนเกี่ยวข้องกับ VEGF ดังนั้นจึงเปลี่ยนการศึกษาจาก VEGFR มาเป็นดังกล่าวแทน ซึ่งจากการศึกษาเบื้องต้นพบความสัมพันธ์ของการแสดงออกของยีน TGF-beta และ Angiopoetin-1 กับการแสดงออกของยีน VEGF

สำหรับการศึกษารูปแบบของยีน VEGF ที่ทำการศึกษาโดย ผศ.พญ.ดร.จงกลณี วงศ์ปิยะบวร นั้นพบว่าทั้งรูปแบบของยีนที่ตำแหน่ง -460C/T และ +405C/G นั้นไม่มีความสัมพันธ์กับการเกิดโรค SLE ในประชากรไทย

ดังนั้นระดับการแสดงออกของยีน VEGF ที่ลดลงและสัมพันธ์กับพยาธิสภาพที่รุนแรงในผู้ป่วย lupus nephritis นั้น อาจจะนำมาใช้ในการจำแนกผู้ป่วย Lupus Nephritis ที่มีความรุนแรงของโรคต่างๆกันได้ รวมทั้งอาจจะสามารถนำมาใช้เป็นตัวทำนายโรคตัวใหม่ได้ (novel prognostic marker)

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Output ที่ได้จากโครงการวิจัย

1. ได้รับการตีพิมพ์เผยแพร่ผลงานลงในวารสารวิชาการระดับนานาชาติ 1 เรื่อง คือ

   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 Feb;69(4):747-53. (ตามเอกสารแนบ)

2. นำเสนอผลงานในที่ประชุมนานาชาติ 2 ครั้ง คือ

   1). Yingyos Avihingsanon, Thitima Benjachat, Vipawee Kittikovit, Nattiya Hirankarn. Decreased intra-renal expression of vascular endothelial growth factor (VEGF) in patients with severe proliferative Lupus nephritis. American society of nephrology: renal week 2006; San Diago,CA,USA,November14 19,2006. (ตามเอกสารแนบ)

   2). Thitima Benjachat, Yingyos Avihingsanon, Patcharin Tangwancharoen, Vipawee Kittikovit , Nattiya Hirankarn. Intra-renal expression of VEGF is associated with poor outcome in lupus nephritis. The 8th International congress on SLE, Shanghai, Chaina, May 23-27, 2007. (ตามเอกสารแนบ)

3. กำลังดำเนินการเขียน manuscript เพื่อส่งตีพิมพ์เผยแพร่ในวารสารวิชาการระดับนานาชาติที่มี Impact factor สูงอีก 1 เรื่อง

Measurement of urinary chemokine and growth factor messenger RNAs: A noninvasive monitoring in lupus nephritis

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Noninvasive molecular tests of urine cells have been developed to monitor the activity of kidney diseases. We evaluate whether measurement of urinary messenger RNA (mRNA) levels of chemokine and growth factor genes could distinguish between diffuse proliferative lupus nephritis (class IV LN) and others and whether it is able to predict the response to therapy. Prebiopsy urine samples were collected from 26 LN patients. Urine specimens were serially collected over a period of 6 months from class IV LN patients who were receiving standard immunosuppressive treatments. Urinary interferon-producing protein 10 and its CXC chemokine receptor (CXCR)3, transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF) mRNA levels were analyzed by quantitative real-time polymerase chain reactions. Levels of chemokine or growth factor mRNAs in urine could distinguish class IV LN from others, with a sensitivity of 85% and a specificity of 94%. The receiver-operative characteristic curve demonstrated that urine mRNA levels of these genes could identify active class IV LN with an accuracy greater than the current available clinical markers, namely systemic lupus erythematosus (SLE) disease activity index, proteinuria, renal function, or urinalysis. A significant reduction of interferon-producing protein 10 (IP-10), CXCR3, TGF-β, and VEGF mRNA levels from baselines was observed in patients who responded to therapy, whereas the levels tended to increase in those who resisted to treatment. Measurement of urinary chemokine and growth factor mRNAs can precisely distinguish class IV LN from others. Temporal association between these markers and therapeutic response is demonstrated. This noninvasive approach serves as a practical tool in diagnosis and management of LN.

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KEYWORDS: lupus nephritis; chemokine; growth factor; interferon-producing protein 10; transforming growth factor-β; vascular endothelial growth factor

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Clinical manifestations of lupus nephritis (LN) could vary from asymptomatic urinary abnormalities to rapidly progressive renal failure. Severity of LN can fluctuate during the course of the disease and treatment; thus, it requires serial monitoring.[1,2] Although urinalysis or quantification of urinary protein levels is simple and may aid the diagnosis, it cannot distinguish among various LN classes. Assessments of serum auto-antibodies and complement levels also have limited value.[3–5] Knowing the nature or type of glomerular pathology is crucial in predicting the prognosis of the patients. For example, a patient with class IV LN usually turns into an advanced state of chronic kidney disease within a few months, whereas a class II or class V patient may maintain nearly normal renal function.[4] Therefore, kidney biopsy is necessary not only for establishing the diagnosis but also for confirmation of relapse.[3,6] Although essential, repeated renal biopsy is not practical due to its invasiveness and potential unacceptable complications. Therefore, a noninvasive tool to monitor relapse as well as to guide treatment decision is extremely warranted.

Substantial evidence suggests that T-helper 1 (Th-1) type chemokines, in particular γ-interferon-inducible protein (IP-10), contribute to the inflammatory cell infiltration of affected organs in SLE patients.[7,8] IP-10 is expressed and secreted by monocytes and endothelial cells. In a lupus mouse model, this chemokine regulates the Th1-cell migration into kidney and lung via interaction with CXC chemokine receptor (CXCR)3.[8] Neutralizing monoclonal antibodies or small-molecule inhibitors that disrupt CXCR3 function markedly attenuates the inflammatory response, resulting in reduction of kidney damages.[9,10] In human SLE, serum levels of IP-10 protein were high, particularly in patients with active nephritis.[11] The increased urinary levels of monocyte chemoattractant protein-1 and IFN-γ proteins in active LN patients support the pathological

roles of these Th-1 type chemokines.[12,13] Growth and sclerosing factors such as transforming growth factor-β (TGF-β) may also play a pivotal role in the initiation of inflammation and the progression of renal fibrosis.[14–16] TGF-β is a potent inducer of vascular endothelial growth factor (VEGF) expression.[17,18] VEGF can increase vascular permeability and lead to proteinuria. Coexpression of both genes has been demonstrated in renal tissue containing accelerated tissue repair and fibrosis.[19] One study has revealed increased VEGF gene expression in both plasma and renal tissue from LN patients.[20]

Determination of cytotoxic T-lymphocyte messenger RNAs (mRNAs) of urine cells has been shown to be useful in the diagnosis of acute renal allograft rejection.[21] Similarly, urinary mRNAs of some Th-1 cytokines, chemokines, and growth factors correlate with SLE disease activity index (SLEDAI) and clinically defined renal flare. However, this approach cannot precisely distinguish diffuse proliferative LN (class IV LN) from other types.[12,13] Importantly, 15–20% of patients with class IV LN respond poorly to the standard therapy and cannot be predicted by currently available markers.[22–24] Therefore, a new surrogate marker is needed.

Measuring mRNA from urine cells can partly reflect the molecular milieu of the whole kidney.[21,25] Thus, the levels of such gene candidates could be employed to study pathophysiologic mechanisms and disease processes. Fluctuating levels of the urinary mRNA of some genes may be markers of the severity or chronicity of the disease. The present study is aimed to determine whether the mRNA levels of chemokine IP-10 or CXCR3 and growth factor TGF-β or VEGF in urine cells could be applied individually or in combination for routine clinical use.

RESULTS

Patient characteristics

We studied urine samples ($n=52$) at baseline from 26 patients. In all, 10 urine samples from healthy females served as controls. The demographic and clinical data of studied patients are summarized in Table 1. There were 14 patients with class IV, three with class II, three with class III, four with class V, and two with class VI LN. The mean $\pm$ standard deviation of baseline pathological activity and chronicity indices for class IV group were 12 ± 6 and 4 ± 4, respectively.

Elevation of urinary chemokine, IP-10, and CXCR3, mRNA levels in class IV LN

The mRNA levels of chemokine, IP-10, and CXCR3 in urinary cells were higher in patients with class IV LN as compared with those in other classes (2.44 ± 0.14 vs 1.06 ± 0.19; $P<0.001$ and 1.81 ± 0.17 vs 0.99 ± 0.16 copies/μg total RNA; $P=0.002$, respectively) (Figure 1a and b). The natural log-transformed chemokine mRNA levels were used as the dependent variable in one-way ANOVA to test the differences between the LN classes. The urinary IP-10 and CXCR3 mRNA levels from patients with class IV LN, measured within 2 weeks before renal biopsy, were significantly higher compared to those of class II, III, V, and VI (Table 2). No significant difference of the housekeeping gene 18s receptor RNA (rRNA) levels was observed among the five

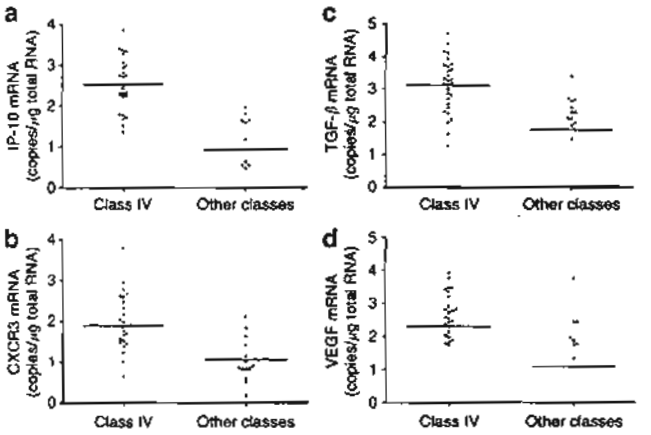

Figure 1 | Levels of mRNA in urine cells. Dots and bars show (log)mRNA levels and means for (**a**) IP-10, (**b**) CXCR3, (**c**) TGF-β, and (**d**) VEGF in urine samples from patients classified as having class IV LN or non-class IV LN. The levels of IP-10 ($P<0.001$), CXCR3 ($P=0.002$), TGF-β ($P<0.001$), and VEGF ($P<0.001$) were significantly higher in patients with class IV than in patients with other classes of LN.

Table 1 | Patient characteristics[a]

	Class IV	Other classes	*P*-value
Number	14	12 (3:3:4:2) (II:III:V:VI)	
Number of urine samples	28	24	
Age (year)	29 ± 1	30 ± 2	0.95
Gender (F/M)	13/1	10	0.90
Serum creatinine (mg/dl)	1.48 ± 0.35	1.44 ± 0.25	0.92
Creatinine clearance (ml/min)	62.9 ± 7.2	48.4 ± 9.4	0.23
Proteinuria (g/day)	2.2 ± 0.5	2.8 ± 0.7	0.43
Urinary erythrocyte count (per high power)	28 ± 9.0	14 ± 6.9	0.22
SLEDAI	6.8 ± 0.9	5.4 ± 1.0	0.32
Steroid dosage (mg/day)	33 ± 6	30 ± 7	0.75

[a]The groups of patients were identified by kidney biopsy based on WHO classification.

Table 2 | Levels of mRNA in urine cells[a,b]

	Class II	Class III	Class IV	Class V	Class VI	*P*-value
IP-10	0.86 ± 0.27	1.55 ± 0.50	2.44 ± 0.14	1.25 ± 0.26	1.1 ± 0.1	<0.001
CXCR3	1.12 ± 0.26	1.53 ± 0.24	1.81 ± 0.17	0.84 ± 0.20	0.5 ± 0.1	0.005
TGF-β	1.64 ± 0.26	2.0 ± 0.52	3.24 ± 0.13	1.40 ± 0.44	3.03 ± 0.38	<0.001
VEGF	0.27 ± 0.27	1.91 ± 0.15	2.39 ± 0.20	0.29 ± 0.29	3.03 ± 0.62	<0.001

[a]Data expressed as mean ± s.e.m. of log copies/μg total RNA.
[b]The groups of patients were identified by kidney biopsy based on WHO classification
IP-10: interferon-producing protein 10, TGF-β: transforming growth factor-β, VEGF: vascular endothelial growth factor.

groups ($P = 0.56$). The urinary mRNA levels of IP-10 and CXCR3 were not detected in the controls (data not shown).

Elevation of urinary growth factor, TGF-β, and VEGF, mRNA levels in class IV and class VI (diffuse glomerulosclerosis) LN

The mRNA levels of growth factor, TGF-β, and VEGF in urine cells were higher in patients with class IV LN as compared with those in other LN classes (3.24 ± 0.13 vs 1.81 ± 0.26; $P < 0.001$ and 2.39 ± 0.20 vs 0.99 ± 0.29 copies/μg total RNA; $P < 0.001$, respectively) (Figure 1c and d). The urinary TGF-β and VEGF mRNA levels from patients with class IV LN, measured within 2 weeks before renal biopsy, were significantly higher compared with class II, III, and V. However, there was no significant difference between the mRNA levels from class IV and class VI LN (Table 2). Interestingly, the two patients of class VI LN formerly had biopsy-proven class IV LN. Both had a history of multiple nephritis flares and had progressive deterioration of renal function despite aggressive immunosuppressive therapy.

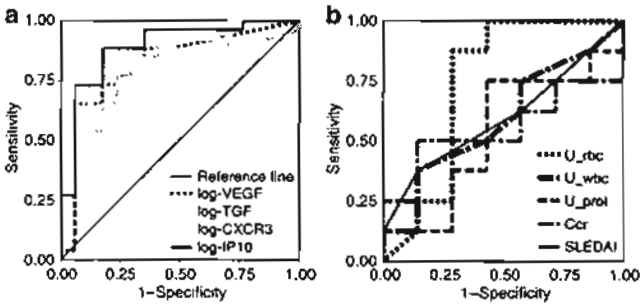

Figure 2 | ROC curves of mRNA levels and other diagnostic markers. The fraction of true-positive results (sensitivity) and that false-positive results (1−specificity) for (**a**) IP-10, CXCR3, TGF-β, and VEGF mRNA levels, and (**b**) other diagnostic markers as markers of class IV LN are shown. The calculated area under the curve was 0.89 for IP-10, 0.79 for CXCR3, 0.87 for TGF-β, 0.82 for VEGF mRNA levels, 0.598 for SLEDAI, 0.554 for creatinine clearance, 0.536 for 24-h urine protein, 0.589 for urine leukocyte count, and 0.741 for urine erythrocyte count. A value of 0.5 (reference line) is no better than expected by chance and a value of 1.0 reflects a perfect indicator.

Urine chemokine and growth factor mRNAs distinguish class IV LN from other LN classes

The receiver-operator characteristic (ROC) curves (Figure 2) depicted the true positive fractions (sensitivity) and false-positive fractions (1−specificity) at various cut points for mRNA levels for IP-10, CXCR3, TGF-β, and VEGF (Figure 2a) and other diagnostic markers: SLEDAI, creatinine clearance, 24-h urine protein excretion, urinary leucocytes, and erythrocytes (Figure 2b). The natural log-transformed cut point (threshold) that maximized the combined sensitivity and specificity for IP10, CXCR3, TGF-β, and VEGF was 2.09, 1.65, 2.50, and 1.82 copies/μg total RNA, respectively. At these thresholds, the sensitivity and specificity were 73 and 94% for IP-10, 65 and 83% for CXCR3, 85 and 83% for TGF-β, and 77 and 76% for VEGF, respectively. The calculated area under the ROC curve for IP-10 was 0.89 (95% confidence interval = 0.78–0.99), for CXCR3 was 0.79 (95% confidence interval = 0.65–0.93), for TGF-β was 0.87 (95% confidence interval = 0.76–0.97), and for VEGF was 0.82 (95% confidence interval = 0.68–0.96). Given the fact that class VI LN can probably be recognized by prebiopsy parameters such as treatment failure and irreversible deterioration of renal function, it might be interesting to assess the usefulness of these markers by leaving out the class VI patients from the analyses. Indeed, removing class VI from the analyses increased diagnostic accuracy of these genes, particularly TGF-β and VEGF. The sensitivity and specificity increased to 85 and 94% for TGF-β and 85 and 84% for VEGF, respectively.

Figure 2b shows the ROC curves for other conventional diagnostic markers with respect to class IV LN. Comparing with the urine mRNA levels, these markers poorly discriminated class IV LN from other classes of LN, as could be demonstrated by the calculated area under the curve at 0.598, 0.554, 0.536, 0.589, and 0.741 for SLEDAI, creatinine clearance, 24-h urine protein excretion, urinary leucocytes, and erythrocytes, respectively.

Pearson correlation analysis revealed that there was a significant correlation between IP-10 and CXCR3 mRNA levels in urine ($R = 0.78$, $P < 0.001$). There was a correlation between TGF-β and VEGF mRNA levels in urine as well (Figure 3a and b) ($R = 0.877$, $P < 0.0001$).

An overlapping of urinary mRNA levels between class IV and other classes (Figure 1) was observed in three patients. Among non-class IV group, two of class VI and one of class III patients showed mRNA levels comparable to those of class IV LN. Using the threshold levels described above, the

patients with class VI LN had high urinary levels of TGF-β and VEGF, but not IP-10 and CXCR3 mRNAs. One patient with class III LN had high urinary levels of all studied genes. Interestingly, this patient had subsequently developed class IV LN despite steroid therapy and required a potent immunosuppressive regimen a few months later.

Serial monitoring of urinary chemokines and growth factor mRNA levels predicts response to therapy

Serial measurements of urine mRNA levels were performed monthly from baseline to the fifth month in 10 responders and four nonresponders of class IV LN according to the criteria described below. The baseline mRNA levels did not significantly differ between these two groups. The IP-10, CXCR3, TGF-β, and VEGF mRNA levels were markedly reduced in the responder group after treatment (Figure 4a) (P-value = 0.01, 0.05, 0.01, and 0.03, respectively). In contrast, the IP-10, CXCR3, TGF-β, and VEGF mRNA levels tended to increase in the nonresponder group (Figure 4b) (P-value = 0.85, 0.66, 0.65, and 0.46, respectively). All patients in the nonresponder group had additional kidney biopsies, which showed worsening of renal pathologies. One of these patients developed crescentic glomerulonephritis with diffuse proliferative nephritis.

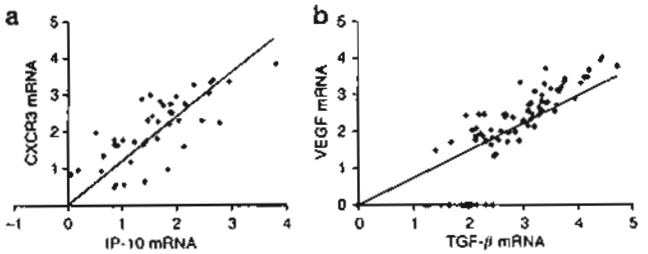

Figure 3 | Coordinated expression of mRNAs for chemokines and growth factors in urine cells. (a) The relationship between the levels of IP-10 and CXCR3 was significant at $P<0.0001$ ($R=0.78$). (b) The TGF-β and VEGF urinary mRNA was significantly correlated at $P<0.0001$ ($R=0.88$).

DISCUSSION

Successful management of LN requires a prompt diagnosis and early treatment. Abnormal urine sediments or proteinuria regardless of its degree or pattern (persistently or episodically) may be found in association with progressive renal pathology despite stable renal functions. Multiple urinary biomarkers have been shown to correlate with clinical criteria such as SLEDAI or clinically defined renal flare.[12,13,26] The present study verifies the potential utility of novel urinary biomarkers in patients who had pathologically confirmed proliferative (class IV) LN. Importantly, the application can be extended to the use in early prediction of therapeutic response in class IV LN patients. It should be emphasized that the class IV LN patients require potent immunosuppressive treatment. This monitoring tool may provide a better optimization of immunosuppressive agents in order to avoid their toxicities.

In this study, the measurement of urinary mRNA levels of IP-10 and CXCR3 chemokine genes, and TGF-β and VEGF growth factor genes is practical and should be useful in diagnosing class IV LN and in predicting clinical therapeutic response with high sensitivity and specificity. IP-10 appears to be the best candidate, followed by TGF-β, despite the fact that some overlapping levels of these mRNA can occur among patients with class IV and others, particularly class VI (diffuse glomerulosclerosis) (Figure 1). It should be noted that the two patients of class VI LN actually transformed from class IV LN and they showed resistance to prior cytotoxic therapy. Interestingly, the patients with class VI LN

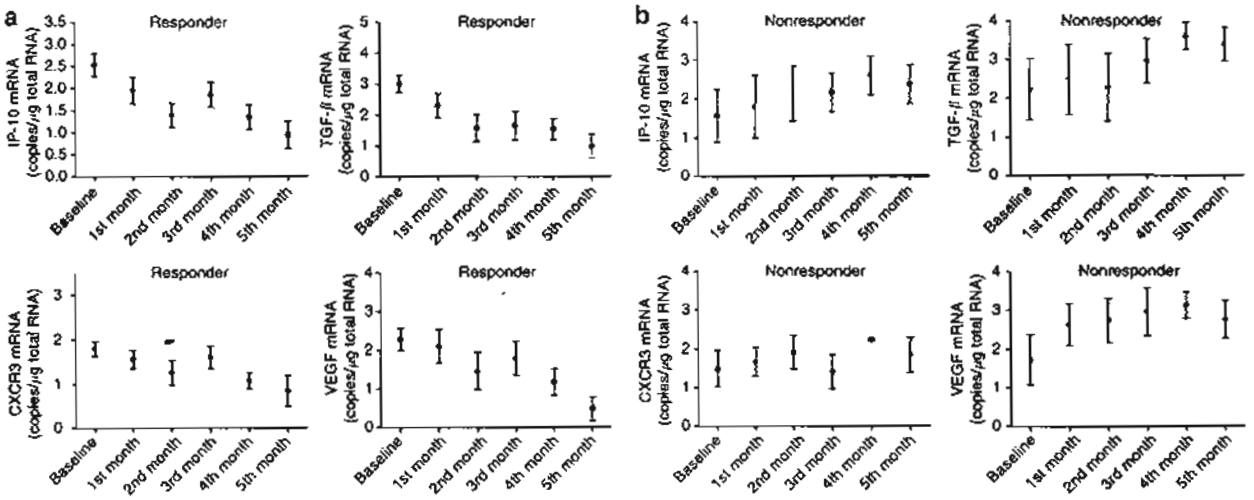

Figure 4 | Changes in urinary mRNA levels of IP-10, CXCR3, TGF-β, and VEGF over the 6-month period of treatment in patients with LN class IV. Dots and bars show means and s.e.m.'s of (log)mRNA levels for IP-10, CXCR3, TGF-β, and VEGF in urine samples from baseline through the fifth month of treatment. (a) The levels of IP-10, CXCR3, TGF-β, and VEGF were significantly decreased in the responder group, (b) whereas the levels of these genes were not changed in the nonresponder group.

had high urinary mRNA levels of TGF-β and VEGF, but not of IP-10 and CXCR3. The above findings suggest that the growth factors, not chemokines, reflect an ongoing process of tissue repair and fibrosis in the kidney. In contrast, the patient with class III (focal proliferative), which subsequently progressed into class IV, LN showed high urinary mRNA levels of both chemokines and growth factors. Taken together, monitoring of urine cells gene expression with combined biomarkers may serve as a reliable tool to distinguish class IV LN from other classes.

Currently, renal histopathologic study is the standard method of determining the presence of inflammatory process in nephritis patients. However, studying renal pathology may not give details on the progression of nephritis over a time span unless the hazardous renal biopsies are repeated. Monitoring gene expression in urine cells over time gives additional information, which would be complementary to the pathological study in understanding the ongoing inflammatory process of nephritis. Increased urinary mRNA levels of IP-10 and CXCR3 in only class IV LN support the role of Th-1 chemokines in intrarenal inflammation. Alternatively, the upregulation of TGF-β and VEGF in class IV and VI (diffuse glomerulosclerosis) may reflect tissue repair and fibrosis. It would be of interest to study whether molecular signaling could predict long-term renal function. Although the present cohort's follow-up period was short, we believe that the highly sustained expression of IP-10 and CXCR3 distinguishes an ongoing renal inflammation. In agreement with the study reported by Rovin *et al.*,[26] serial measurement of urine chemokine, monocyte chemoattractant protein-1 protein, predicts clinical renal flare and response to therapy in their prospective study. It remains intriguing as to which parameters could predict long-term renal function. Upregulation of TGF-β and VEGF found in class IV and VI in this report may reflect tissue repair and fibrosis. Alternatively, such persistent expression may predict poor outcome in the future, with sclerosis and fibrosis of the entire kidney.

Multiple conventional markers as shown here, such as proteinuria and SLEDAI, ineffectively discriminate class IV LN from others. Only urine erythrocyte count can reliably distinguish class IV renal pathology. Nevertheless, variable numbers of urine erythrocyte counts could be found in most female patients during each visit. Therefore, the presence of erythrocyte in urine cannot predict the response to treatment. There are pressing needs for a short-term LN treatment trial to compare the outcome, since current management relies on more rigid criteria, such as renal or patient survival, which require at least 5 years to complete. Given the key functions of the studied genes, the measurement of their gene transcripts may replace the conventional markers being used as surrogate markers in the clinical trials of molecularly targeted therapy such as cytokine or growth factor blockade.

Repeated attacks of LN, particularly diffuse nephritis, is the worst prognostic factor in the development of end-stage renal disease.[5] Most SLE patients, however, die from complications and infections partly from overuse of immunosuppressive agents. The monitoring of urine cell chemokines and growth factors described herein may be another useful approach for early detection of the critical stage of inflammation in the kidney of SLE patients and for tailoring immunosuppressive therapy.

MATERIALS AND METHODS

Patients

In total, 26 patients who had been diagnosed with SLE according to the American College of Rheumatology diagnostic criteria for SLE were recruited between August 2002 and September 2004. A total of 52 urine samples were obtained. Two urine samples were collected from each individual patient approximately 2 weeks and 1 day before renal biopsy. Renal involvement was documented by having one of the following criteria: a total urinary protein level of more than 0.5 g/day, an increment of serum creatinine levels of more than 0.5 mg/dl during 1 month period of follow-up, or presence of pyuria, hematuria, or urinary cast by microscopic examination. All biopsy specimens were examined by one pathologist (VK) who was not aware of the results of the molecular study. Using the classification of the histologic types of LN by World Health Organization,[27,28] 14 of 26 biopsy specimens were identified as class IV, whereas the remaining 12 specimens were categorized into other classes. All 26 patients received prednisolone (0.5 mg/kg/day) without other cytotoxic drugs, prior to renal biopsy for at least 1 month. Four patients received 5–10 mg per day of enalapril. In all, 10 urine samples from healthy women served as controls.

This study has been approved by the Ethics Committee for Human Research of the Faculty of Medicine, Chulalongkorn University, and written informed consents were obtained from all subjects.

Of the 26 patients, 14 patients with class IV LN were prospectively followed. These patients received oral prednisolone at the dosage of 0.5 mg/kg/day plus a 6-month regimen of either monthly intravenous pulse cyclophosphamide or oral mycophenolate mofetil. Urine samples were collected monthly from pretreatment baseline to the fifth month of treatment. Therapeutic response was defined either by the improvement of pathological scores of activity and chronicity based on repeated kidney biopsies, or by the following clinical criteria, including: (1) stabilization or improvement in renal function; (2) $\geqslant$50% decrease in hematuria to less than 10 RBC per high-power field; and (3) significant reduction in proteinuria ($\geqslant$50% decrease to less than 3 g/day if baseline nephrotic range, $\leqslant$1 g/day if baseline non-nephrotic) for at least 3 months.[29]

Collection of urine sample and RNA isolation

Urine was immediately centrifuged after collection at 1000 *g* 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 as described previously.[21]

Analysis of urinary mRNAs

The mRNA levels of IP-10, CXCR3, TGF-β, VEGF, and the housekeeping gene, 18s rRNA, were measured by a Light Cycler machine (Roche Molecular Biochemicals, Indianapolis, IN). The sequences of primers and fluorescence probes are as follows: IP-10

sense 5'att ttg tcc acg tgt tga gat ca3', IP-10 antisense 5'tgg cct tcg att ctg gat tc3' and IP-10 probe 5' 6-carboxy-fluorescein (FAM) aca tct ctt ctc acc ctt ctt ttt cat tgt agc a 6-carboxy-tetramethylrodamine (TAMRA)3'; CXCR3 sense 5'acc cag cag cca gag cac3', CXCR3 antisense 5'caa cct cgg cgt cat tta gc3', and CXCR3 probe 5'FAM ctt ggt ggt cac tca cct caa gga cca t TAMRA3'; TGF-β sense 5'ccc tgc ccc tac att tgg ag3', TGF-β antisense 5' ccg ggt tat gct ggt tgt aca3', and TGF-β probe 5'FAM cac gca gta cag caa ggt cct ggc c TAMRA3'; VEGF sense 5'cct aca gca caa caa atg tga atg3', VEGF antisense 5' caa atg ctt tct ccg ctc tga3', and VEGF probe 5'FAM caa gac aag aaa atc cct gtg ggc ct TAMRA; 18s rRNA sense 5'gcc cga agc gtt tac ttt ga3', 18s rRNA antisense 5'tcc att att cct agc tgc ggt atc3', and 18s rRNA probe 5'FAM aaa gca ggc ccg agc cgc c TAMRA3'. The probes were labeled with FAM at the 5' end, and with TAMRA at the 3' end. FAM serves as the reporter dye, and TAMRA serves as the quencher dye. All primer pairs were designed to span across an intron-exon boundary to distinguish an amplification of genomic DNA. Each polymerase chain reaction was set up for 20 μl reaction volume comprising of 18 μl of polymerase chain reaction mastermix (Roche Molecular Biochemical, Mannheim, Germany), 2 μl of complementary DNA template (diluted 1:1000 for 18s rRNA), 600 nM of primer, and 200 nM of probe. Polymerase chain reaction amplification included an initial denature at 95°C for 2 min, followed by heating at 95°C for 10 s and 60°C for 10 s repeated for 40 cycles. The TGF-β and VEGF plasmids (kindly provided by Karumanchi SA, Harvard Medical School, Boston, MA) and polymerase chain reaction amplicon for 18s rRNA (kindly provided by Ding R, Cornell University, New York, NY) were used for developing standard curves. The standard curves were based on the principle that a plot of the log of the initial target copy of a standard vs threshold cycles results in a straight line. Levels of mRNA were expressed as number of copies per microgram of total RNA isolated from the urine cells.

Kidney biopsy

Kidney biopsy specimens were scored for activity and chronicity index. The activity index was calculated from the sum of semiquantitative manual scores (0–3 each) of the following parameters: endocapillary hypercellularity, leucocyte infiltration, subendothelial hyaline deposits, interstitial inflammation, necrosis, and cellular crescents. Scores of the last two parameters were counted double, yielding the total range for the activity index of 0–24. The chronicity index was the sum of scores of the following parameters: glomerular sclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis.[30] The maximum score of the chronicity index was 12.

Statistical analysis

Statistical analysis was performed using the SPSS software (version 11.5). The levels of all studied mRNAs deviated significantly from normal distribution ($P \leq 0.001$), which was substantially reduced by the use of a log transformation. The natural log mRNA levels were used as dependent variables in comparison for any variable difference by Student's *t*-test (between two groups) and one-way analysis of variance with Bonferrini's correction (between groups). The relationship between the mRNA levels of each gene was estimated with Pearson's correlation. To distinguish class IV LN from others, receiver operator characteristic curve analysis of mRNA levels was used to determine the cutoff levels that maximized the combined sensitivity and specificity. The area under the curve was calculated, and sensitivity and specificity at the selected cutoffs were determined.

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investigated 14 kidney biopsies of scleroderma renal crisis by immunohistochemistry, ET-1 and anti-vWF antibodies. Results were compared to renal biopsies in the following renal pathologies : typical hemolytic and uremic syndrome (HUS, n=5), anti-phospholipid syndrome (n=6), diabetic nephropathy (n=5), Minimal Change Disease with cyclotoxicity (n=5), and ischemia (pre-transplant biopsies with nephroangiosclerosis, n=5).

ET-1 staining was increased only in glomeruli with microangiopathic lesions in scleroderma and HUS cases. In scleroderma, it was also increased in vascular lesions (mucoid endarteritis, « onion skin » arteries). In the other pathologies tested, vascular ET-1 expression was less increased, and even decreased in HUS compared to normal controls (non-tumoral part of nephrectomy for malignancies, n=5).

In conclusion, this is the first study showing ET-1 overexpression in glomeruli and vessels in scleroderma renal crisis. Our finding suggests that ET-1 may be an interesting target for the treatment of scleroderma renal crisis, as already used in scleroderma-associated pulmonary hypertension, which improves under endothelin receptor blockade (*bosentan, tracleer®*).

SA-FC103

Evidence for Activation of the IFN Pathway in Kidneys of Proliferative Lupus Glomerulonephritis (LGN). Kyriakos A. Kirou,[1] Kleio Mavragani,[1] Dee Dee Wu,[1] Pamela Cole,[1] Surya V. Seshan,[2] Mary K. Crow.[1] *[1]Hospital for Special Surgery, New York, NY; [2]New York-Presbyterian Hospital, Weill Medical College of Cornell University, New York, NY.*

Our previous data have indicated that peripheral blood mononuclear cells (PBMC) from SLE patients overexpress IFNα-inducible genes (IFIG), and that activation of the IFN pathway is associated with active disease and renal involvement. In this study, we investigated whether activation of the IFN pathway can be detected locally in kidneys of patients with lupus nephritis and whether it is associated with the clinically more active proliferative form of LGN.

Fresh frozen kidney biopsies from 28 SLE patients with nephritis, and 3 normal kidney tissue (controls), were assessed by real-time PCR for expression of IFN-inducible genes (IFIT1, IFI44, MCP1, etc), several IFN gene subtypes, and inflammatory genes (IL8, IL1b, IL-10, TNF, defensin-alpha1, etc).

There were 4 cases of pure class V (membranous), and 24 cases of proliferative GN (9, 10, and 5 cases of ISN/RPS class IV-Global, IV-Segmental, and III respectively) with or without membranous lesions.

Expression of IFIG was not elevated in any of the class V cases, but was increased in 6 (IV-G), 4 (IV-S), and 3 (III) cases of proliferative nephritis. In contrast to our previous PBMC data, kidneys with high IFIG also expressed type I (several IFNα subtypes, IFNβ, iFNτ), and type II iFN (IFNγ) genes. Notably, expression of inflammation-associated cytokines IL8, IL10, TNF, (but not IL1b) and defensin-alpha1 (neutrophil microbicidal peptide) correlated with IFIG. Only two cases (III and IV-G) demonstrated isolated type I IFN gene without evidence for IFIG or other inflammatory gene expression.

These data demonstrate that activation of the IFN pathway may be associated primarily with proliferative versus membranous lupus nephritis. These renal tissues appear to coexpress various other inflammation-associated genes, perhaps indicating (sequential or parallel) activation of multiple inflammatory pathways. Unlike PBMC, several type I and II IFN genes were detected in the tissue, suggesting local production by infiltrating or activated resident IFN-producing cells.

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SA-FC104

Decreased Intra-Renal Expression of Vascular Endothelial Growth Factor (VEGF) in Patient with Severe Proliferative Lupus Nephritis. Yingyos Avihingsanon,[1,2] Thitima Benjachat,[1,2] Vipawee Kittikovit,[1,3] Nattiya Hirankarn.[1,4] *[1]Lupus Research Unit, Faculty of Medicine; [2]Medicine, Faculty of Medicine; [3]Pathology, Faculty of Medicine; [4]Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.*

VEGF plays a pivotal role in mechanism of albuminuria in many glomerular diseases. Intra-renal gene and protein expression of VEGF in lupus nephritis remains controversy. Our aim is to study the correlation between intra-renal gene and protein expression of VEGF and severity of renal pathology. Forty-one renal biopsy samples from lupus nephritis patients were studied by real-time polymerase chain reaction and immunohistochemistry for VEGF. The same renal biopsy core was used to study both VEGF expression and histopathology. All patients had received the same immunosuppressive treatment including prednisolone and cyclophosphamide. The VEGF gene expression was normalized by 18s RNA. All data were expressed as median and inter-quartile range (IQR). The median age (IQR) of patients was 27 (23-34) years. The 24-hour urinary protein excretion was 2.0 (1.0-3.3) gram/day and erythrocyturia was 17 (2-48) cell/HPF. The serum creatinine was 0.9 (0.7-1.8) mg/dl. All biopsy samples were classified in focal or diffuse proliferative lupus nephritis. The activity and chronicity indices were 6 (1-11) and 2 (1-5), respectively. VEGF gene and protein expression were significantly decreased as compared to control (transplant donor biopsy) (-1.2 ± 0.7 vs. -0.5 ± 0.4 log copies; p=0.006). VEGF gene expression was inversely associated with high percentage (≥20%) of glomerular endocapillary proliferation (p=0.006), crescentic formation (p=0.01), high (>7.5) activity index (p=0.02) and high (>4.5)chronicity index (p=0.02). Conclusion. Intra-renal expression of VEGF is negatively associated with severe pathologic changes of lupus nephritis. VEGF may be used as a new molecular prognostic marker in proliferative lupus nephritis.

SA-FC105

Pregnancy, Chimerism, and Lupus Nephritis: A Multi-Center Study. M. Koopmans,[1] I. C. Kremer Hovinga,[1] C. Grootscholten,[2] A. M. van der Wal,[1] M. Bijl,[3] R. H. W. M. Derksen,[2] A. E. Voskuyl,[3] E. de Heer,[1] J. A. Bruijn,[1] J. H. M. Berden,[2] I. M. Bajema.[1] *[1]Pathology, LUMC, Leiden, Netherlands; [2]Nephrology, UMCN, Nijmegen, Netherlands; [3]Clinical Immunology, UMCG, Groningen, Netherlands; [4]Clinical Immunology, UMCU, Utrecht, Netherlands; [5]Rheumatology, VUMC, Amsterdam; [6]on Behalf of the Dutch Working Party on SLE.*

Chimerism may be involved in the pathogenesis of systemic lupus erythematosus (SLE). We demonstrated that chimeric cells are present twice as often in kidneys of women with lupus nephritis as in normal kidneys (JASN 2004;38A). Pregnancy is considered the most important source of chimerism, but the exact relationship between pregnancy, persistence of chimeric cells and the development of SLE has thusfar not been investigated.

Renal biopsies and clinical data came from patients included in the First Dutch Lupus Nephritis Study. Chimeric cells were identified in tissue by in situ hybridization of the Y-chromosome. A questionnaire was used to obtain detailed information on reproductive data including pregnancy history and miscarriages.

Chimerism was found in 12 of 26 (46%) renal biopsies, which is consistent with our previous results in another study group. Of 12 women who were chimeric, 5 reported a pregnancy; of 14 women who were not chimeric, 8 reported a pregnancy (not significant). Chimeric women who had been pregnant reported significantly more pregnancies than non-chimeric women who had been pregnant (p=0.04). The median age of the youngest child was higher in chimeric women (19 yrs) than in non-chimeric women (6 yrs). Chimeric and non-chimeric women did not differ with respect to age, age at diagnosis, time since diagnosis, and number of miscarriages.

Despite the attention that has been paid to pregnancy histories with respect to chimerism and SLE, this study shows that a clear-cut relationship is not apparent. There was a considerable number of chimeric women without reported pregnancies: in these women, other sources of chimerism must be considered, e.g. unrecognized pregnancies. Our data provide evidence for the chimeric cell population in patients with SLE being heterogeneous, with other sources than reported pregnancies contributing to the presence of chimerism as well.

SA-FC106

Chimerism in Childhood Lupus Nephritis. I. C. L. Kremer Hovinga,[1] M. Koopmans,[1] H. J. Baelde,[1] D. Cohen,[1] R. Goldschmeding,[2] T. Q. Nguyen,[2] K. Cransberg,[3] E. de Heer,[1] J. A. Bruijn,[1] I. M. Bajema.[1] *[1]Pathology, LUMC, Leiden, Netherlands; [2]Pathology, UMCU, Utrecht, Netherlands; [3]Nephrology, EMCR, Rotterdam, Netherlands.*

Pregnancy-derived Y-chromosome-positive chimeric cells are significantly more often present in kidneys of women with lupus nephritis than in kidneys of normal women (JASN, 2004;38A), pointing towards an important role of chimeric cells in this disease. If chimeric cells are really essential for the development of lupus nephritis, they would also have to be present in patients who have never been pregnant, and in whom chimeric cells would be derived from other sources. Therefore, we investigated whether in childhood lupus nephritis, chimeric cells were also significantly more often present than in normal controls.

In situ hybridization of the Y-chromosome was performed on 36 kidney biopsies of 29 girls (age: 4-16) with SLE and on 11 control kidneys autopsy specimens of girls (age: 1-11). Results are shown in table. For comparison, results on adult patients and adult controls are also given.

	children: SLE (N=36)	children: controls (N=11)	adults: SLE (N=57)	adults: controls (N=51)
chimerism present	12 (33%)	4 (36%)	29 (51%)	13 (25%)
chimerism absent	24 (67%)	7 (64%)	28 (49%)	38 (75%)

Y-chromosome-positive chimeric cells were not significantly more often present in girls with SLE than in normal controls, and chimerism was not related to clinical or histological parameters. Our data indicate that Y-chromosome-positive chimeric cells are not pathogenic in childhood lupus nephritis. This is in contrast to our findings that in adult lupus nephritis, chimeric cells could be involved in a host-versus-graft-like reaction (JASN 2005:51A). In adult lupus nephritis, Y-chromosome-positive cells are most likely pregnancy derived. In childhood lupus, Y-chromosome-positive cells are probably derived from brothers (transferred from the maternal circulation in utero). Apparently, sibling to sibling chimerism has a different pathogenic potential than chimerism from child to mother. This is probably due to a complex interaction of inherited and non-inherited HLA antigens, leading to a host-versus-graft reaction in only a selected number of combinations, like in transplantation pathology.

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PO250-Intra-renal expression of vascular endothelial growth factor is associated with poor outcome in lupus nephritis

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Background: Vascular endothelial growth factor (VEGF) plays a pivotal role in delayed progression of renal diseases such as remnant kidney model and thrombotic microangiopathy. It protects vascular endothelial and tubular epithelial cells from apoptosis, hypoxia and inflammation.

Purpose: This study determines an association between intra-renal VEGF gene expression and short-term renal outcome in patient with active lupus nephritis.

Method: Forty-one renal biopsy samples from lupus nephritis patients were studied by real-time polymerase chain reaction for VEGF. The same renal biopsy core was used to study both VEGF expression and histopathology. Most patients had received the same immunosuppressive treatment including prednisolone and cyclophosphamide or mycophenolate mofetil capsules. The VEGF gene expression was normalized by 18s rRNA. Receiver operating characteristic (ROC) curves analyze was used to define the cutoff level of VEGF that give the positive predictive value (PPV) and negative predictive value (NPV) for the outcomes. The outcomes at 6-month post-treatment were doubling serum creatinine and end-stage renal disease (ESRD).

Result: All data were expressed as median and inter-quartile range (IQR). The median age (IQR) of patients was 27 (23-34) years. The 24-hour urinary protein excretion was 2.0 (1.0-3.3) gram/day and erythrocyturia was 17 (2-48) cell/HPF. The serum creatinine was 0.9 (0.7-1.8) mg/dl. Eighty percent biopsy samples were classified in focal or diffuse proliferative lupus nephritis. The activity and chronicity indices were 6 (1-11) and 2 (1-5), respectively. VEGF gene expression were significantly decreased as compared to healthy control (transplant donor biopsy) ($-1.2 + 0.7$ vs. $-0.5 + 0.4$ log copies; $p = 0.006$). VEGF gene expression was inversely associated with high percentage (>20%) of glomerular endocapillary proliferation ($p = 0.006$), crescentic formation ($p = 0.01$), high activity index ($p = 0.02$) and high chronicity index ($p = 0.02$). The levels of intra-renal VEGF mRNAs at biopsy could predict ESRD ($p = 0.005$) and trend toward doubling serum creatinine ($p = 0.23$). The calculated area under the ROC curve for ESRD was 0.76 (95% confidence interval = 0.61–0.91). The PPV and NPV for the ESRD was 38 and 100%, respectively.

Conclusions: Intra-renal expression of VEGF is negatively associated with severe pathologic changes of lupus nephritis. Consequently, decreased intra-renal expression of VEGF could predict patient at risk for ESRD.

PO251-Progression of renal disease is associated with impaired clearance of EPCR

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Purpose: Candidate biomarkers for renal disease in systemic lupus erythematosus (SLE) include a subset which reflect involvement of the endothelium. We have previously reported that soluble endothelial protein C receptor (sEPCR) levels and EPCR gene polymorphisms may predict and/or reflect vasculopathy in SLE. This study sought to correlate plasma and urine levels of sEPCR and disease manifestations/severity with a focus on lupus nephritis.

Methods: 121 patients who fulfilled at least 4 ACR criteria for SLE and 51 healthy controls were recruited. eGFR was calculated by the Modification of Diet in Renal Disease equation. Plasma and urine specimens from subjects were assessed for sEPCR (ELISA). Circulating endothelial cells (CEC, isolated by immunomagnetic separation); and DNA were isolated for assessment of EPCR gene polymorphisms (PCR-RFLP). Patients were assigned to four clinical categories: 1) active renal disease (≥ 2 + protein spot urine or > 500 mg protein/24 h) 2) patients with active extrarenal disease (≥ 4 points on SELENA-SLEDAI (not serologies alone); < 500 mg protein/24 h; inactive sediment) 3) patients with inactive disease but history of renal involvement: < 4 points on SELENA-SLEDAI (not serologies alone); ≤ 1+ protein spot urine (< 500 mg protein/24 h); inactive urinary sediment and 4) patients with inactive disease and no renal history.

Results: Mean sEPCR in plasma was significantly higher in active nephritis than inactive SLE without nephritis ($p = 0.009$). In controls and nonrenal patients, urine sEPCR positively correlated with plasma values, suggesting that sEPCR is eliminated via a renal pathway. In renal patients, eGFR <70 is associated with low sEPCR in urine (<100 ng/mg creatinine). For these patients, plasma sEPCR negatively correlated with urine sEPCR, independent of proteinuria. In patients with active renal disease but normal renal function, sEPCR was elevated in plasma and in urine, independent of proteinuria. The high-shedding SNP of EPCR was more frequent in renal SLE than nonrenal or healthy controls. CECs were evident at significantly higher levels in active renal patients compared to inactive nonenal patients (39 ± 7 versus 9 ± 2, $p = 0.05$).

Conclusion: High levels of sEPCR in patients with active nephritis may increase the extent of vasculopathy as reflected by high levels of CECs. Shedding of EPCR may contribute to the progression of renal injury in patients with a filtration abnormality.

PO252-Irbesartan for the treatment of hepatitis B associated glomerulonepritis associated with quiescent lupus nephritis and persistent proteinuria

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Object: To investigate the effects of Irbesartan on persistent proteinuria in hepatitis B virus-associated glomerulonephritis (HBV-GN) associated with quiescent lupus nephritis.

Methods: Nine cases of HBV-GN associated with quiescent lupus nephritis diagnosed by the clinical data and renal biospy were included, the proteinuria of the patients was above 3.5 g/d after 24 weeks of treatment with prednisone