ความหลากหลายของโปรโมเตอร์ทูเมอร์เนคโคซิสแฟคเตอร์แอลฟายีนและความรุนแรงของภาวะไตวายฉับพลัน

นางสาวปวีณา สุสัณฐิตพงษ์

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาชีวเวชศาสตร์ (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2556
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย
The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository (CUIR)
are the thesis authors' files submitted through the University Graduate School.

TUMOR NECROSIS FACTOR ALPHA PROMOTOR POLYMORPHISM AND SEVERITY OF ACUTE KIDNEY INJURY

Miss Paweena Susantitaphong

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biomedical Sciences
(Interdisciplinary Program)
Graduate School
Chulalongkorn University
Academic Year 2013

Copyright of Chulalongkorn University

Thesis Title	TUMOR NECROSIS FACTOR ALPHA PROMOTOR POLYMORPHISM AND SEVERITY OF ACUTE KIDNEY INJURY
By	Miss Paweena Susantitaphong
Field of Study	Biomedical Sciences
Thesis Advisor	ProfessorSomchai Eiam-ong, M.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

....Dean of the Graduate School

(Associate Professor Amorn Petsom, Ph.D.)

THESIS COMMITTEE

....Chairman

(ProfessorApiwat Mutirangura, M.D.,Ph.D.)

....Thesis Advisor

(ProfessorSomchai Eiam-ong, M.D.)

....Examiner

(ProfessorVorasuk Shotelersuk, M.D.)

....Examiner

(ProfessorNattiya Hirankarn, M.D., Ph.D.)

....External Examiner

(ProfessorWichittra Tassaneeyakul, Ph.D.)

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

ปวีณา สุสัณฐิตพงษ์ : ความหลากหลายของโปรโมเตอร์ทูเมอร์เนคโคซิสแฟคเตอร์แอลฟายีนและความรุนแรงของภาวะไตวายฉับพลัน. (TUMOR NECROSIS FACTOR ALPHA PROMOTOR POLYMORPHISM AND SEVERITY OF ACUTE KIDNEY INJURY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. นพ.สมชาย เอี่ยมอ่อง, 127 หน้า.

ที่มา สารทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาเป็นไซโตไคน์ชนิดหนึ่งที่อยู่ในกลุ่มโปรอินแฟลมเมอร์เทอรี่ไซโตไคน์ซึ่งเชื่อว่ามีส่วนเกี่ยวข้องกับพยาธิกำเนิดของภาวะไตวายฉับพลัน

ขั้นตอนการทำวิจัย เป็นการศึกษาไปข้างหน้าในการหาความสัมพันธ์ระหว่างความหลากหลายของโปรโมเตอร์ยีนทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาในตำแหน่ง rs1800629 กับความรุนแรงของภาวะไตวายฉับพลัน โดยวัดค่าการทำงานของไตจากระดับซีรั่มซิสเตตินซีและระดับครีอะตรีนิน รวมถึงความรุนแรงของการบาดเจ็บที่ท่อไต โดยวัดระดับเอ็นอะซีทิลเบต้าดีกลูโคซามินิเดสในปัสสาวะ ระดับคิดนี่อินจูรีย์โมเลกุลวันในปัสสาวะ ระดับแอลฟากลูตาไทโอนแอสทรานเฟอร์เรสในปัสสาวะ ระดับพายกลูตาไทโอนแอสทรานเฟอร์เรสในปัสสาวะของผู้ป่วยที่มีภาวะไตวายฉับพลันในระหว่างนอนรักษาตัวในโรงพยาบาลจำนวน 262 ราย

ผลการศึกษา กลุ่มที่มีทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาจีโนไทป์จีเอและเอเอมีแนวโน้มที่จะมีระดับค่าครีอะตรีนินสูงสุดในระหว่างนอนโรงพยาบาลและระดับค่าครีอะตรีนินในวันที่กลับบ้านสูงกว่าในกลุ่มที่มีทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาจีโนไทป์จีจี โดยมีค่าความน่าจะเป็น 0.004 กลุ่มที่มีทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาจีโนไทป์จีเอและเอเอมีระดับซีรั่มซิสเตตินซี ระดับคิดนี่อินจูรีย์โมเลกุลวันและระดับพายกลูตาไทโอนแอสทรานเฟอร์เรสในปัสสาวะในวันที่เข้าร่วมการศึกษาสูงกว่าในกลุ่มที่มีทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาจีโนไทป์จีจี โดยมีค่าความน่าจะเป็น 0.04, 0.03 และ 0.03 ตามลำดับ ภายหลังการปรับค่าที่อาจจะรบกวนการแปลผลด้วยเพศ อายุ เชื้อชาติ พื้นฐานของระดับการทำงานของไต ภาวะติดเชื้อในกระแสเลือด และการฟอกเลือด พบว่าทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาจีโนไทป์จีเอและเอเอมีระดับค่าครีอะตรีนินสูงสุดในระหว่างนอนโรงพยาบาล และระดับคิดนี่อินจูรีย์โมเลกุลวันในปัสสาวะในวันที่เข้าร่วมการศึกษาสูงกว่ากลุ่มที่มีทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาจีโนไทป์จีจี ทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาจีโนไทป์จีเอและเอเอยังมีระดับความรุนแรงของการบาดเจ็บในหลาย ๆ อวัยวะสูงกว่าทูเมอร์เนคโครซิสแฟคเตอร์แอลฟาจีโนไทป์จีจี ภายหลังการปรับค่าที่อาจจะรบกวนการแปลผลด้วยเพศ อายุ เชื้อชาติ และภาวะติดเชื้อในกระแสเลือด

สรุป ความหลากหลายของโปรโมเตอร์ยีนทูเมอร์เนคโครซิสแฟคเตอร์ยีนตำแหน่ง rs1800629 มีความสัมพันธ์กับความรุนแรงของภาวะไตวายฉับพลันและการบาดเจ็บของอวัยวะต่างๆ ในร่างกายของผู้ป่วยที่มีภาวะไตวายฉับพลันในระหว่างนอนโรงพยาบาล

สาขาวิชา ชีวเวชศาสตร์ ลายมือชื่อนิสิต ....

ปีการศึกษา 2556 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....

5587828620 : MAJOR BIOMEDICAL SCIENCES
KEYWORDS: TNFA GENE / POLYMORPHISM / ACUTE KIDNEY INJURY / KIM-1 / NAG / PI-GST

PAWEENA SUSANTITAPHONG: TUMOR NECROSIS FACTOR ALPHA PROMOTOR POLYMORPHISM AND SEVERITY OF ACUTE KIDNEY INJURY. ADVISOR: PROF.SOMCHAI EIAM-ONG, M.D., 127 pp.

Background: Tumor necrosis factor alpha (TNFA) is a pro-inflammatory cytokine that has been implicated in the pathobiology of acute kidney injury (AKI).

Methods: We explored the association of a functional polymorphism in the promoter region (rs1800629) of the TNFA gene with severity of AKI, as defined by levels of glomerular filtration rate (GFR) [serum cystatin C (using immunonephelometry technique) and serum creatinine (using Modified Jaffe method)] and tubular injury [urinary N-acetyl-β-D-glucosaminidase (NAG, using colorimetric assay) , kidney injury molecule-1 (KIM-1,using microsphere-based Luminex assay), alpha-glutathione s-transferase (alpha-GST, using sandwich ELISA), and pi-glutathione s-transferase (pi-GST ,using sandwich ELISA)] markers, in 262 hospitalized AKI adults.

Results: In unadjusted analyses, TNFA GA- and AA-genotype groups had significantly higher peak (P=0.004), and discharge serum creatinine level (P=0.004), and enrollment serum cystatin C level (P=0.04) than TNFA GG-genotype. TNFA GA- and AA-genotype groups also had significantly higher urinary KIM-1 level (P=0.03), and urinary pi-GST level (P=0.03) when compared with the GG-genotype. After adjustment for sex, race, age, baseline estimated GFR, sepsis, and dialysis requirement, TNFA GA- and AA-genotype groups had a higher peak serum creatinine of 1.03 mg/dl (0.43, 1.63; P=0.001) and a higher urinary KIM-1 level (relative ratio 1.73; 95% CI 1.16, 2.59; P=0.008) when compared with the GG-genotype. TNFA GA- and AA-genotype groups also had a higher multiple organ failure score of 0.26 (95% CI 0.03, 0.49; P=0.024) after adjustment for sex, race, age, and sepsis when compared with the GG-genotype.

Conclusions: The TNFA rs1800629 gene polymorphism is associated with markers of kidney disease severity and distant organ dysfunction among patients with AKI. Both monoclonal antibodies to TNF-alpha as well as soluble TNF receptors that can neutralize this cytokine and result in its biologically inactive form might be the novel treatment for AKI. Larger studies are needed to confirm these relationships and effect of new treatment on surrogate outcomes

Field of Study: Biomedical Sciences   Student's Signature ....

Academic Year: 2013   Advisor's Signature ....

ACKNOWLEDGEMENTS

I would like to thank everyone who encouraged and supported me to complete this dissertation. This dissertation could not be accomplished without the support of Dr. Bertrand L. Jaber, St. Elizabeth’s Medical Center, Tufts University School of Medicine, Boston, USA, Dr. Nattiya Hirankarn, Dr. Apiwat Mutirangura and Dr. Somchai Eiam-Ong, King Chulalongkorn University Hospital, Chulalongkorn University, Bangkok, Thailand.

This study was funded in part by grants from the National Institutes of Health: DK065102 and DK077751 (to Dr. Jaber), and DK083428 (to Dr. Perianayagam). This work was also feasible in part through an investigator-initiated research study funded by Argutus Medical, Ltd, Dublin, Ireland (to Dr. Jaber), and an International Society of Nephrology funded Fellowship (to Dr. Susantitaphong).

CONTENTS

Page

THAI ABSTRACT iv

ENGLISH ABSTRACT v

ACKNOWLEDGEMENTS vi

CONTENTS vii

LIST OF TABLES x

LIST OF FIGURE xi

LIST OF ABBREVIATIONS xiii

CHAPTER I INTRODUCTION 1

1. Background and rationale 1

1.1 Acute kidney injury 1

1.2 Incidence of AKI 5

1.3 Early detection of AKI 6

1.3.1 Serum and Urine Biomarkers of AKI 6

A) Functional markers 7

B) Up-regulated proteins 8

C) Tubular enzymes 10

1.3.2 Genetic polymorphism in AKI 11

2. Research questions 12

3. Research hypotheses 13

4. Research objectives 13

CHAPTER II REVIEW OF RELATED LITERATURE 14

1. Incidence and Prognosis of AKI 14

2. Etiology of AKI 26

3. The pathophysiology change in AKI 30

3.1 Microvascular change in glomerular and medullary 31

3.1.1 Vascular reactivity and endothelial Injury 31

3.1.2 Inflammation, leukocytes, and adhesion molecules 33

Page

3.2 The tubulular cell as a contributor to the inflammatory response. 34
4. The immune response in acute inflammatory states 34
4.1 SIRS 35
4.2 CARS 35
5. Role of the inflammatory response in AKI 36
6. Role of oxidative stress in AKI 37
7. Role of nitric oxide in AKI 39
8. Organ crosstalk during AKI 39
9. Early detection of AKI 43
9.1 Serum and Urine Biomarkers of AKI 43
9.1.1 Cystatin C 43
9.1.2 N-acetyl-B-(D)-glucosaminidase (NAG) 43
9.1.3 Kidney injury molecule-1 (KIM-1) 43
9.1.4 Glutathione S-transferase (GST) enzyme 44
9.2 Role of Genetic Polymorphism in AKI 45
9.2.1 Gene and genome 45
9.2.2 Human genetic variability 47
9.2.3 Type of human gene polymorphism 48
9.2.4 Identifying human disease genes and susceptibility factors 50
9.2.5 Genetic Approaches for Identifying Disease Genes 55
9.2.6 Methods Used for the Detection of Mutations 57
9.2.7 Genetic Polymorphism of Acute Inflammatory Response 59
9.2.8 Genetic Polymorphism in AKI 65
9.2.9 The TNF Locus 67
CHAPTER III MATERIALS AND METHODS 68
1. Study design 68
2. Sample size calculation 68

Page

3. Study population 69

4. Data collection 70

5. DNA Extraction and Genotyping Analyses 72

6. Serum Measurement of Glomerular Filtration Markers 73

7. Urinary Measurement of Tubular Injury Markers 73

8. Statistical Analysis 74

CHAPTER IV RESULTS 76

1. First part 76

1.1. Study Characteristics and Quality Assessment 76

1.2. Pooled Incidence Rate of AKI 77

1.3. AKI- Associated Mortality Rate 79

2. Second part 81

2.1. Characteristics of the cohort Stratified by *TNFA* genotypes 81

2.2. Association of *TNFA* genotypes with filtration and tubular injury markers. 85

CHAPTER V DISCUSSION 93

REFERENCES 102

VITA 127

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

LIST OF TABLES

TABLE PAGE

1 Criteria for acute kidney injury 3

2 Biomarkers for AKI 7

3 Odds ratios for death in patients with and without AKI according to KDIGO... 17

4 Excess lengths of stay and costs associated with AKI by the KDIGO definition 18

5 Incidence of AKI in high income as well as low and middle income countries...20

6 Prevention, assessment and management of AKI 24

7 Causes of prerenal AKI 27

8 Causes of parenchymatous AKI 28

9 Causes of obstructive AKI 30

10 Genetic Approaches for Identifying Disease Genes................................ 56

11 Methods used for the detection of mutations 58

12 Selected list of polymorphism of immune response genes in humans 60

13 Positive associations between polymorphism of immune response 62
genes and acute infectious and inflammatory disorders

14 Characteristics of the cohort according to the *TNFA* rs1800629 genotypes ... 84

15 Association of the *TNFA* rs1800629 polymorphism with filtration markers.... 87

16 Association of the *TNFA* rs1800629 polymorphism with tubular injury markers. 90

LIST OF FIGURE

FIGURE PAGE

1 Hospital mortality rates in renal replacement therapy patients and matched … 4
control patients.

2 Risk of chronic dialysis in association with acute kidney injury and dialysis… 5

3 Incidence of various organ failures among critically ill patients………………… 14

4 Incidence and stages of AKI according to the RIFLE, AKIN, KDIGO, and CK…15
definition

5 Incidence of AKI according to the KDIGO definition across clinical settings… 16

6 Population incidence rate of dialysis-requiring AKI and in-hospital incidence…19
of dialysis-requiring AKI by regions from 2007-2009

7 Population incidence rate of dialysis-requiring AKI versus case fatality rate… 20
By regions from 2007-2009

8 The global burden of AKI ………………………………………………………… 21

9 Estimated burden of AKI with progression to CKD and death across the world 22

10 Conceptual framework and targeted approach for raising awareness of AKI… 23

11 Outcome of AKI or ARF……………………………………………………………… 25

12 Characteristics of AKI…………………………………………………………… 26

13 Pathophysiology change of AKI……………………………………………………32

14 Hypothetical model of the role of chemokines and chemokine receptors 33
in renal disease

15 Inflammatory response in AKI……………………………………………… 36

16 Key pathways involved in the generation and degradation of oxidants … 38
in AKI

17 AKI and organ crosstalk ……………………………………………………… 40

18 Pathological mechanisms between AKI and ALI. ….…………………………… 41

FIGURE PAGE

19 The central Dogma of Molecular Biology.. 45

20 Flow of genetic information from DNA to protein 46

21 Structure of a human gene, sites of polymorphism, and functional relevance. 48

22 Types of gene polymorphism 49

23 Representative DNA gels of the different TNF-α –308 G to A.............. 73

24 Pooled incidence rate of AKI in studies that used KDIGO-equivalent...... 78

25 Pooled incidence rate of AKI by world zone in studies that used ...... KDIGO-equivalent 78

26 Pooled AKI-associated mortality rate in studies that used KDIGO-equivalent 80

27 Pooled unadjusted odds ratios for all-cause mortality in patients with AKI relative to patients without AKI in studies that used KDIGO-equivalent 81

28 Hardy-Weinberg equilibrium test 82

29 Multiple organ failure (MOF) score stratified by the .... *TNFA* rs1800629 gene polymorphism 83

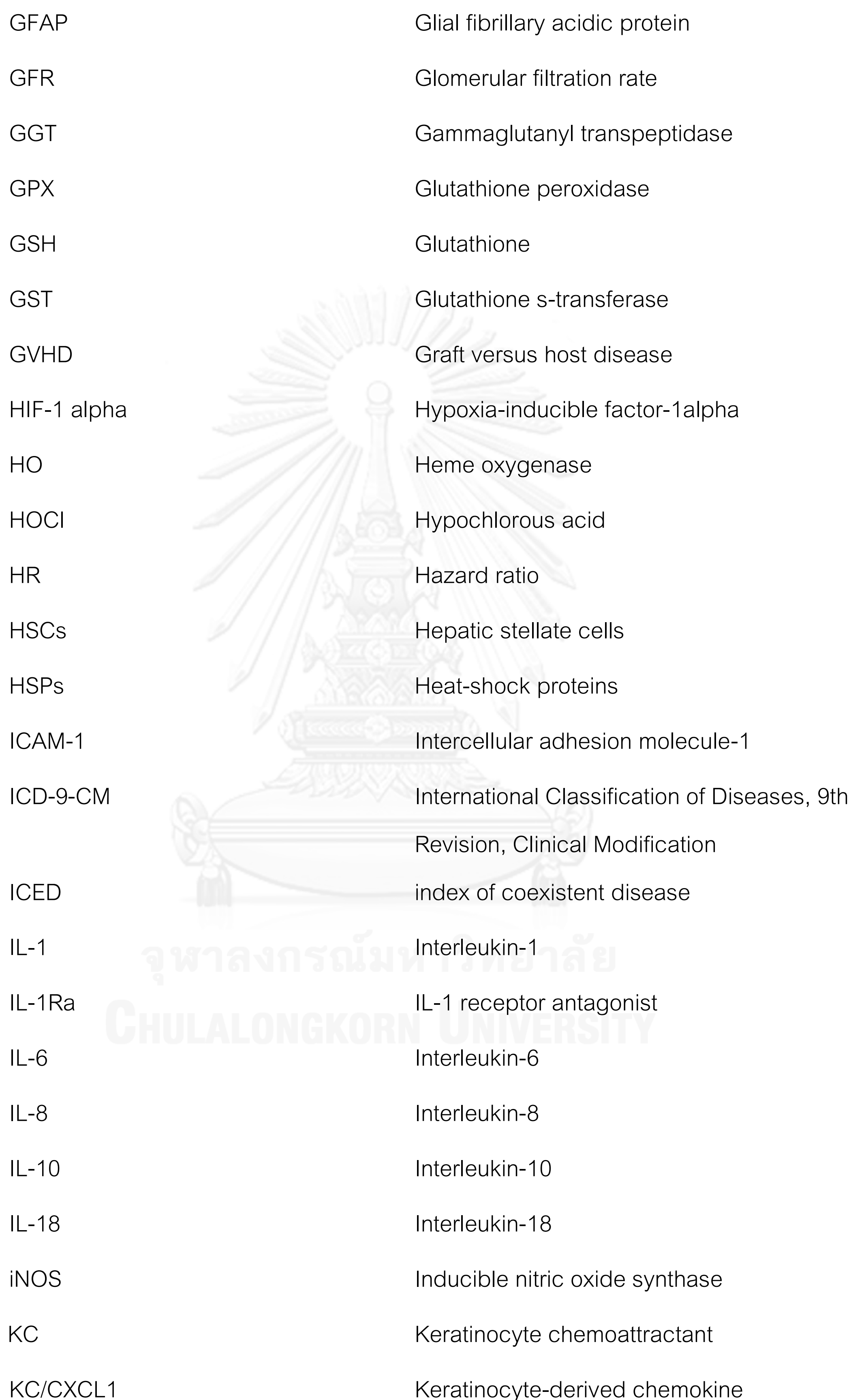
จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

LIST OF ABBREVIATIONS

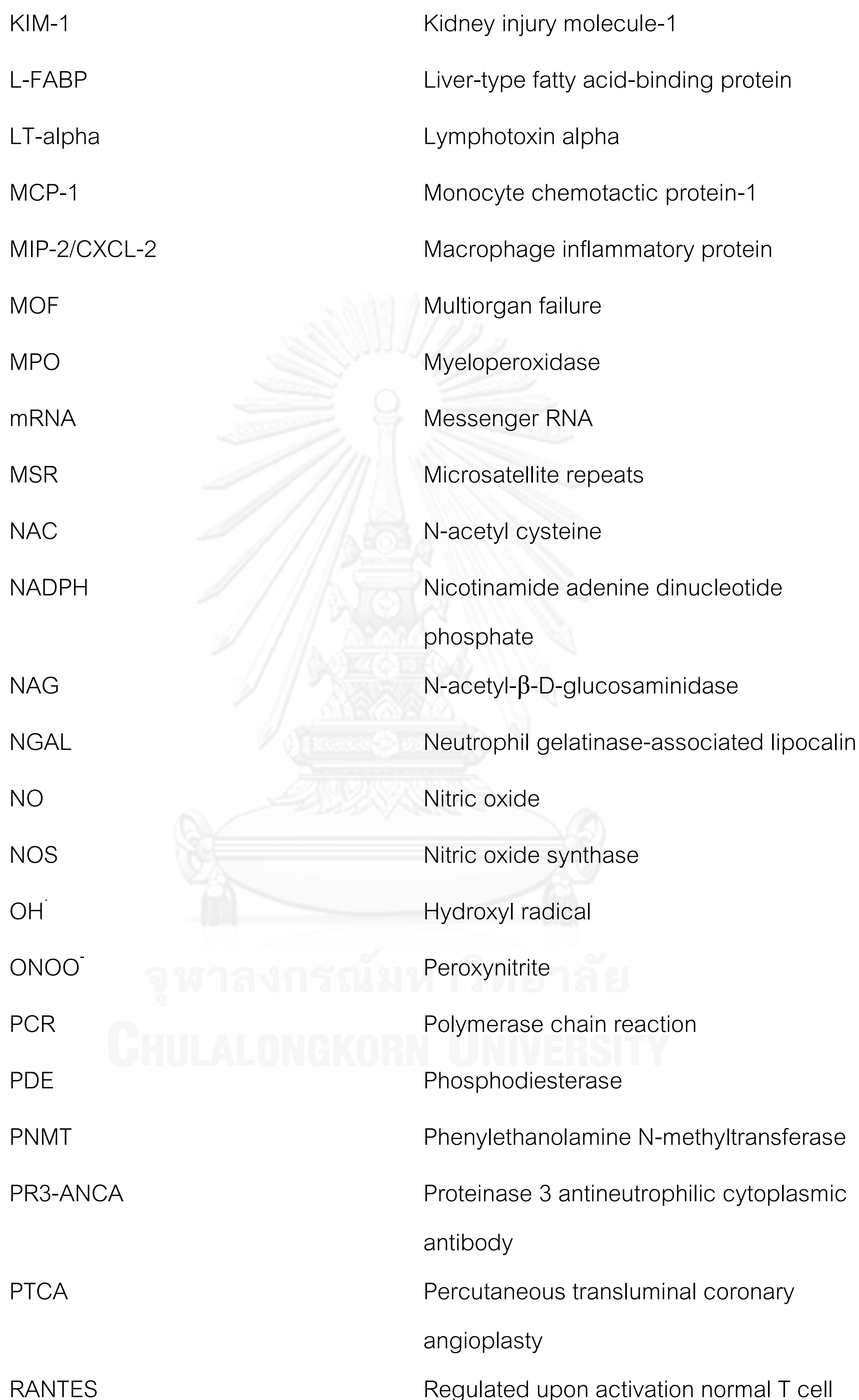
ADQI	Acute Dialysis Quality Initiative
AKI	Acute kidney injury
AKIN	Acute kidney injury network
ALI	Acute lung injury
AP	Alkaline phosphatase
APACHE II	Acute Physiology and Chronic Health Evaluation II
AUC	Area under curve
BMI	Body mass index
CARS	Compensatory anti-inflammatory response syndrome
cGMP	Cyclic guanosine monophosphate
CKD	Chronic kidney disease
CPB	Cardiopulmonary bypass
Cr	Creatinine
CRP	C-reactive protein
CX3CL1	Chemokine (C-X3-C motif) ligand 1
CYBA	Cytochrome
CysC	Cystatin C
DRG	Diagnosis-related group
eGFR	Estimated glomerular filtration rate
eNOS	Endothelial nitric oxide synthase
ESRD	End-stage renal disease
G-CSF	Granulocyte colony-stimulating factor

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

GFAP	Glial fibrillary acidic protein
GFR	Glomerular filtration rate
GGT	Gammaglutanyl transpeptidase
GPX	Glutathione peroxidase
GSH	Glutathione
GST	Glutathione s-transferase
GVHD	Graft versus host disease
HIF-1 alpha	Hypoxia-inducible factor-1alpha
HO	Heme oxygenase
HOCl	Hypochlorous acid
HR	Hazard ratio
HSCs	Hepatic stellate cells
HSPs	Heat-shock proteins
ICAM-1	Intercellular adhesion molecule-1
ICD-9-CM	International Classification of Diseases, 9th Revision, Clinical Modification
ICED	index of coexistent disease
IL-1	Interleukin-1
IL-1Ra	IL-1 receptor antagonist
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-18	Interleukin-18
iNOS	Inducible nitric oxide synthase
KC	Keratinocyte chemoattractant
KC/CXCL1	Keratinocyte-derived chemokine



KIM-1	Kidney injury molecule-1
L-FABP	Liver-type fatty acid-binding protein
LT-alpha	Lymphotoxin alpha
MCP-1	Monocyte chemotactic protein-1
MIP-2/CXCL-2	Macrophage inflammatory protein
MOF	Multiorgan failure
MPO	Myeloperoxidase
mRNA	Messenger RNA
MSR	Microsatellite repeats
NAC	N-acetyl cysteine
NADPH	Nicotinamide adenine dinucleotide phosphate
NAG	N-acetyl-β-D-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
NO	Nitric oxide
NOS	Nitric oxide synthase
$OH^{\cdot}$	Hydroxyl radical
$ONOO^{-}$	Peroxynitrite
PCR	Polymerase chain reaction
PDE	Phosphodiesterase
PNMT	Phenylethanolamine N-methyltransferase
PR3-ANCA	Proteinase 3 antineutrophilic cytoplasmic antibody
PTCA	Percutaneous transluminal coronary angioplasty
RANTES	Regulated upon activation normal T cell


จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

	expressed
RIFLE	Risk –Injury-Failure-Loss-End stage renal disease
ROS	Reactive oxygen species
RRT	Renal replacement therapy
SCr	Serum creatinine
SIRS	Systemic inflammatory response syndrome
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
sTNF-R	soluble TNF receptors
TGF-β	Transforming growth factor beta
TLR	Toll-like receptors
TNF	Tumor necrosis factor
TNFA	Tumor necrosis factor-alpha
UO	Urine output
3'-UTR	3'-untranslated
VNTR	Variable number of tandem repeats

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER I
INTRODUCTION

1. Background and rationale

1.1 Acute kidney injury

Kidney is the important organ that plays a role in maintaining fluid and electrolyte as well as excreting metabolite waste products. When kidney function is declined, resulting in the retention of urea, other nitrogenous waste products, and dysregulation of extracellular volume and electrolytes. Acute kidney injury (AKI) has been defined as the abrupt loss of kidney function within a week. Serum creatinine is used as a surrogate marker for determining kidney function due to ease of measurement and low cost. However, varying definitions for AKI were used in the literature making it difficult to compare result between studies. Some definitions used in clinical studies yield extreme complexity with graded increments in serum creatinine for different baseline serum creatinine values[1,2]. As an example, the epidemiology of hospital-acquired AKI in a classic study, AKI was defined as a 0.5 mg/dL increase in serum creatinine if the baseline serum creatinine was $\leq$ 1.9 mg/dL, an 1.0 mg/dL increase in serum creatinine if the baseline serum creatinine was 2.0 to 4.9 mg/dL, and a 1.5 mg/dL increase in serum creatinine if the baseline serum creatinine was $\geq$ 5.0 mg/dL[2].

In 2004, the Acute Dialysis Quality Initiative (ADQI) was created by a group of expert intensivists and nephrologists to develop consensus and evidence-based guidelines for the treatment and prevention of AKI [3]. Recognizing the need for a uniform definition for AKI, the ADQI group proposed a consensus graded definition, called the RIFLE criteria (Risk, Injury, Failure, Loss, End-stage renal disease)[4].

. The RIFLE criteria consist of three graded levels of injury (Risk, Injury, and Failure) based upon either the magnitude of elevation in serum creatinine or urine output, and two outcome measures (Loss and End-stage renal disease).The severity of AKI using RIFLE criteria correlated with worsening outcome [5]. A systematic review of 13 studies demonstrated a stepwise increase in the relative risk of death in patients who met the RIFLE criteria for various stages of AKI patients in the RIFLE stages of "risk", "injury" , and "failure" [2.4 (95%CI 1.9-3.0), 4.2 (95%CI 3.1-5.5), and 6.4 (95%CI 5.1-7.9), respectively], when compared with patients who did not have AKI [6].

However, most investigators and clinicians who use the RIFLE criteria to stratify patients with AKI do not use the GFR, because of the change in serum creatinine concentrations do not correlate with the percent decrease in GFR that is cited in the RIFLE classification, for example, a 1.5-fold increase in serum creatinine corresponds to a 33 rather than 25 percent decrease in GFR even among patients in steady state such as serum creatinine 1.0 mg/dL [7].

A modification of the RIFLE criteria was subsequently proposed by the Acute Kidney Injury Network (AKIN), including the ADQI group as well as representatives from other nephrology and intensive care societies, and called the AKIN criteria [8]. In the RIFLE criteria, the classification or staging system for AKI is composed of three stages of increasing severity, which correspond to risk (stage 1), injury (stage 2), and failure (stage 3). Loss and ESRD are removed from the staging system and defined as outcomes.

However, the timing for diagnosis is still controversial, therefore, Kidney Disease: Improving Global Outcomes (KDIGO) work group revised the definition and staging of AKI to harmonize previous RIFLE and AKIN criteria in 2011. In term of KDIGO criteria, AKI is defined as any of the following an increase in SCr by ≥ 0.3 mg/dL (≥ 26.5 μmol/l) within 48 hours; or increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or urine volume < 0.5 mL/kg/h for 6

hours. AKI is staged for severity according to the following criteria. The criteria for diagnosis AKI are summarized in Table1.

Table 1: Criteria for acute kidney injury

		Serum creatinine criteria	Urine output criteria
WRF	Definition	Increase in SCr by ≥ 0.3 mg/dL at any time during admission	
CK	Definition	Increase in SCr by ≥ 0.3 mg/dL within 24 hours or ≥ 0.5 mg/dL within 48 hours	
	Staging	1: Increase in SCr by ≥ 0.3 mg/dL within 24 hours or ≥ 0.5 mg/dL within 48 hours	
		2: Increase in SCr by ≥ 0.5 mg/dL within 24 hours or ≥ 1.0 mg/dL within 48 hours	
		3: Increase in SCr by ≥ 1.0 mg/dL within 24 hours or ≥ 1.5 mg/dL within 48 hours	
RIFLE	Definition	Increase in SCr by ≥ 1.5 times baseline within 7 days	
	Staging	R (Risk): Increase in SCr by 1.5 - < 2.0 times baseline	Urine output < 0.5 mL/kg/h x 6 hr
		I (Injury): Increase in SCr by 2.0 - < 3.0 times baseline	Urine output < 0.5 mL/kg/h x 12 hr
		F(Failure): Increase in SCr by ≥ 3.0 times baseline	Urine output <0.3 mL/kg/h x 24 hr or anuria x 12 hrs
AKIN	Definition	Increase in SCr by 0.3 mg/dL or ≥ 1.5 times baseline within 48 hours	
	Staging	1: Increase in SCr by ≥ 0.3 mg/dL or ≥ 1.5 - <2.0 times baseline	Less than 0.5 mL/kg per hour for more than 6 hours
		2: Increase in SCr by ≥ 2.0 - < 3.0 times baseline	Less than 0.5 mL/kg per hour for more than 12 hours
		3: Increase in SCr by ≥ 3.0 times baseline	Less than 0.3 mL/kg per hour for 24 hours or anuria for 12 hours
KDIGO	Definition	Increase in SCr by ≥ 0.3 mg/dl (≥ 26.5 μmol/l) within 48 hours; or increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or urine volume < 0.5 ml/kg/h for 6 hours	
	Staging	1: Increase in SCr 1.5-1.9 times baseline or ≥ 0.3 mg/dl (≥ 26.5 μmol/l) increase	Less than 0.5 ml/kg/h for 6–12 hours
		2: Increase in SCr 2.0-2.9 times baseline	Less than 0.5 mL/kg per hour for more than 12 hours
		3: Increase in SCr by ≥ 3.0 times baseline or increase in serum creatinine to ≥ 4.0 mg/dl (≥ 353.6 μmol/l) or initiation of renal replacement therapy or in patients <18 years, decrease in eGFR to < 35 ml/min per 1.73 m^2	Less than 0.3 mL/kg per hour ≥ 24 hours or anuria ≥ 12 hours

WRF- worsening renal function, CK- creatinine kinetics

Over the last decade, in multiple studies, the presence and severity of AKI using these various classification systems including RIFLE, AKIN, KDIGO have been shown to correlate with clinical outcomes, including in-hospital mortality, hospital length of stay, recovery of kidney function, and health resource utilization [8-10]. AKI has also been variably linked to a long-term risk of chronic kidney disease, end-stage renal disease, and death[11,12] (Figure 1-2).

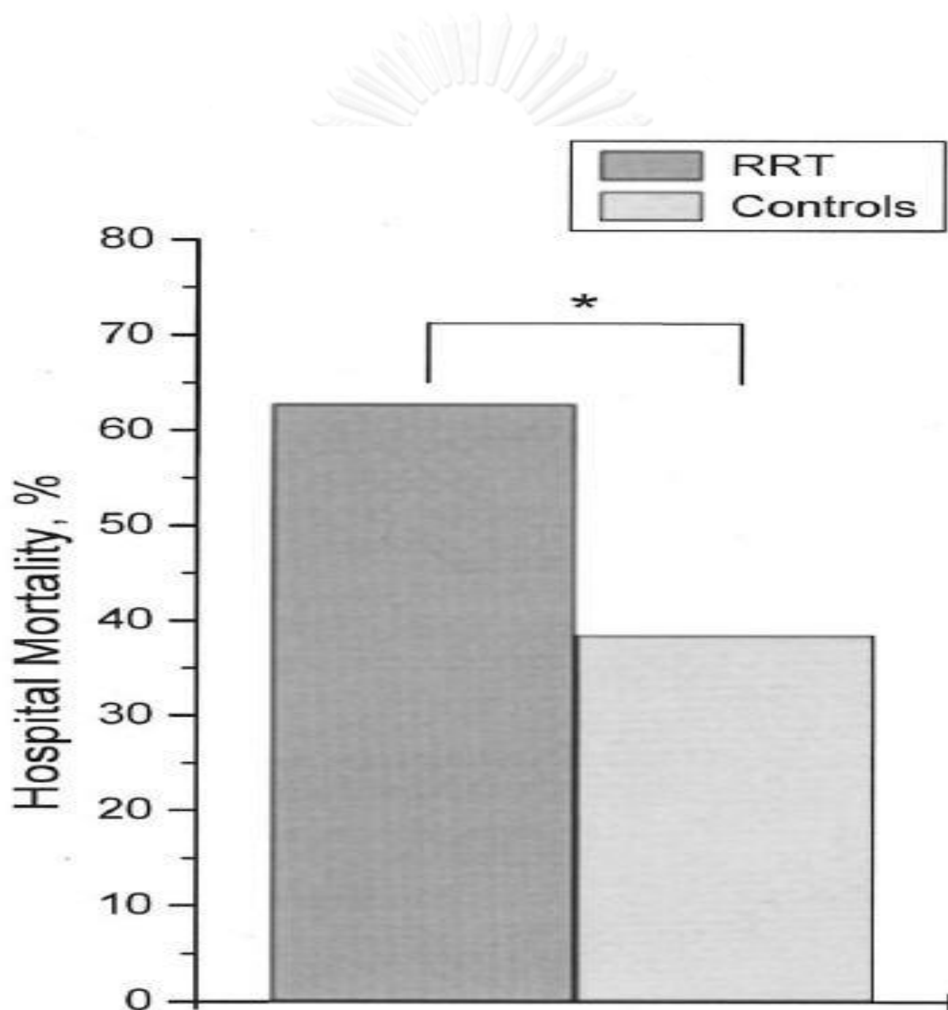


Figure 1. Hospital mortality rates in renal replacement therapy (*RRT, dialysis*) patients and matched control patients (11).

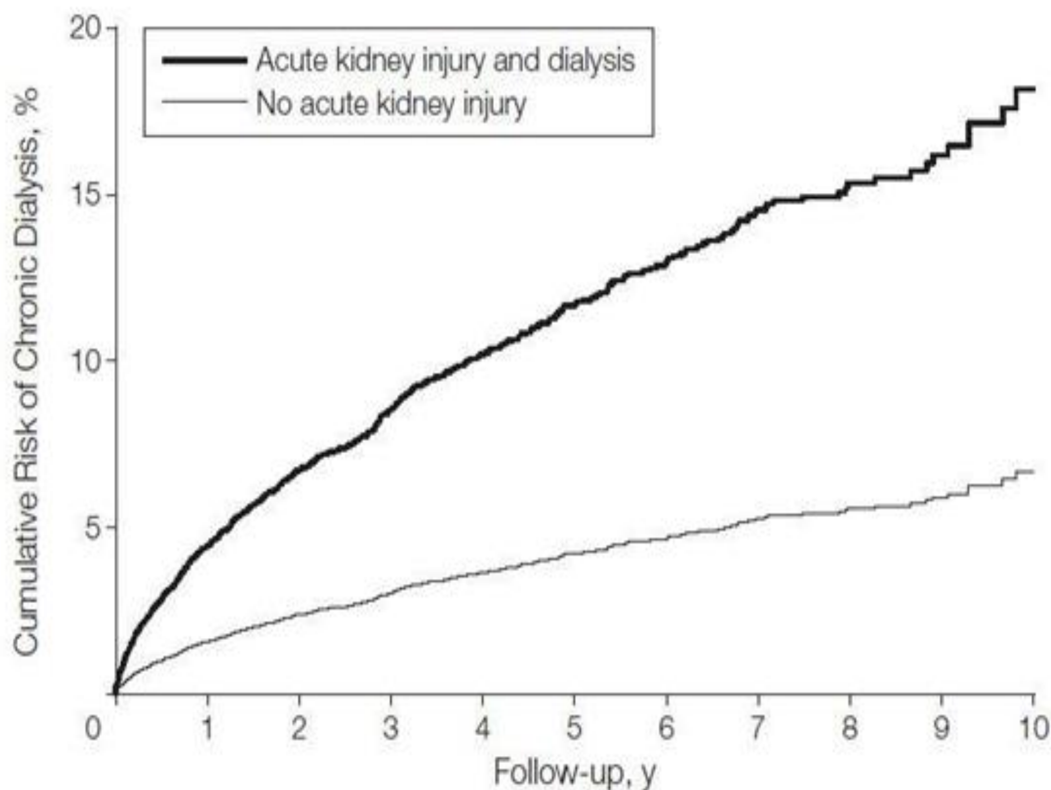


Figure 2 Risk of chronic dialysis in association with acute kidney injury and dialysis [12]

1.2 Incidence of AKI

The incidence of AKI is 5-7% in hospitalized patients [13], but it is more common in the critically ill, ranging from 29-36% [14]. The mortality rate in critically ill patients increases from 25% among those without AKI [15] to 57-63% among those who develop AKI [14,16]. Of surviving patients, 5-20 % remain dialysis-dependent at hospital discharge [17]. Zeng et al.[18] explored the incidence of AKI by using the Acute Dialysis Quality Initiative's RIFLE criteria, the Acute Kidney Injury Network (AKIN) criteria, KDIGO criteria, and a definition based on a model of creatinine kinetics (CK). Authors reported that AKI incidence was highest according to the KDIGO definition (18.3%) followed by the AKIN (16.6%), RIFLE (16.1%), and CK (7.0%) definitions. All definitions of AKI were associated with a significantly higher risk of death and higher resource utilization. The adjusted odds ratios for in-hospital mortality in those with AKI were highest with the CK

definition (5.2; 95% confidence interval [95% CI], 4.1 to 6.6), followed by the RIFLE (2.9; 95% CI, 2.2 to 3.6), KDIGO (2.8; 95% CI, 2.2 to 3.6), and AKIN (2.6; 95% CI, 2.0 to 3.3) definitions.

However, the burden of AKI around the globe has not been systematically examined. The publication of studies that used the RIFLE or AKIN classification and staging systems over the past decade provides an opportunity to generate more congruent estimates on the incidence of AKI and its associated outcomes. First meta-analysis that is the first part of this thesis will be conducted to estimate the incidence of AKI in the world, and describe geographic variations according to countries, regions and their economies. In addition, to improve the prognosis of AKI, the strategy for prevention, early detection and individualized treatment seems to be more important issue. Furthermore, risk assessment such as identification of susceptibility patients including genetic risk as well as early detection by using novel urine and serum biomarkers will be the further step to explore in this thesis, hopefully leading to individual approach and management in AKI patients as well as improving the prognosis of AKI in future.

1.3 Early detection of AKI

1.3.1 Serum and Urine Biomarkers of AKI

In general practice, although AKI is most easily detected by the serum creatinine measurement, the use of serum creatinine to quantitatively define AKI is still not a perfect marker especially in a patient who is not in steady state. In the early stages of severe AKI, the serum creatinine may be in normal range even though the actual renal function is markedly decreased because of no sufficient time for creatinine accumulation. In addition, substantial amount of creatinine is removed by dialysis in patients receiving any form of dialysis. As a result, it is usually not possible to accurately assess kidney function by measuring the serum creatinine once dialysis is initiated. One exception is when the serum creatinine continues to fall on the days when hemodialysis is not performed, indicating recovery of renal function.

Therefore, the performance of biomarkers for AKI predictor has been studied intensely in several different clinical settings. For the clinical application of a new biomarker, it should be proved to be more accurate with earlier detectability than the current gold standard serum creatinine, which is not so early marker of AKI. Several biomarkers including functional markers, up-regulated proteins, and enzymes were also explored (Table 2).

Table 2: Biomarkers for AKI

Biomarker types	Biomarkers
Functional markers	Serum creatinine and plasma/serum cystatin C
Up-regulated proteins	NGAL, KIM-1, L-FABP and IL-18
Enzymes	NAG, alpha-GST, pi-GST, GGT and AP

A) Functional markers

Some surrogate biomarkers, including serum creatinine, and serum cystatin C, are used as functional markers due to both of them are excreted through glomerular filtration.

1) Serum creatinine

Serum creatinine is a degradation product of muscle cells and represents a surrogate for the efficiency of GFR. Even though it has poor predictive accuracy for renal injury, particularly, in the early stages of AKI [19]. In critical illness setting, serum creatinine concentrations may largely fluctuate due to dilutional volume status, the catabolic effects of critical illness, and the increased tubular excretion. Furthermore, the rise in serum creatinine is slow after injury insult. Therefore, other plasma or urinary biomarkers should be used for the earliest AKI detection.

2) Serum cystatin C

Cystatin C (CyC) is a 13-kilodalton non-glycosylated cysteine protease inhibitor produced by all nucleated cells at a constant rate. In healthy subjects, serum CyC (sCyC) is excreted through glomerular filtration and metabolized completely by the proximal tubules. Furthermore, there is no evident of tubular secretion. Therefore, serum CyC was used as alternative marker of the GFR in AKI with good area under the curve (AUC) [20].

B) Up-regulated proteins [21]

After kidney injury insults, some biomarkers including Neutrophil gelatinase-associated lipocalin (NGAL), Kidney injury molecule-1 (KIM-1), Liver-type fatty acid-binding protein (L-FABP) and interleukin-18 (IL-18) are up-regulated and excreted in the urine.

1) Neutrophil gelatinase-associated lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein linked to neutrophil gelatinase in specific leukocyte granules [22]. In the normal kidney, NGAL is expressed only in the distal tubules and collecting ducts whereas proximal tubule cells also show staining for NGAL proteins, which is explained by megalin–cubilin-mediated re-uptake of NGAL present in the glomerular filtrate [23,24]. A previous meta-analysis published by Haase et al. [25] revealed that serum NGAL can predict the development of AKI across different settings with an overall Receiver Operating Characteristic -Area Under the Curve ROC-AUC of 0.782 (CI 0.689–0.872).

2) Kidney injury molecule-1 (KIM-1)

Kidney injury molecule-1 (KIM-1) is a type I transmembrane glycoprotein with a cleavable ectodomain (90 kilodalton) which is localized in the apical membrane of dilated tubules in acute and chronic injury [26,27]. KIM-1 is believed to play a role in regeneration processes after epithelial injury and in the removal of dead cells in the tubular lumen through phagocytosis [26,28].

จุฬาลงกรณ์มหาวิทยาลัย CHULALONGKORN UNIVERSITY

Susantitaphong et al. performed meta-analysis to summarize the performance of urinary KIM-1 in AKI settings, and demonstrated that the estimated sensitivity for prediction of AKI development was 66.6% (95% CI 53.1, 77.9%, P=0.017), and the specificity was 81.2% (95% CI 75.7, 85.6%, P<0.001) (unpublished data).

3) Liver-type fatty acid-binding protein (L-FABP)

Liver-type fatty acid-binding protein (L-FABP) is a 14-kDa protein expressed in the proximal tubular epithelial cells [29]. This endogenous antioxidant promotes free fatty acid metabolism by binding to long-chain fatty acid oxidation products [30].The L-FABP gene is responsive to hypoxic stress, which occurs in the setting of kidney ischemia-reperfusion injury. As a consequence, urinary excretion of L-FABP reflects the stress of proximal tubular epithelial cells, correlating with the severity of ischemic tubular injury [31-33].

A recent meta-analysis was published by Susantitaphong et al. to assess urinary L-FABP performance as AKI predictor, the estimated sensitivity of urinary L-FABP level for the diagnosis of AKI was 74.5% (95% CI, 60.4%-84.8%) and specificity was 77.6% (95% CI, 61.5%-88.2%)[34] .

4) Interleukin-18

Urinary interleukin-18 is also released after kidney injury insults. In critical illness, urine IL-18 had moderate performance for AKI predictor (ROC-AUC 0.55, 95%CI 0.47-0.62) [35]. Therefore, urine IL-18 was used as a biomarker of AKI, as well as seemed to be associated with sepsis [36]. In addition, urine IL-18 could predict AKI progression within 24 h in patients with acute lung injury with an accuracy of ROC-AUC 0.731[37]. In cardiopulmonary bypass (CPB) patients, 2 hours after CPB time, the urine IL-18 had optimal performance to yield an AUC 0.66 (95%CI 0.49–0.83) [38]. Of note, urine IL-18 seems to be one of the biomarkers of AKI predictors in several settings.

C) Tubular enzymes

Some enzymatic biomarkers including N-acetyl-β-(D)-glucosaminidase (NAG), alpha-glutathione s-transferase (alpha-GST), pi-glutathione s-transferase (pi-GST), gamma glutanyl transpeptidase (GGT), and alkaline phosphatase (AP) are excreted in the urine after kidney injury insults and also were used for early detection in several AKI settings.

1) N-acetyl-β-(D)-glucosaminidase (NAG)

N-acetyl-β-(D)-glucosaminidase (NAG) is a lysosomal enzyme (>130 kilodalton) that is localized in the renal tubules. Due to its large molecular weight, it precludes glomerular filtration, implying that urinary elevations have a tubular origin. Increased activity suggests injury to its cells but may also reflect increased lysosomal activity without cell disruption. NAG catalyses the hydrolysis of terminal glucose residues in glycoproteins. Increasing of urinary activity is correlated with severity of renal tubular damage[39-41]. Recently, urinary NAG has been utilized as diagnostic marker for the early detection of AKI [38,42,43].

2) Alpha-glutathione s-transferase (alpha-GST) and pi-glutathione s-transferase (pi-GST)

Alpha -GST and pi-GST are both members of a multigene family of detoxification enzymes present in many organs including the kidney. Distribution across the entire nephron of structurally and functionally distinct isoforms has been demonstrated. In normal condition, these enzymes are not present in urine. However, alpha-GST is primarily detected in the proximal tubular cells, whereas pi-GST is observed in the distal parts after kidney injury[44].

Both of these enzymes are used as urine biomarker for diagnosis AKI. In patients undergoing cardiopulmonary bypass (CPB), Susantitaphong et al. demonstrated that the 2-hour post-CPB pi-GST level modestly predicted the development of AKI including higher stages of AKI severity [45]. A single urinary pi-GST

measurement also performed better than alpha-GST at predicting dialysis requirement or death, but neither marker had good prognostic discrimination In patients with an established diagnosis of AKI [46].

3) Gamma glutanyl transpeptidase and alkaline phosphatase

Gamma glutanyl transpeptidase (GGT) and alkaline phosphatase (AP) are tubular brush border enzymes that are released into urine when there has been significant damage to the brush border membrane with loss of the microvillus structures. However, few clinical studies are available. In critical illness, urine GGT and urine AP had moderate predictive performance for AKI development with AUC 0.57 (95% CI 0.50–0.64) and AUC 0.56 (95%CI 0.49–0.63), respectively [35].

1.3.2 Genetic polymorphism in AKI

Gene polymorphism is the variations of human genome, which are markers of biologic diversity. It has been observed in the representing of 0.1% in general population. Genetic polymorphism might be demonstrated at any sites of human gene such as the promoter or 5'-flanking region, the exon(s) or the gene coding sequences, the intron(s) or the gene intervening sequences and the 3'-untranslated (3'-UTR) region. Genetic polymorphisms from all of these sites are potential result in different functional effect.

Recently, there has been an interest in deciphering the role of genetic polymorphisms as potential determinants of adverse outcomes in patients with AKI including the oxidative stress and inflammatory gene such as NADPH oxidase, myeloperoxidase (MPO), hypoxia-inducible factor-1alpha (HIF-1alpha), and phenylethanolamine N-methyltransferase (PNMT) [47-54].

In addition, ischemia-reperfusion and nephrotoxic injury induce the generation of pro-inflammatory cytokines, which result in morphological and functional changes in glomerular endothelial and tubular epithelial cells [55-57] in experimental settings. Cytokines can also mediate distant organ injury such as acute lung injury [58,59].

The TNF-alpha and TNF-beta genes are located on the short arm of chromosome 6. Genetic variations in transcriptional sites of the tumor necrosis factor (TNF) gene are associated with TNF synthesis. Several polymorphisms have been identified in the TNF gene promoter that affected on TNF levels [60,61].

Wilson et al. identified a biallelic G to A transition polymorphism located at position -308 in the TNF promoter, which were defined as TNF1 (-308G) and TNF2 (-308A) alleles[60]. The less common TNF-alpha gene at position –308 (G to A) [TNF2] have been associated with high promoter activity [79–81] via promoted spontaneous and stimulated TNF-alpha production in both *in vitro* [82, 83] and *in vivo* [62-66].

High circulating levels of tumor necrosis factor alpha (TNF-alpha) have been associated with adverse clinical outcomes in patients with AKI [67]. Functionally-relevant polymorphisms within the promoter region of the TNF-alpha (*TNFA*) gene, which affect transcriptional activity [68], have previously been linked to adverse clinical outcomes in critically ill patients [69-71].

Moreover, the high TNF-alpha producer and low IL-6 producer genetic variants were associated with an increased risk for AKI in low-birth-weight infants [72]. Among patients with AKI who require dialysis, TNF-alpha high producer genotype (-308 A-allele carrier) was associated with a higher risk of death after adjustment for the APACHE II score [47]. Of note, the TNF-alpha high producer genotype was associated with increased odds for a higher the index of coexistent disease (ICED) score (an index of comorbidity), lower Karnofsky Index (a measure of functional status), and lower serum albumin compared with patients with the low producer genotype for this cytokine [73].

2. Research questions

1) What is the incidence of AKI defined by KDIGO criteria around the world by using meta-analysis?

2) Is functional polymorphism in the promoter region of the *TNFA* gene (position-308, rs1800629) associated with kidney disease severity, including glomerular filtration markers and urinary tubular injury markers?

3) Can this genetic polymorphism be used as early biomarkers of AKI?

3. Research hypotheses

1) High incidence of AKI is demonstrated by using meta-analysis approach.

2) The *TNFA* rs1800629 gene polymorphism is associated with markers of kidney disease severity and distant organ dysfunction among patients with AKI.

3) Genetic polymorphism can be used as early biomarkers of AKI.

4. Research objectives

1) Primary objective

To systematically estimate the incidence of AKI in the world by meta-analysis, describe geographic variations according to countries, regions, and their economies as well as to provide a platform to raise awareness of AKI with the public, government, and healthcare professionals across the globe and a resource of incidence rate estimates for future development of public health policies, and planning of clinical trials for AKI.

2) Secondary objective

To explore the association between a functional polymorphism in the promoter region of the *TNFA* gene (position -308) ,rs1800629) and kidney disease severity, including glomerular filtration markers and urinary tubular injury markers in hospitalized adults with AKI and hope to use as early genetic biomarker and guide for individual treatment.

CHAPTER II
REVIEW OF RELATED LITERATURE

1. Incidence and Prognosis of AKI

The incidence of AKI is increasing and is also associated with high morbidity and mortality [74]. AKI has been developed in hospitalized patients between 3.2% and 20% [75] as well as has been reported between 22% and as high as 67% in intensive care units (ICUs) [76]. Interestingly, the incidences of organ dysfunction for four vital organ systems including kidney, lung, cardiovascular, and central nervous system are actually quite not different in critically ill patients, between one-third and one-half of patients have each type of organ system failure (Figure 3). Although, the incidence of AKI seems to be underestimated by using traditional measures that is likely incomplete application of the diagnostic criteria.

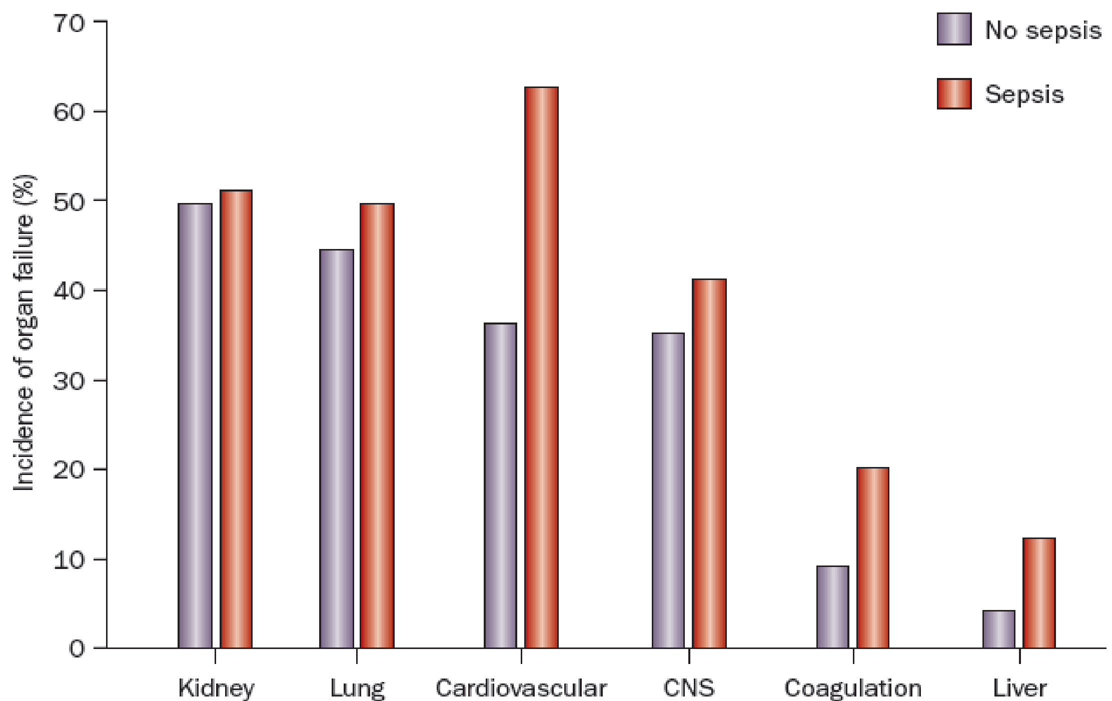


Figure 3 Incidence of various organ failure among critically ill patients. Rates of organ dysfunction in 3,417 adults with or without sepsis treated in 198 intensive care units in 24 European countries [77] Abbreviation: CNS, central nervous system.

Zeng et al.[18] explored the incidence of AKI by using the Acute Dialysis Quality Initiative's RIFLE criteria, the Acute Kidney Injury Network (AKIN) criteria, KDIGO criteria, and a definition based on a model of creatinine kinetics (CK). The authors reported that the incidence of AKI was highest according to the KDIGO definition (18.3%) followed by the AKIN (16.6%), RIFLE (16.1%), and CK (7.0%) definitions (Figure 4).

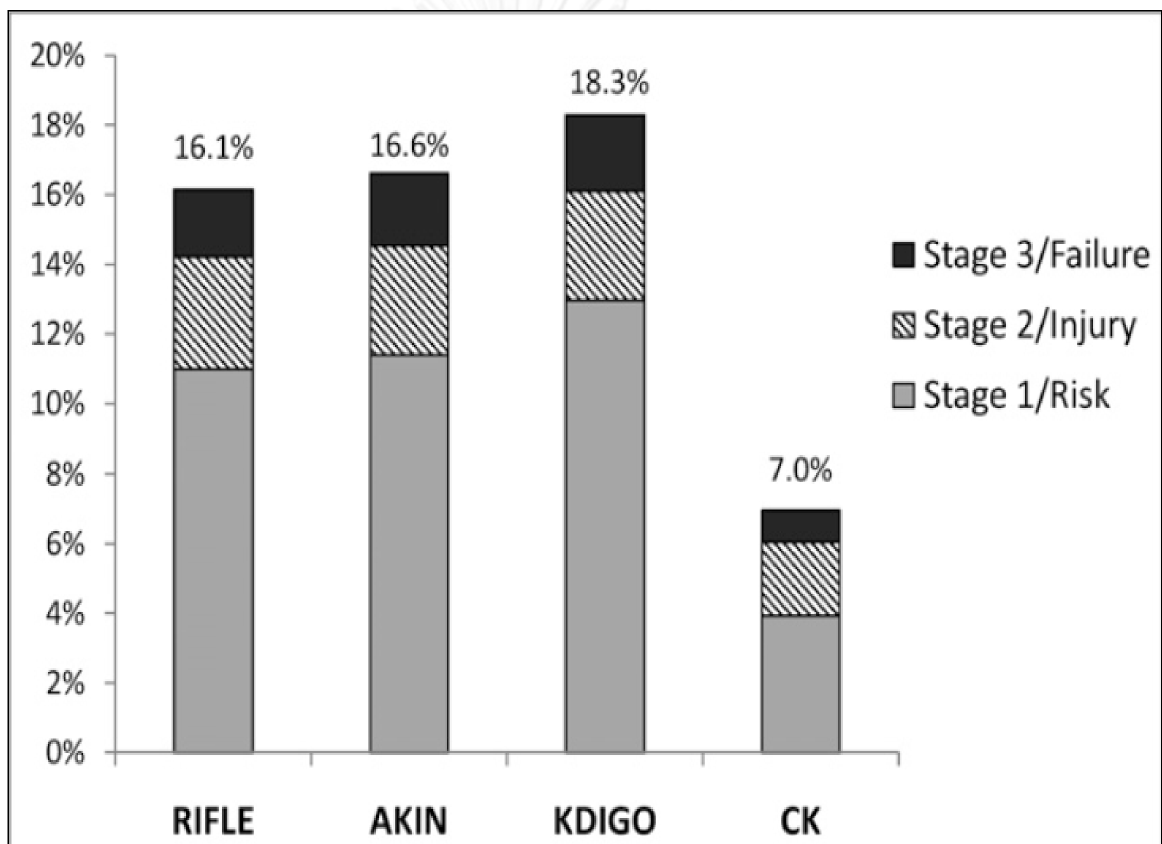


Figure 4 Incidence and stages of AKI according to the RIFLE, AKIN, KDIGO, and CK definitions (18).

The clinical settings with the highest incidence of AKI were sepsis (68.4%), mechanical ventilation (63.9%), critical care (60.3%), hematopoietic stem cell transplantation (55.9%), cardiac surgery (52.2%), and vascular surgery (50.2%) (Figure 5).

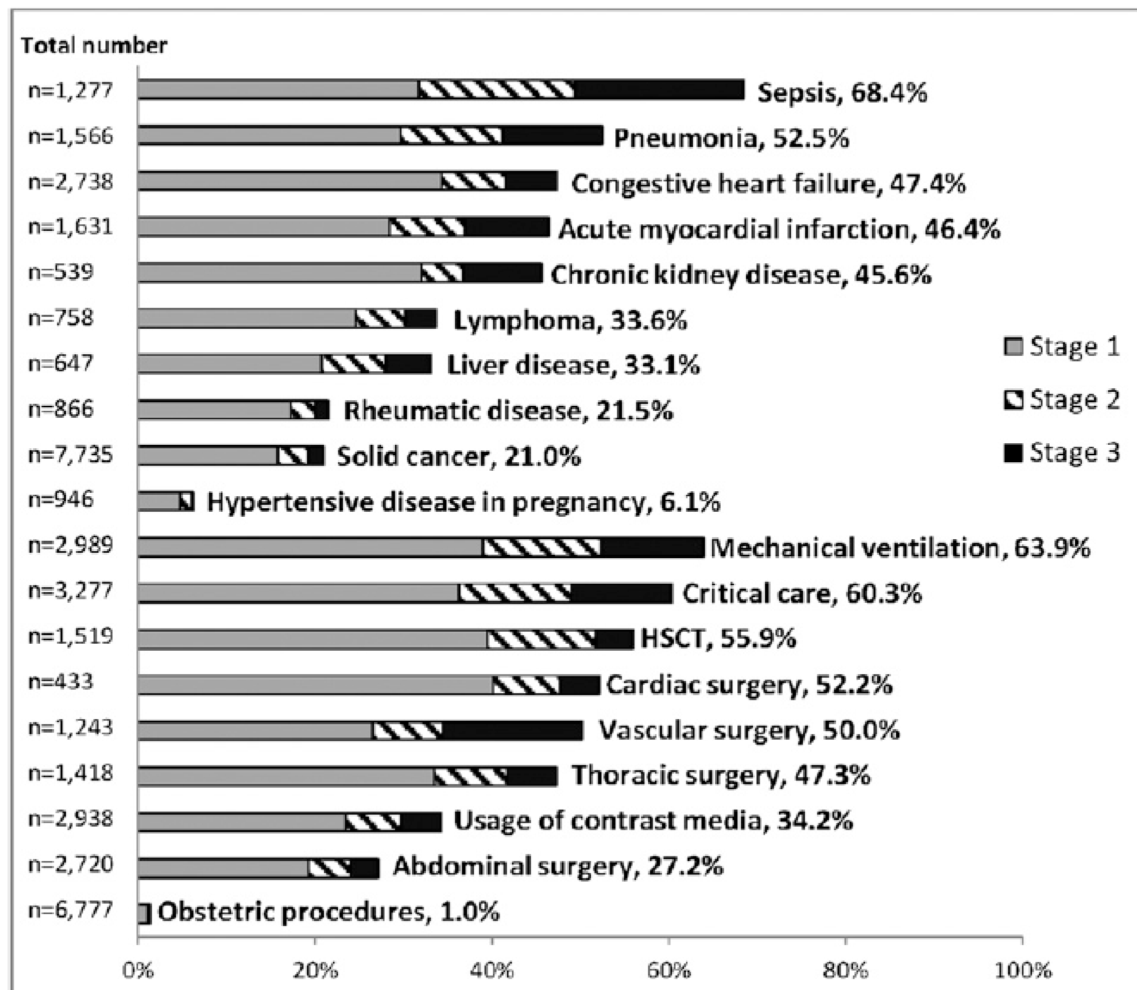


Figure 5 Incidence of AKI according to the KDIGO definition across clinical settings (18).

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

All definitions of AKI were associated with a significantly higher risk of death and higher resource utilization. The adjusted odds ratios for in-hospital mortality in those with AKI were highest with the CK definition (5.2; 95% confidence interval [95% CI], 4.1 to 6.6), followed by the RIFLE (2.9; 95% CI, 2.2 to 3.6), KDIGO (2.8; 95% CI, 2.2 to 3.6), and AKIN (2.6; 95% CI, 2.0 to 3.3) definitions.

The association between AKI and in-hospital mortality persisted but was attenuated after additional adjustment in multivariable models (Table 3). After adjusted for age, sex, race, procedures, diagnoses, diagnosis-related group weights, and

laboratory values including estimated glomerular filtration rate, AKI stages 1, 2, and 3 were associated with 2.0-, 3.4-, and 10.1-fold higher odds of death, respectively, compared with those without AKI.

Table 3: Odds ratios for death in patients with and without AKI according to KDIGO

Model	No AKI	All AKI stages Odds ratios (95%CI)	Stage 1 Odds ratios (95%CI)	Stage 2 Odds ratios (95%CI)	Stage 3 Odds ratios (95%CI)
Unadjusted	1.0 (Ref)	18.1 (14.9-22.0)	8.9 (7.0-11.2)	24.4 (18.6-32.1)	86.4 (67.4-110.6)
Model 1	1.0 (Ref)	12.9 (10.6-15.8)	6.0 (4.7-7.6)	18.0 (13.7-23.8)	65.6 (50.9-84.6)
Model 2	1.0 (Ref)	3.4 (2.6-4.3)	2.4 (1.8-3.1)	4.0 (2.8-5.6)	13.3 (9.4-18.7)
Model 3	1.0 (Ref)	2.8 (2.2-3.6)	2.0 (1.5-2.7)	3.4 (2.4-4.9)	10.1 (7.1-14.4)

Model 1: Adjusted for age, sex, race, Model 2: Adjusted for age, sex, race, procedures, and diagnosis-related group weights, Model 3: Adjusted for age, sex, race, procedures, diagnoses, diagnosis-related group weights, and laboratory values including estimated glomerular filtration rate.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Hospital length of stay was significantly higher in patients with AKI and significantly increasing following severity of AKI. Table 4 demonstrates the multivariable models in hospital length of stay and cost at the median, 10th, and 90th percentiles for all stages of AKI.

Table 4: Excess lengths of stay and costs associated with AKI by the KDIGO definition according to quantile regression analyses.

Percentile	All AKI stages	Stage 1	Stage 2	Stage 3
Length of stay, d				
10 th	1.5 (1.4-1.6)	1.3 (1.2-1.5)	2.0 (1.7-2.3)	2.0 (1.5-2.5)
50 th	2.8 (2.6-2.9)	2.5 (2.3-2.7)	4.2 (3.5-4.9)	6.4 (5.3-7.5)
90 th	6.2 (5.4-7.0)	4.7 (4.1-5.4)	10.5 (8.6-12.4)	17.6 (14.3-21.0)
Cost, $1,000 USD				
10 th	3.5 (3.1-3.8)	3.1 (2.8-3.5)	4.2 (3.2-5.3)	5.2 (3.8-6.5)
50 th	7.1 (6.4-7.8)	5.4 (4.7-6.1)	15.2 (13.3-17.2)	27.3 (22.6-32.0)
90 th	18.7 (16.4-21.0)	13.1 (10.9-15.4)	35.4 (26.0-44.9)	88.8 (72.6-105.0)

Hsu et al. [78] also explored the regional variation in the incidence of dialysis requiring AKI in the United States and reported that the overall population incidence rate of dialysis-requiring AKI in the United States from 2007 to 2009 was 492 cases/million person-year (95% CI 465-519 cases/million person-year).The population incidence rates of dialysis-requiring AKI differed across the four Census designated regions. Incidence was highest in the Midwest (523 cases/million person-year, 95%CI 483 - 568) and lowest in the Northeast (457 cases/million person-year, 95% CI 426-492). On contrary, the West region had the highest incidence of dialysis requiring AKI per hospitalization because hospitalization rates are lowest in the West (Figure 6).

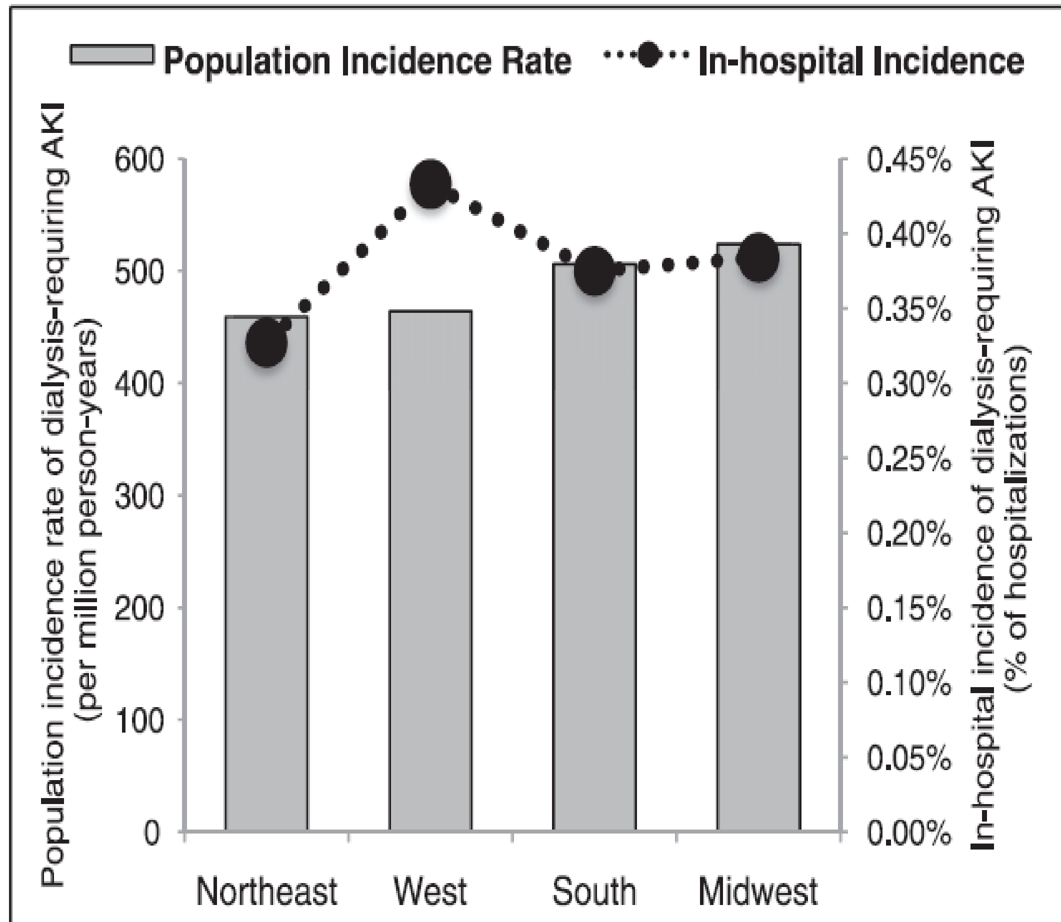


Figure 6 Population incidence rate of dialysis-requiring AKI and in-hospital incidence of dialysis-requiring AKI by regions from 2007 to 2009 (78).

In-hospital mortality associated with dialysis-requiring AKI differed across the four regions, with the highest case fatality in the Northeast and the lowest case fatality in the Midwest. Of note, the ranking order in case fatality rate associated with dialysis-requiring AKI across the four regions is directly opposite of the ranking order of the population incidence of dialysis-requiring AKI across the four regions (Figure 7).

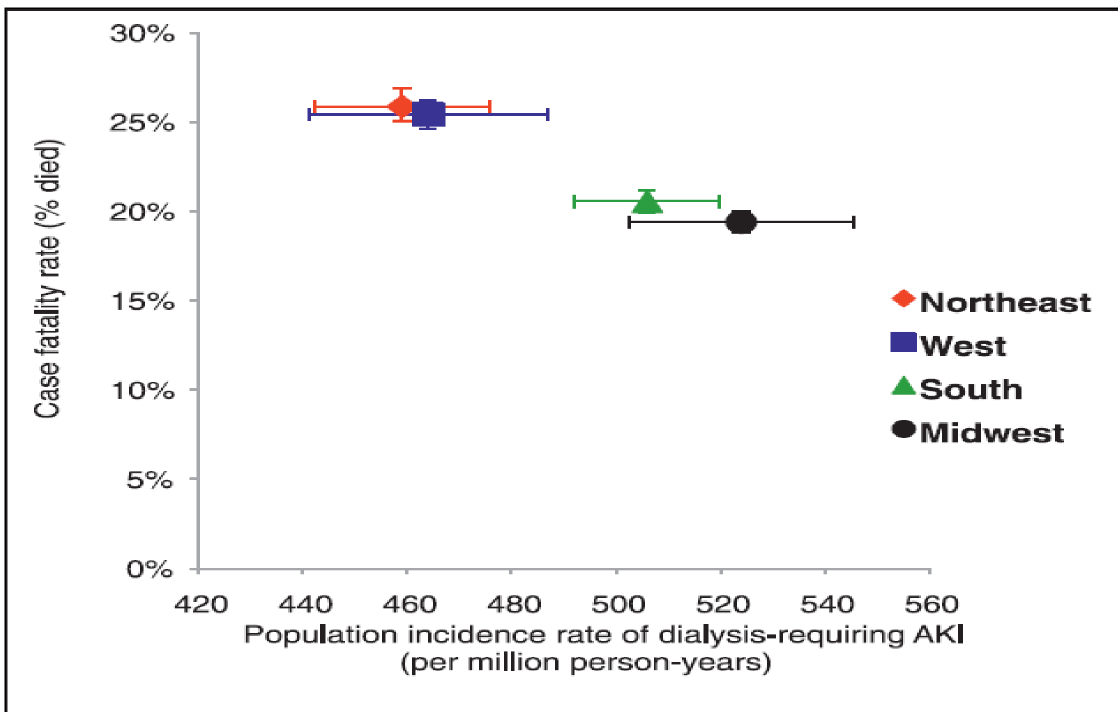


Figure 7 Population incidence rate of dialysis-requiring AKI versus case fatality rate by regions from 2007 to 2009 (78).

Moreover, the incidence of AKI worldwide also varies widely across different settings and is largely dependent on the setting, hospital acquired (h-AKI) vs. community-acquired (c-AKI). It affects between 7 and 18% of hospital inpatients and ranges from 20 to 200 per million populations in the community. Incidence of AKI in high income as well as low and middle income countries were demonstrated in Table 5

Table 5 : Incidence of AKI in high income as well as low and middle income countries [79]

	Community acquired	Change in incidence	Hospital acquired	Change in incidence
High income countries	200 PMP	51-62%	60-288/100,000 population	6.8 times increase, 11% / year increase
Low and middle income countries	20 PMP	No significant change	5.4/100,00 population	1.06 increase over 5 years

In low and middle income countries, insufficient and late recognition is even more problematic issue in both community and hospital settings. Late recognition results in delayed management and thus, associated high morbidity and mortality. Late recognition also may account for the apparently 10-fold lower incidence in low and middle income countries. It is likely that AKI cases are underreported. Several factors, including access to appropriate medical care, lack of knowledge, and no availability of standard tests such as serum creatinine are the important concerns.

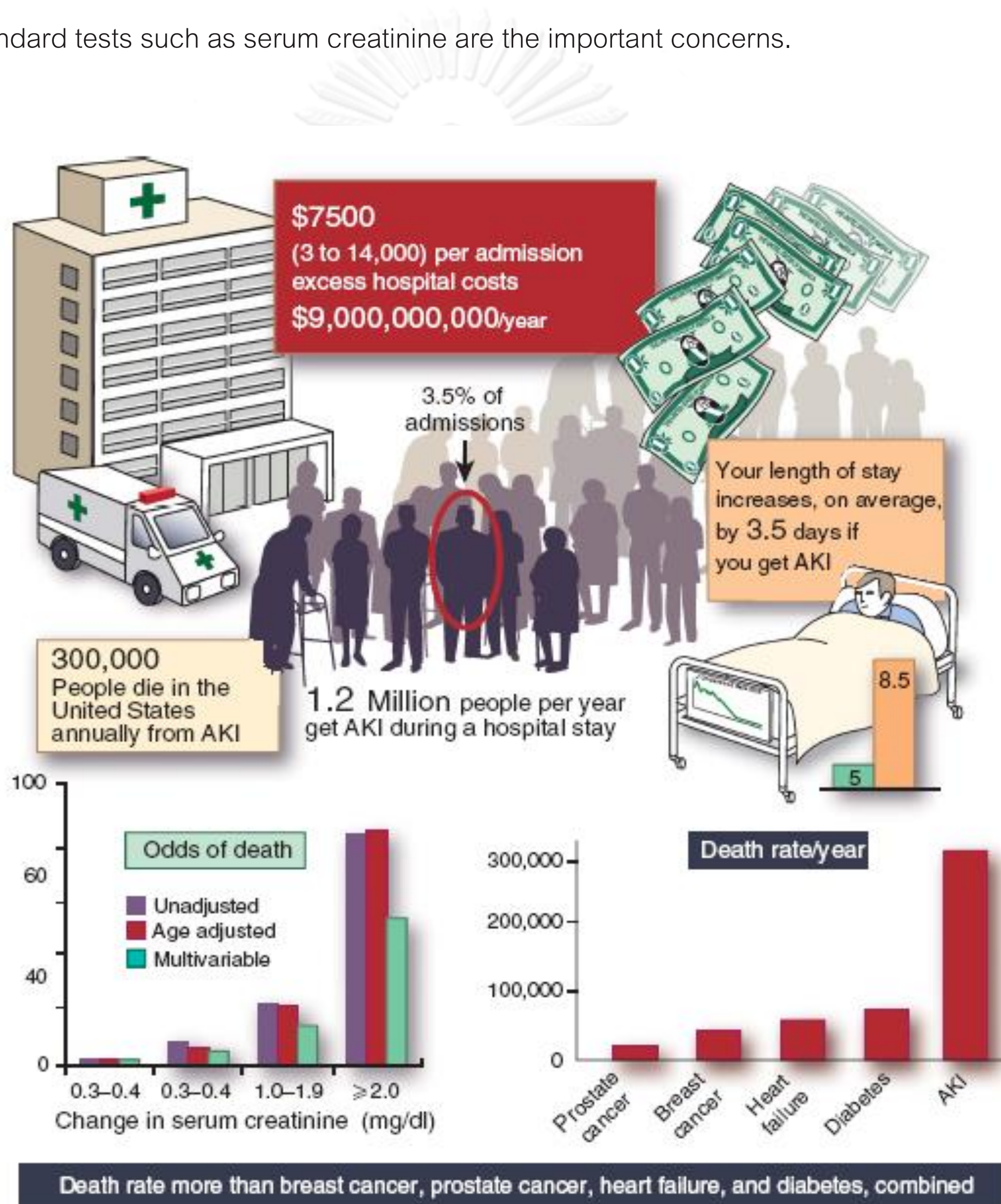


Figure 8 The global burden of acute kidney injury (AKI)[9].

Therefore, worldwide AKI seems to be now well established as a common condition, usually underrecognized disorder, which is associated with development of CKD and a high risk for mortality. This condition leads to increased resource utilization [80].There is increasing recognition of both the effect of AKI on the individual patient and the resulting societal burden from its long-term effects, including development of CKD and advanced CKD requiring dialysis [81] (Figure 8,9).

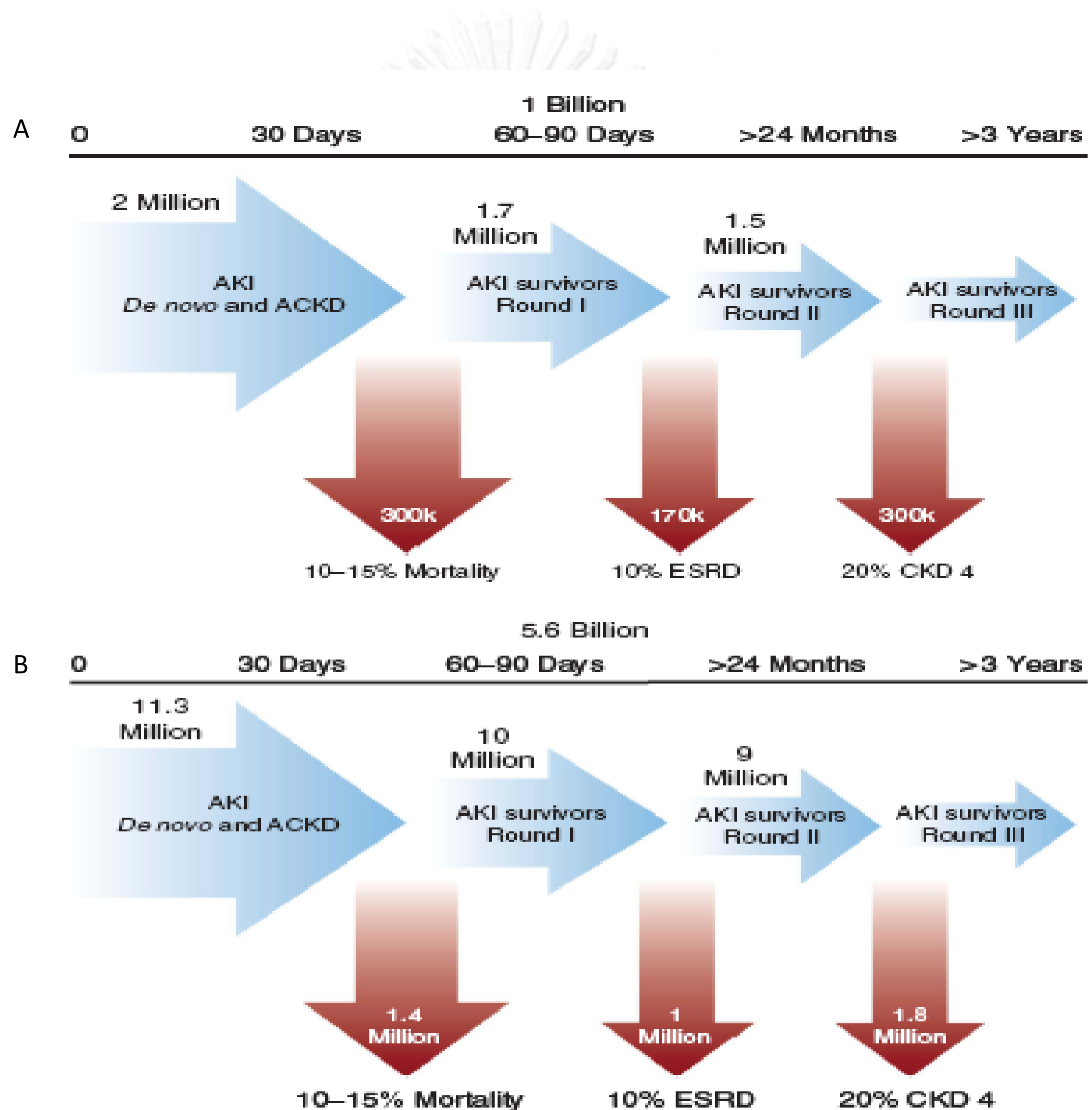


Figure 9 Estimated burden of AKI with progression to CKD and death across the world. (a) High-income (HI) countries. (b) Low- and middle-income (LMI) countries. The burden of cases of acute kidney injury (AKI), deaths, and progression to chronic kidney disease (CKD) in HI and LMI countries is shown[82].

AKI can occur in multiple settings and is commonly first approached by no specialized health-care providers in both community and hospital setting. Since AKI is not associated with any specific symptoms, therefore the diagnosis is usually based on measurement of laboratory parameters. It is more important for giving education for caregivers on the risks for AKI and knowledge for early recognition, timely intervention, and effective follow-up because AKI is preventable, treatable, and reversible disease.

The current conceptual framework of AKI was proposed (Figure 10). First, AKI is a disease that evolves from early injury through severe damage, resulting in kidney failure and the need for RRT. Second, the natural course can vary from complete renal recovery to dialysis dependency or death. Finally, leads to an individual's transition from one state to another during the course of the disease

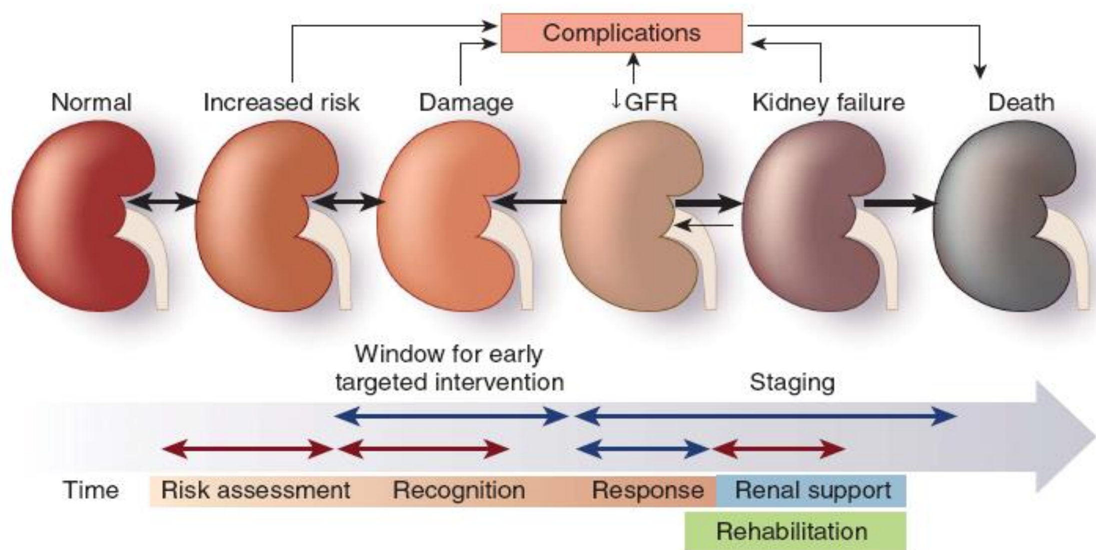


Figure 10 Conceptual framework and targeted approach for raising awareness of acute kidney injury (AKI) (81).

This conceptual framework also recognizes that AKI can occur in individuals who have normal kidney function or have preexisting kidney damage, thus allowing risk assessment. New strategy for raising awareness that emphasizes five areas of focus: risk assessment, recognition, response, renal support, and rehabilitation are proposed (Table 6).

Table 6: Prevention, assessment and management of AKI

Category	Component	Areas of focus
Risk assessment		
	Susceptibility	Genetics, clinical risk scores
	Surveillance	E-alerts, drug dosing modifications
	Primary prevention	High-risk patients and situations (for example , contrast exposure)
Recognition		
	Diagnosis	Functional changes (urine output, biomarkers)
	Staging	AKIN, KDIGO, duration of AKI
Response		
	Reversible factors	Hydration, hemodynamics, relieve obstruction, remove nephrotoxic medications
	Avoid nephrotoxins	Drug dose adjustments
	Referral	Nephrology consultation in high-risk patients and at recognition.
	Therapy	Emerging molecules targeting different pathways
Renal support		
	Dialytic modalities	Dosing, duration, and timing of initiation and withdrawal
Rehabilitation		
	Follow up	Team approach (primary care, specialist, nursing, social worker, patient family)
	Recovery	Targeted interventions (for example, hypertension management)
	Functional assessment	Quality of life

Whereas, in another concept, two groups of factors which play a role on AKI outcome are proposed (Figure 11).

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

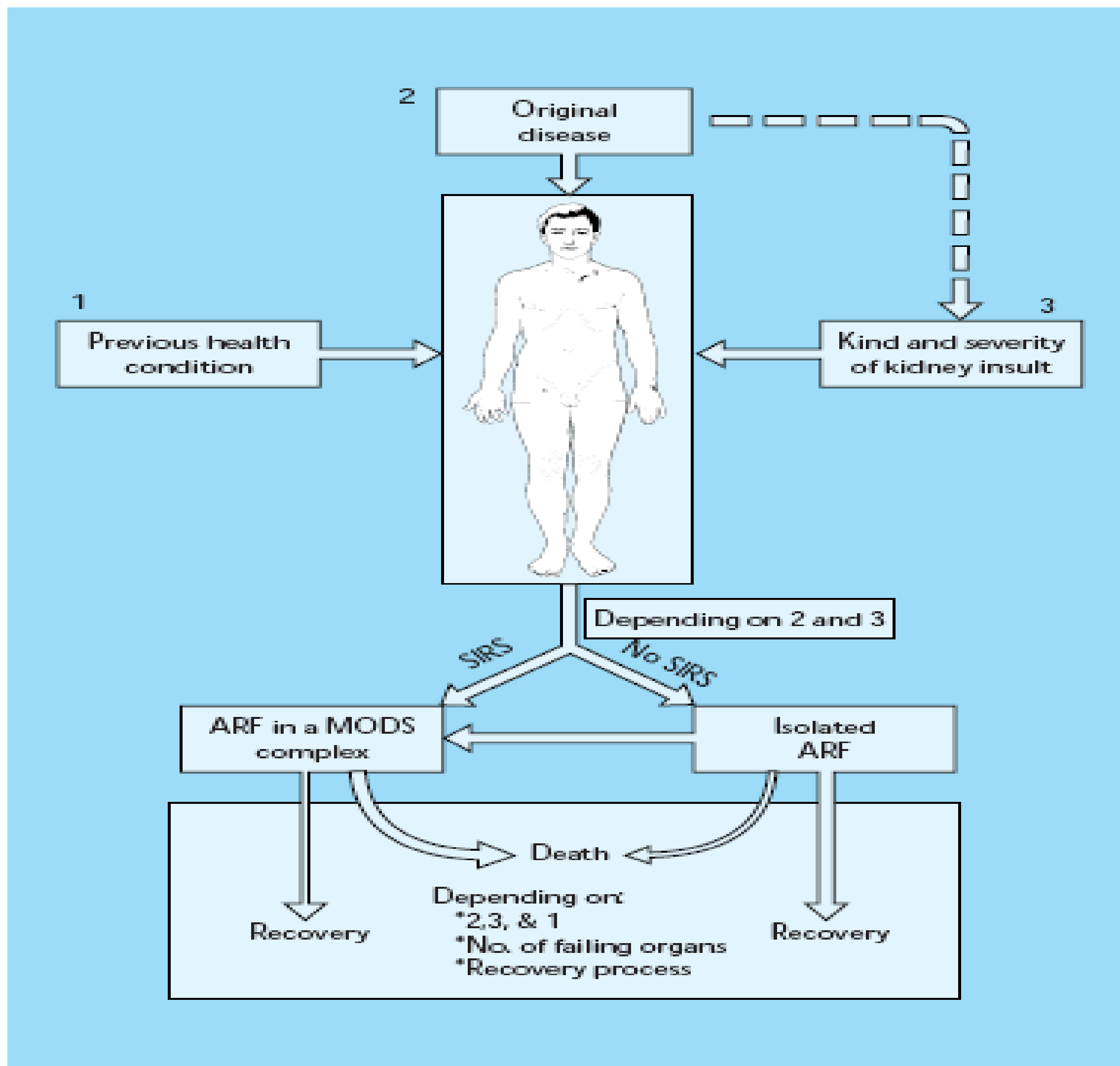


Figure 11: Outcome of acute kidney injury (AKI) or acute renal failure (ARF)[83].

The first includes factors that affect the patient: 1) previous health condition and/or genetic predisposing factor; 2) initial disease—usually, the direct or indirect (*eg*, treatments) cause of kidney failure; 3) the kind and severity of kidney injury. While 1 is a conditioning element, 2 and 3 trigger the second group of factors: the response of the patient to the insult. If this response includes a systemic inflammatory response syndrome (SIRS) like that usually seen in intensive care patients (*eg*, sepsis, pancreatitis, burns), a multiple organ dysfunction syndrome (MODS) frequently appears and consequent outcome is associated with a higher fatality rate (*thick line*). On the contrary, if SIRS does not develop and isolated AKI predominates, death (*thin line, right*)

is less frequent than survival (*thick line*). This concept seems to highlight the patient's risk factors and the cause of AKI.

2. Etiology of AKI

Depending on the localization or the nature of the renal insult, etiology of AKI is classified as prerenal, parenchymatous, or obstructive (postrenal) (Figure 12)

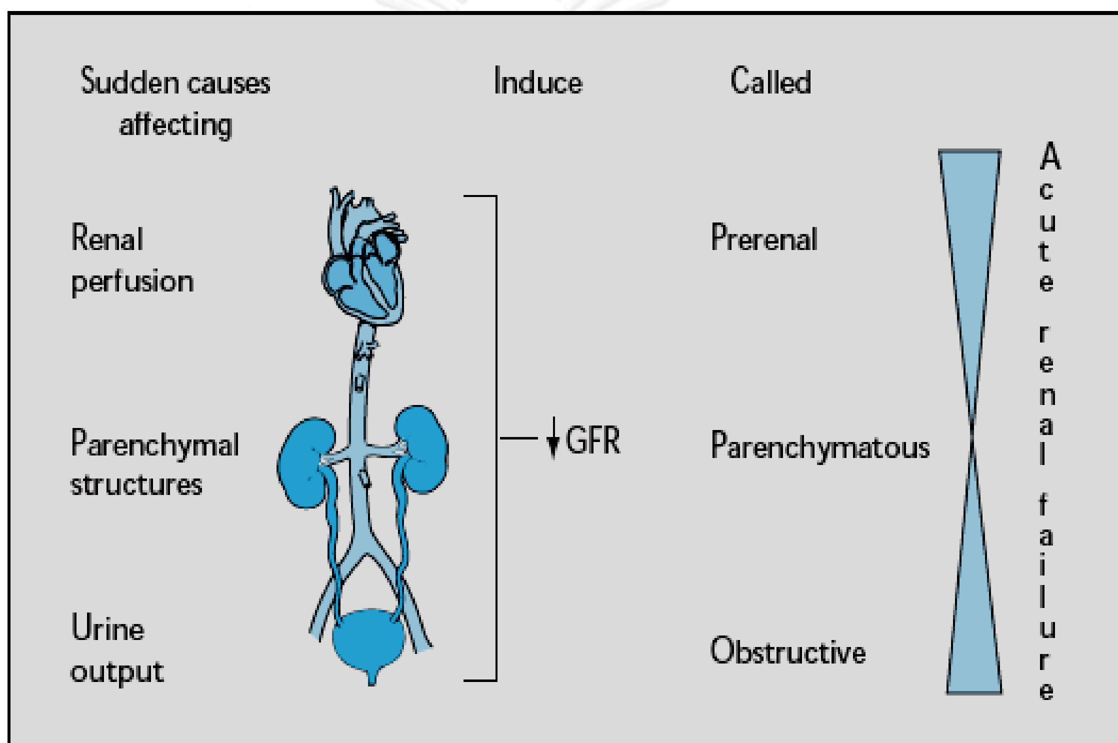


Figure 12 Characteristics of AKI.[83]

Chulalongkorn University

Prerenal AKI, also known as prerenal uremia, supervenes when glomerular filtration rate falls as a consequence of decreased effective renal blood supply. The condition is reversible if the underlying disease is resolved. Causes of prerenal AKI are summarized in Table 7

Table 7: Causes of prerenal AKI[83]

- Decreased effective extracellular volume
- Renal losses: hemorrhage, vomiting, diarrhea, burns, diuretics
- Redistribution: hepatopathy, nephrotic syndrome, intestinal obstruction, pancreatitis, peritonitis, malnutrition
- Decreased cardiac output: cardiogenic shock, valvulopathy, myocarditis, myocardial infarction, arrhythmia, congestive heart failure, pulmonary emboli, cardiac tamponade
- Peripheral vasodilation: hypotension, sepsis, hypoxemia, anaphylactic shock, treatment with interleukin L2 or interferons, ovarian hyperstimulation syndrome
- Renal vasoconstriction: prostaglandin synthesis inhibition, α-adrenergics, sepsis, hepatorenal syndrome, hypercalcemia
- Efferent arteriole vasodilation: converting-enzyme inhibitors

When the sudden decrease in glomerular filtration rate that are characterized as AKI, leading to intrinsic renal damage mainly affecting tubules, interstitium, glomeruli and/or vessels that are characterized as parenchymatous AKI. Multiple causes have been described in Table 8, some of them constituting the most frequent ones are marked with an asterisk.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table 8 Cause of parenchymatous AKI[83]

- Acute tubular necrosis
  - Hemodynamic: cardiovascular surgery,* sepsis,* prerenal causes*
  - Toxic: antimicrobials,* iodide contrast agents,* anesthesics, immunosuppressive or antineoplastic agents,* Chinese herbs, Opiaceous, Extasis, mercurials, organic solvents, venoms, heavy metals, mannitol, radiation
  - Intratubular deposits: acute uric acid nephropathy, myeloma, severe hypercalcemia, primary oxalosis, sulfadiazine, fluoride anesthesics
  - Organic pigments (endogenous nephrotoxins): Myoglobin rhabdomyolysis: muscle trauma; infections; dermatopolymyositis; metabolic alterations; hyperosmolar coma; diabetic ketoacidosis; severe hypokalemia; hyper- or hyponatremia; hypophosphatemia; severe hypothyroidism; malignant hyperthermia; toxins such as ethylene glycol, carbon monoxide, mercurial chloride, stings; drugs such as fibrates, statins, opioids and amphetamines; hereditary diseases such as muscular dystrophy, metabolopathies, McArdle disease and carnitine deficit
  - Hemoglobinuria: malaria; mechanical destruction of erythrocytes with extracorporeal circulation or metallic prosthesis, transfusion reactions, or other hemolysis; heat stroke; burns; glucose-6-phosphate dehydrogenase; nocturnal paroxystic hemoglobinuria; chemicals such as aniline, quinine, glycerol, benzene, phenol, hydralazine; insect venoms
- Acute tubulointerstitial nephritis
  - Antimicrobials: Penicillin, Ampicillin, Rifampicin, Sulfonamides
  - Analgesics, anti-inflammatories: Fenoprofen, Ibuprofen, Naproxen,
  - Other drugs: Cimetidine, Allopurinol
  - Immunological: Systemic lupus erythematosus, Rejection
  - Infections (at present quite rare)
  - Neoplasia: Myeloma, Lymphoma, Acute leukemia

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

- Vascular occlusion
  - Principal vessels: bilateral (unilateral in solitary functioning kidney) renal artery thrombosis or embolism, bilateral renal vein thrombosis
  - Small vessels: atheroembolic disease, thrombotic microangiopathy, hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura, postpartum acute renal failure, antiphospholipid syndrome, disseminated intravascular coagulation, scleroderma, malignant arterial hypertension, radiation nephritis, vasculitis
- Acute glomerulonephritis
  - Postinfectious: streptococcal or other pathogen associated with visceral abscess,endocarditis, or shunt
  - Henoch-Schonlein purpura
  - Essential mixed cryoglobulinemia
  - Systemic lupus erythematosus
  - ImmunoglobulinA nephropathy
  - Mesangiocapillary
  - With antiglomerular basement membrane antibodies with lung disease (Goodpasture is syndrome) or without it
  - Idiopathic, rapidly progressive, without immune deposits
  - Cortical necrosis, abruptio placentae, septic abortion, disseminated intravascular coagulation

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Obstruction at any level of the urinary tract frequently leads to obstructive AKI. Most frequent causes are summarized in Table 9.

Table 9: Causes of obstructive AKI[83]

• Congenital anomalies	• Acquired uropathies
- Ureterocele	- Benign prostatic hypertrophy
- Bladder diverticula	- Urolithiasis
- Posterior urethral valves	- Papillary necrosis
- Neurogenic bladder	- Iatrogenic ureteral ligation
• Malignant diseases	• Retroperitoneal fibrosis
- Prostate	- Idiopathic
- Bladder	- Associated with aortic aneurysm
- Urethra	- Trauma
- Cervix	- Iatrogenic
- Colon	- Drug-induced
- Breast (metastasis)	
• Gynecologic non-neoplastic	• Infections
- Pregnancy-related	- Schistosomiasis
- Uterine prolapse	- Tuberculosis
- Endometriosis	- Candidiasis
- Acute uric acid nephropathy	- Aspergillosis
	- Actinomycosis
• Drugs	• Other
- €-Aminocaproic acid	- Accidental urethral catheter occlusion
- Sulfonamides	

3. The pathophysiology change in AKI

Ischemic, nephrotoxic, and sepsis are the most important causes of hospital-induced AKI. Ischemic AKI has been known as vasomotor nephropathy because there is increased basal tone, increased reactivity to vasoconstrictive substances, and decreased vasodilatory responses in arterioles. However, sepsis and nephrotoxic AKI

จุฬาลงกรณ์มหาวิทยาลัย CHULALONGKORN UNIVERSITY

are well-established known as inflammatory nephropathy because they can induce the release of cytokines and several substances such as oxygen-free radicals, arachidonic acid metabolites, and nitric oxide (NO)[84,85]. This proinflammatory cascade contributes to endothelial injury of the renal microvascular bed [55], leading to the development of tubular dysfunction and progression of AKI.

3.1 Microvascular change in glomerular and medullary

3.1.1 Vascular reactivity and endothelial Injury

Declined GFR has been attributed to persistent vasoconstriction and endothelial injury. As a result, cell swelling and enhancement of expression of cell adhesion molecules and the leukocyte activation have been developed, resulting in enhanced leukocyte–endothelial interactions that can induce more injury and swellng of the endothelial cell, physically obstruct blood flow, and also contribute to the production of local factors promoting vasoconstriction. This condition leads to additional effects of vasoconstriction on local blood flow and induces tubule cell injury as vicious cycle [86,87] (Figure 13).

After an ischemic insult, the disorganized actin cytoskeleton has been demonstrated in the arteries, arterioles, and mural cells or pericytes of vasa recta of the kidney [88]. It has been proposed to contribute an important role in the loss of autoregulation of renal blood flow and abnormal vascular reactivity.

Under normal circumstances, NO generated from the endothelial cell plays an important vasodilatory role and decreases endothelin expression [89]. Whenever endothelial damage occurs, the endothelial nitric oxide synthase (eNOS) is inhibited that results in the abnormal vascular tone. On the contrary, the changes in the vasoconstrictors including angiotensin II, thromboxane A2, leukotrienes C4 and D4, endothelin-1, adenosine, endothelium-derived prostaglandin H2, and sympathetic nerve stimulation also have been implicated in abnormal vascular tone. Systemic endothelin-1 levels increase with ischemia. Administration of either anti-endothelin antibodies or

endothelin receptor antagonists has been demonstrated to protect against ischemia-reperfusion injury [90].

Although enhanced vasoconstriction is a fundamental contributor to the pathophysiology of ischemic AKI, vasodilators such as low-dose dopamine and atrial natriuretic peptide have not been demonstrated to be useful in prevention or treatment of AKI[91,92].

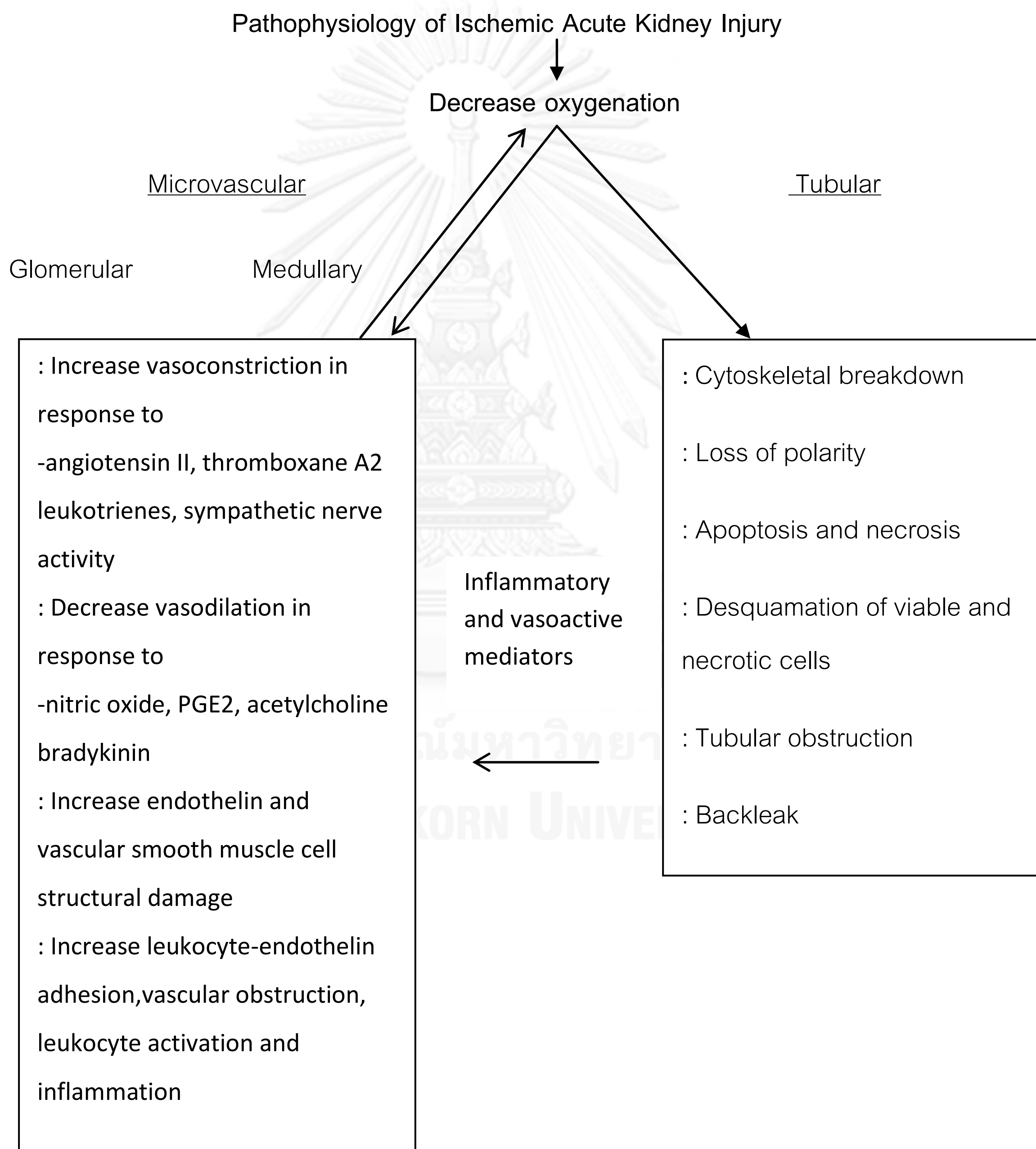


Figure 13. Pathophysiology change of AKI (84)

3.1.2 Inflammation, leukocytes, and adhesion molecules

Subsequent renal blood flow reduction results in the reduced medullary blood flow and oxygen delivery to the tubular structures. An imbalance between oxygen delivery and demand induces cellular injury, upregulated adhesion molecules, and endothelial cells injury, leading to cell swelling, loss of the patency of the endothelial barrier. Finally potentiate induce the interactions with leukocytes and platelets and mechanical obstruction to the small vessels. [86].

The activated leukocytes sequestered several local factors including cytokines, chemokines, eicosanoids, and reactive oxygen species (ROS) that result in upregulation of adhesion molecules. In addition, ROS and eicosanoids can also further the inflammatory responses and enhance vascular tone [93].

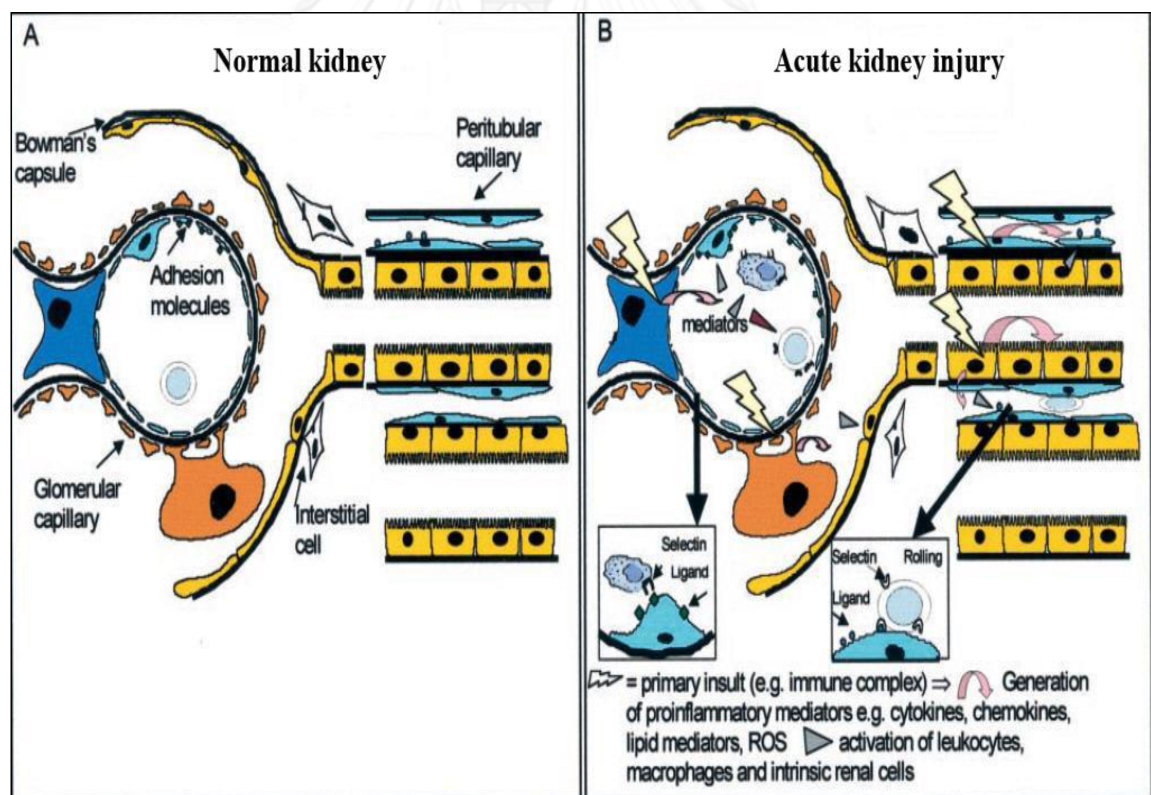


Figure 14. Hypothetical model of the role of chemokines and chemokine receptors in renal disease (A) Normal renal tissue (B) Acute kidney injury[94]

Chemokines, which are chemotactic and immunomodulatory substances for leukocytes, are upregulated by inflammatory cytokines, such as IL-1 and TNF-alpha [95]. Renal TNF-alpha mRNA is increased after only 30 min of ischemia, while the TNF-alpha transcription factor is activated after 15 min of ischemia [96]. Infusion of a TNF-alpha–binding protein decreases TNF-alpha bioactivity and neutrophil infiltration and preserves renal function, suggesting that local TNF-alpha synthesis may be an early and pivotal event in renal ischemia/reperfusion injury [96]. (Figure 14)

3.2 The tubulular cell as a contributor to the inflammatory response.

The renal epithelial cell can produce fractokine [Chemokine (CX3-C motif) ligand 1, CX3CL1], a transmembrane protein with a CX3 chemokine motif attached to a mucin stalk [97]. Fractokine can induce adhesion and migration of leukocytes and induced cell injury. Renal tubular cells can also produce a number of proinflammatory cytokines, including TNF-alpha, interleukin-6 (IL-6), TGF-β, and chemotactic cytokines (chemokines) such as RANTES, monocyte chemotactic protein-1 (MCP-1), and IL-8. [94,98,99]

In experimental settings, ischemia-reperfusion and nephrotoxic injury induce the generation of pro-inflammatory cytokines, resulting in morphological and functional changes in glomerular endothelial and tubular epithelial cells [55-57].

4. The immune response in acute inflammatory states

Under an acute inflammatory state, proinflammatory and anti-inflammatory response play an important role in the host response. Proinflammatory response called as SIRS results in the recruitment of inflammatory cells to sites of injury whereas anti-inflammatory response, named as compensatory anti-inflammatory response syndrome (CARS) plays a role to limit tissue injury as well as promote tissue healing.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

4.1 SIRS

SIRS activates several inflammatory cascades leading to organ dysfunction, including AKI via the release of biologically active mediators by monocytes and neutrophil [100]. Monocytes release proinflammatory cytokines such as TNF-alpha, IL-1beta and IL-6 causes generalized vascular endothelial cell injury. In addition, TNF-alpha and IL-1beta also interact with the vascular endothelium, and stimulate platelet-activating factor, prostanoids, and nitric oxide synthesis, resulting in arterial vasodilatation, hypotension, and organ dysfunction. IL-6 also induces the hepatic synthesis of positive acute-phase proteins, such as C-reactive protein (CRP) as well as suppresses synthesis of negative acute-phase proteins, such as albumin [101].

TNF-alpha and IL-6 also lead to protein catabolism which induces negative nitrogen balance and significant loss of lean body mass [102]. Furthermore, interleukin-8 (IL-8), a potent chemokine, promoted the recruitment of neutrophils to inflammatory sites, as well as induces releasing ROS and proteloytic enzymes.

.

4.2 CARS

On the contrary, CARS plays an important role in counterbalancing the proinflammatory responses associated with SIRS via producting immunomodulatory molecules for controlling proinflammatory cytokine response. Major anti-inflammatory monocyte-derived molecules include IL-10, IL-1 receptor antagonist (IL-1Ra), and soluble TNF receptors (sTNF-R). IL-10 is the most potent anti-inflammatory cytokine that is released from monocytes. It plays a role to inhibit TNF-alpha, IL-1b, and IL-6 production [103]. The IL-1Ra is a member of the IL-1 family, is mainly secreted by monocyte, and binds with IL-1 receptors [104]. IL-1Ra plays an important role in host defense against endotoxin-induced injury and inflammatory states [104,105].

The balance between proinflammatory cytokines, such as TNF-alpha and IL-1b, and anti-proinflammatory cytokines, such as IL-10 and IL-1Ra, is an important factor for determining the extent of the inflammatory response, as well as influencing on patient's outcomes.

5. Role of the inflammatory response in AKI

Sepsis is the most important cause of AKI and is the well-established known condition to induce cytokines release, activate adhesion molecules expression, and produce several substances, including oxygen-free radicals, arachidonic acid metabolites, platelet-activating factor, nitric oxide, endothelins, and heat-shock proteins (HSPs)[84,85] (Figure 15).

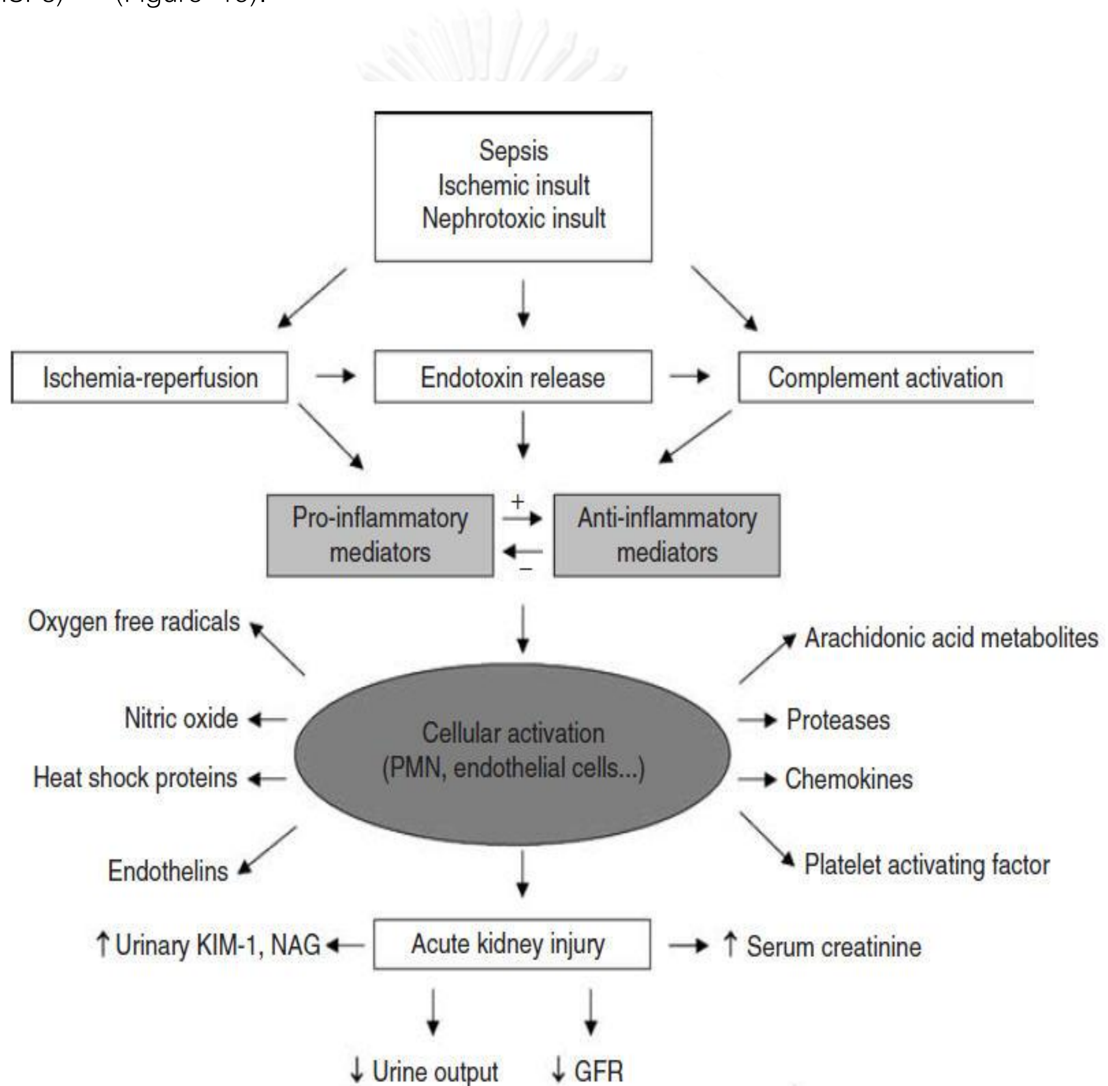


Figure 15: Inflammation response in AKI. Abbreviations: GFR, glomerular filtration rate; NAG, N-acetyl-b-D-glucosaminidase; KIM, kidney injury molecule 1

This proinflammatory cascade contributes to endothelial injury of the renal microvascular bed [55], leading to the development of tubular dysfunction and AKI.
A predominate of neutrophils in vasa recta and the interstitium was demonstrated in histology of acute tubular necrosis [55]

Despite sepsis induced AKI, ischemic [55] and nephrotoxic [106,107] are the important causes of AKI that can also induce inflammatory responses. Increased proinflammatory cytokine including TNF-alpha and IL-1b mRNA, chemokines such as monocyte chemotactic peptide-1 (MCP-1) as well as increased gene expression of intercellular adhesion molecule-1 (ICAM-1) and MPO were demonstrated [108].

On the other hand, anti-inflammatory response also plays a role in ischemic and nephrotoxic AKI. In experimental model, the exogenous administration of IL-10 can inhibit TNF-alpha and ICAM-1 mRNA expression [106], and antibody to ICAM-1 can protect the kidney against ischemic and nephrotoxic injury [109,110].

The changes in plasma and urinary cytokines such as IL-8, IL-10, TNF-alpha and IL-1b are correlated with urinary N-acetyl-β-D-glucosaminidase(NAG)level which is a biomarker of renal proximal tubular dysfunction following surgery with cardiopulmonary bypass (CPB) [111,112]. In addition, plasma cytokine levels including IL-6 and IL-10 have been correlated with the risk of AKI and mortality [113-115].

High circulating levels of TNF-alpha have been associated with adverse clinical outcomes in patients with AKI [67]. Functionally-relevant polymorphisms within the promoter region of the TNF-alpha (*TNFA*) gene, which affect transcriptional activity [68], have previously been linked to adverse clinical outcomes in critically ill patients [69-71], including those with AKI requiring dialysis [47].

6. Role of oxidative stress in AKI

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are enzymes which highly express in neutrophils and endothelial cells. They play a role to catalyze superoxide product and they are highly expressed in neutrophils and endothelial cells [116]. In sepsis and possibly AKI, there are several sources of ROS

including the mitochondrial respiratory electron transport chain, xanthine oxidase, and neutrophil-associated respiratory burst [117]. Activated neutrophils and monocytes also released MPO which catalyzed the production of hypochlorous acid which is a potent oxidant (Figure 16).

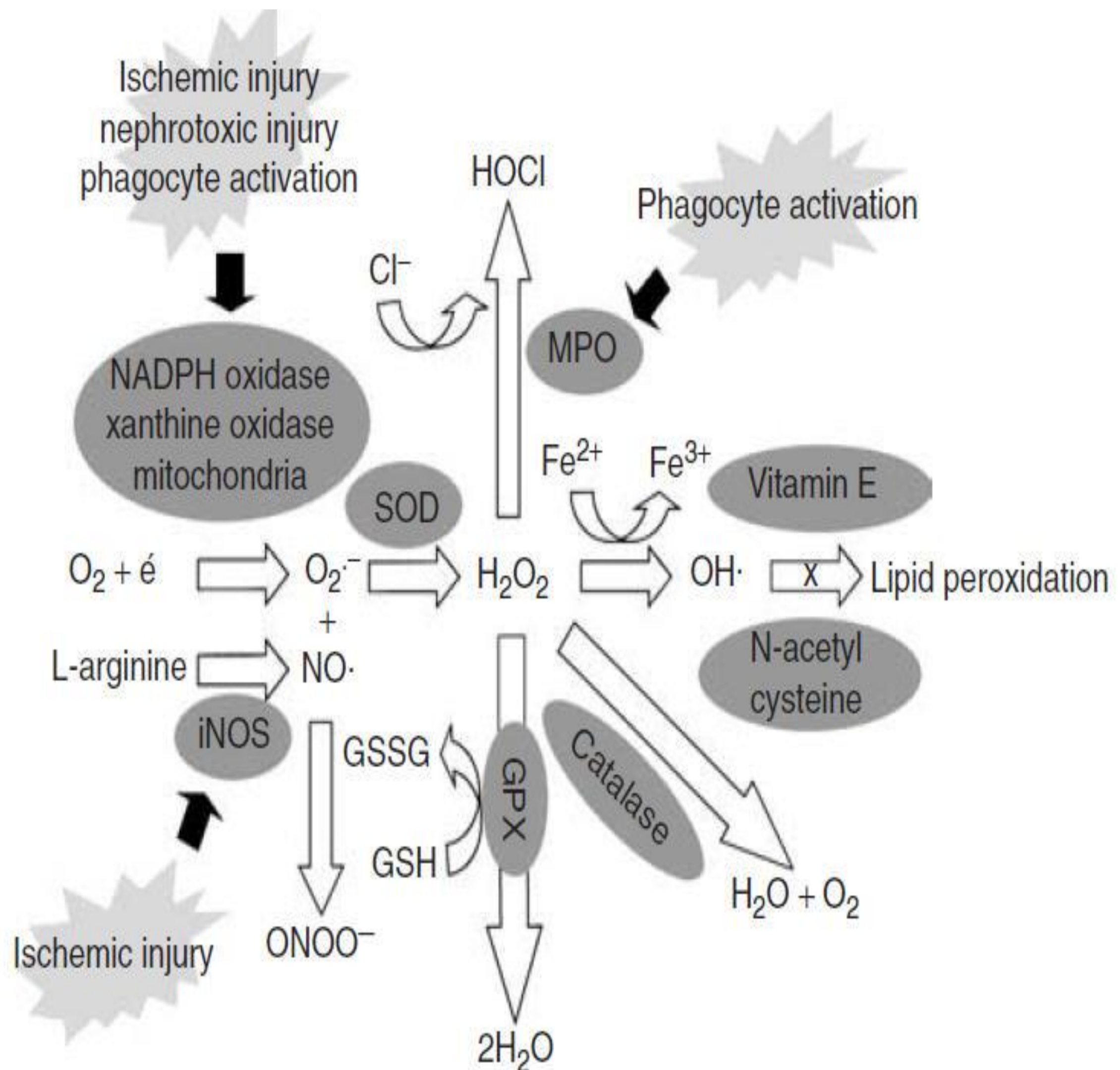


Figure 16: Key pathways involved in the generation and degradation of oxidants in acute kidney injury. Abbreviations are: $O2^-$, superoxide anion; SOD, superoxide dismutase; MPO, myeloperoxidase; NO, nitric oxide; $ONOO^-$, peroxynitrite. OH·, hydroxyl radical; GPX, glutathione peroxidase; HOCl, hypochlorous acid; iNOS, inducible nitric oxide synthase.

In addition, glutathione and the glutathione peroxidases (GPX) are the important antioxidant defense system by using glutathione (GSH) to reduce hydrogen peroxide to water [118]. There are four different (GPX1-4). GPX1 is particularly abundant in the kidney [119]. Superoxide dismutase (SOD) also catalyzes superoxide anion to hydrogen peroxide [120]. Finally, incrimination of free oxygen radicals and ROS play a role in the pathogenesis of ischemic and nephrotoxic AKI [121] . The use of N-acetyl cysteine (NAC) as anti-oxidative stress has been demonstrated the benefit on preventing contrast-induced nephropathy [122] .

7. Role of nitric oxide in AKI

NO is an important substance, derived from L-arginine by NOS. NO induces smooth muscle relaxation and vasodilatation by stimulating cyclic guanosine monophosphate (cGMP) production

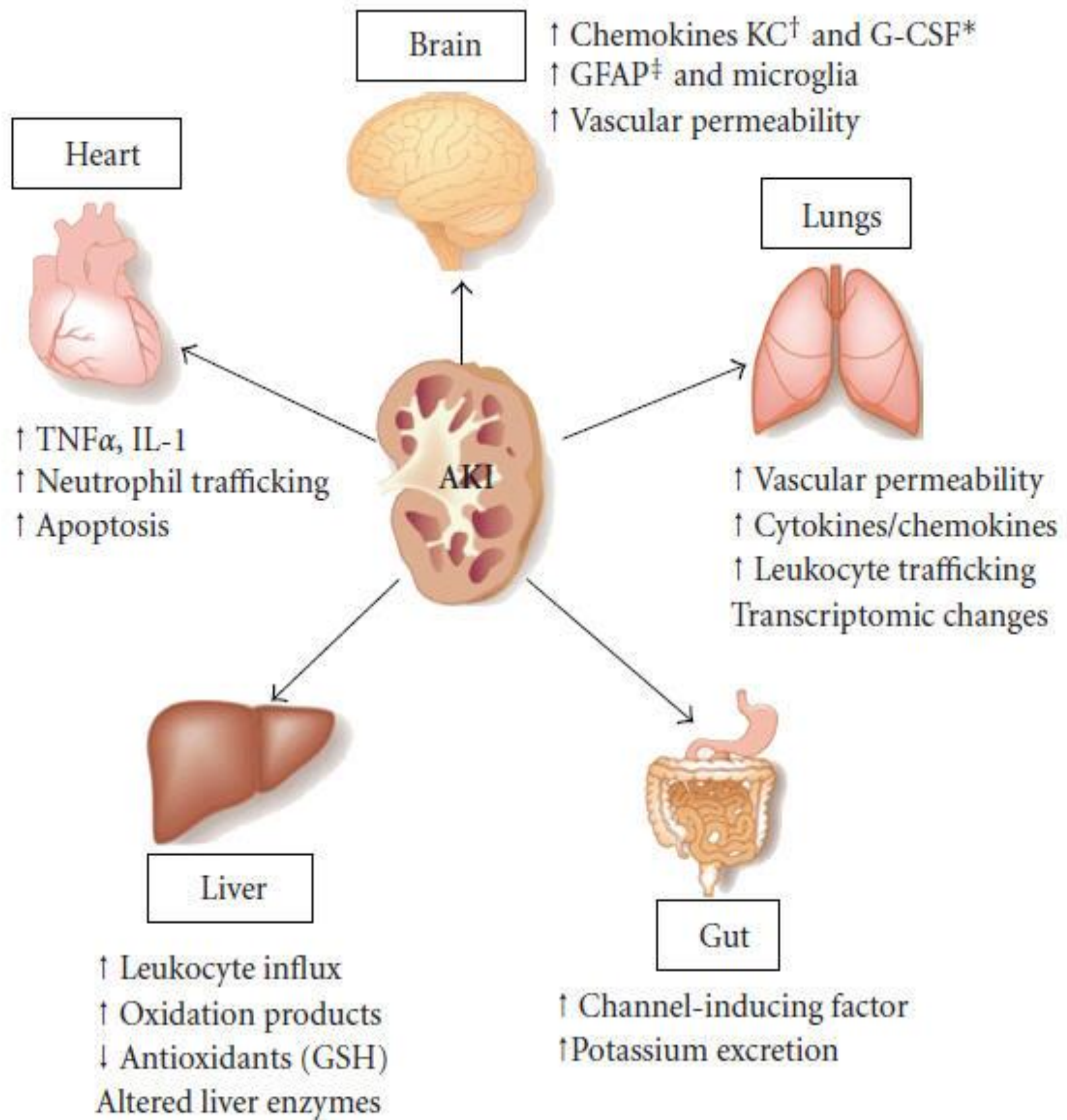
In ischemia/reperfusion injury model, tubular and interstitial cells are the major sources of NO production that play a role as a strong vasodilator as well as inhibit production of vasoconstrictors and reduce inflammation [123]. The use of L-arginine, the substrate for iNOS-dependent NO generation can reduce severity of AKI.

In summary, incrimination of cytokines, chemokines, ROS, and NO seem to be the important roles in the pathophysiology of AKI (Figure 16). However, the association between genetic polymorphism of these substances and susceptibility of AKI development as well as adverse related outcomes should be explored.

8. Organ crosstalk during AKI [59]

Clinical and translational laboratory studies have demonstrated the relevance of interactions between AKI and distant organs including lungs, liver, heart, gut, and brain. Complex mechanisms including immune response, activation of proinflammatory cascades, and an alteration of transcriptional events in remote organs during ischemic AKI have been proposed (Figure 17).

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY



Chulalongkorn University

Figure 17: AKI and organ crosstalk.AKI induces remote organ injury involving multiple inflammatory pathways, including increased expression of soluble proinflammatory mediators, innate and adaptive immunity, cellular apoptosis, physiologic derangements and genomic changes. †A brain IL-8 homologue, *Granulocyte colony-stimulating factor, ‡Glial fibrillary acidic protein Glutathione [59]

In the setting of multi-organ failure, AKI and acute lung injury (ALI) developed more frequently together than any other combination of organ systems which are

associated with high mortality [124]. Although, the mechanisms for organ crosstalk between AKI and ALI are still not well understood, activation of proinflammatory and proapoptotic pathways have been proposed (Figure 18).

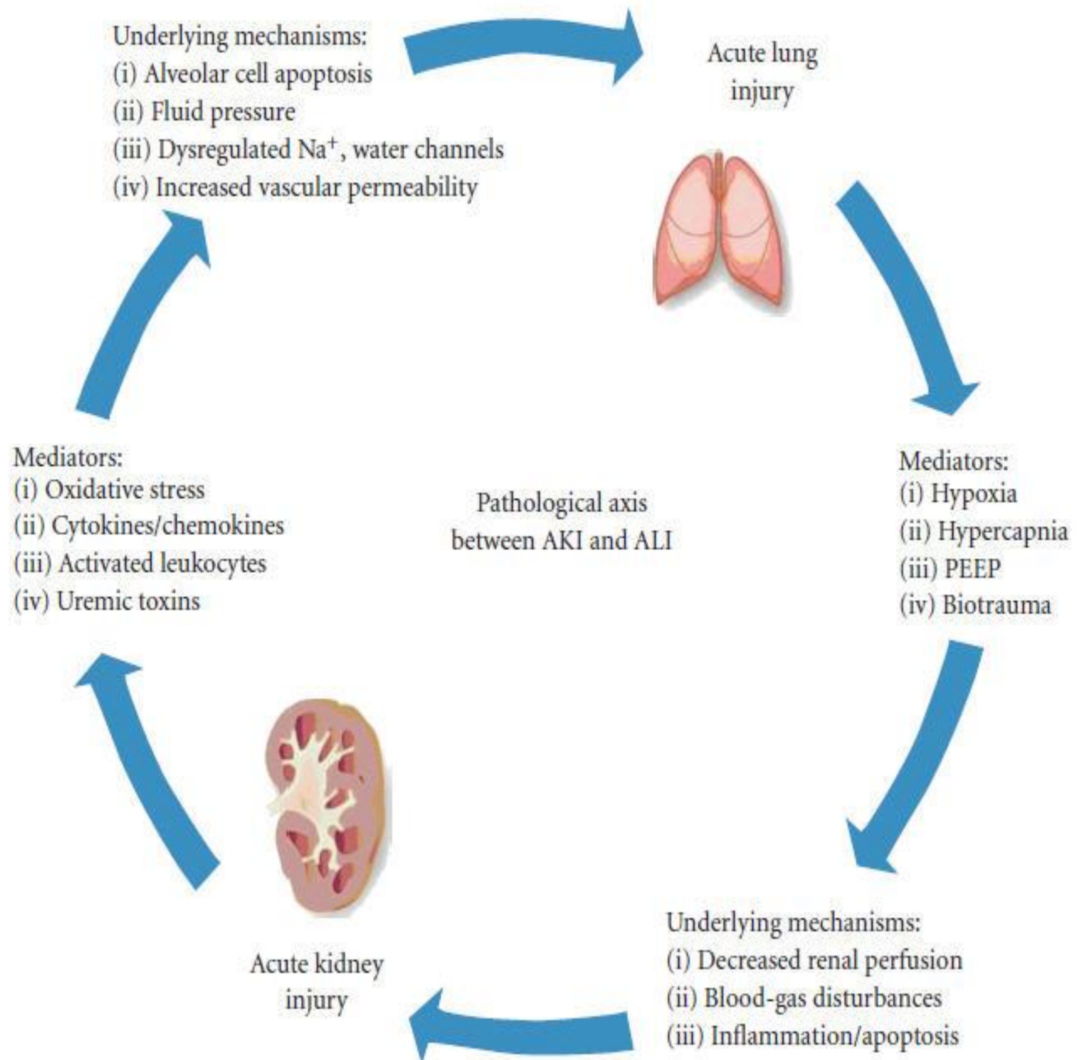


Figure 18 Pathological mechanisms between AKI and ALI. AKI induces pathophysiologic effects on the lung via cellular and soluble mediators. ALI, in turn, exacerbates kidney dysfunction through metabolic and biochemical derangements (122).

AKI results in lung injury and inflammation. Whereas ALI and its attendant hypoxemia and hypercapnia that are worsened by mechanical ventilation with high positive end-expiratory pressure, leading to diminish renal hemodynamics and function. In addition, lung injury during ischemic AKI manifestates increased pulmonary vascular permeability, erythrocyte sludging in lung capillaries, interstitial edema, focal alveolar hemorrhage, and infiltration of inflammatory cell[125-127].

Regarding the cytokines, early pulmonary inflammatory response with rapid activation of transcription factors NF-κB and activator protein-1 (AP-1) are demonstrated during ischemic AKI. TNF-alpha and ICAM-1 result in an accumulation of pulmonary neutrophils within 4 hours. Therefore, leukocytes play an important role in the development of ALI.[128]. In addition, early expression of the neutrophil chemokine keratinocyte-derived chemokine (KC/CXCL1) and macrophage inflammatory protein (MIP-2/CXCL-2) along with increased pulmonary myeloperoxidase activity and pulmonary microvascular permeability are also demonstrated during ischemic AKI[129].

In the heart, increased expression of TNF-alpha and interleukin-1 (IL-1) are associated with myocyte apoptosis. In the brain, increased expression of chemokines including keratinocyte chemoattractant (KC, a brain IL-8 homologue), granulocyte colony-stimulating factor (G-CSF) and glial fibrillary acidic protein (GFAP) are seen with increased vascular permeability.

AKI is also implicated in oxidative stress, inflammation, apoptosis, and tissue damage in hepatocytes. Hepatic stellate cells (HSCs) regulate leukocyte trafficking and the secretion of chemokines such as IL-8, and crosstalk between HSCs likely occurs via a c-Jun N-terminal kinase pathway[130]. Oxidative stress during ischemic AKI results in increased hepatic malondialdehyde, an index of lipid peroxidation whereas increased total glutathione, an antioxidant. Proinflammatory cytokine TNF-alpha expression and hepatic cellular apoptosis also occur during ischemic AKI [131]. In summary, cytokines can also mediate distant organ injury[58,59].

9. Early detection of AKI

9.1 Serum and Urine Biomarkers of AKI

Serum creatinine is used as an overall marker for renal function. However, this marker is not perfect, especially in AKI. There is no ideal marker for kidney injury similar to troponin for myocardial injury which can be used as an early marker for ischemia in the heart. The ideal biomarkers of kidney injury should be early detected in the blood or urine. Several serum and urinary proteins have been evaluated as potential non-invasive markers of renal injury [21].

9.1.1 Cystatin C

CysC is a 13-kD cysteine protease inhibitor that is synthesized in all nucleated cells. It is freely filtered by the glomerulus, not secreted by renal tubules, and completely metabolized at the renal tubules. Therefore, it is utilized as alternative marker of the GFR in chronic kidney disease[132]. Recently, serial measurements of serum CysC were highly correlated with the development of AKI in cardiopulmonary bypass surgery[133]. In addition, serum CysC level was used for predicting dialysis requirement or in-hospital death in established AKI[134].

9.1.2 N-acetyl-B-(D)-glucosaminidase (NAG)

NAG is the most active glycosidase which is a lysosomal brush border enzyme of proximal renal tubular cells. Increasing of urinary activity is correlated with severity of renal tubular damage[39-41]. Recently, tubular enzymuria as urinary NAG has been utilized as a diagnostic marker for the early detection of acute kidney injury[38,42,43].

9.1.3 Kidney injury molecule-1 (KIM-1)

Using a genomic approach, KIM-1 was cloned from the post-ischemic kidney [135]. Human KIM-1, a type 1 transmembrane protein, is not detectable in normal kidney and in normal urine but it is expressed at very high levels after acute kidney

injury. KIM-1 has been proposed as an early biomarker for diagnosis AKI in few years ago[38,136].

From meta-analysis by Susantitaphong et al. (unpublished data), the estimated sensitivity of urinary KIM-1 was 66.6% (95% CI 53.1, 77.9%, P=0.017), the specificity 81.2% (95% CI 75.7, 85.6%, P<0.001) for prediction of AKI development. For prediction of dialysis requirement, the estimated sensitivity of urinary KIM-1 was 42.6% (95% CI 25.7, 61.5%, P=0.445) and the specificity was 91.0% (95% CI 49.2, 99.1%, P=0.053). Finally, the estimated sensitivity of urinary KIM-1 was 71.5% (95% CI 6.4, 98.9%, P=0.617), and the specificity was 77.9% (95% CI 62.0, 88.4%, P<0.001) for predicting mortality. Therefore, urinary measurement of KIM-1 provides good diagnostic and prognostic discrimination for predicting development of AKI, dialysis requirement, and in-hospital mortality.

9.1.4 Glutathione S-transferase (GST) enzyme

GST is a ubiquitous enzyme that takes part in the detoxification of free radicals. The human kidney expresses two cytosolic GST subtypes, alpha–GST that has specificity for the proximal tubule, and pi-GST with specificity for the distal tubule [137]. Tubular cell injury results in leakage of cytosolic content into the urine, including alpha-GST and pi-GST [138]. Several cohort studies have evaluated the potential role of urinary alpha-GST and pi-GST for the early detection of AKI and for predicting its severity in various clinical settings, including cardiac surgery [45,139-141] and critical illness [43,46].

The performance of six candidate urinary biomarkers, KIM-1, NAG, NGAL, IL-18, CysC and α-1 microglobulin were explored for the early detection of acute kidney injury in cardiopulmonary bypass (CPB). Urinary KIM-1 performed the highest area under-the-receiver-operator-characteristic curve (AUC 0.78, 95% confidence interval 0.64–0.91), followed by IL-18 and NAG. After adjustment for a preoperative AKI prediction score (Cleveland Clinic Foundation score) or CPB perfusion time, only urinary KIM-1 remained independently associated with AKI[38].

9.2 Role of Genetic Polymorphism in AKI

9.2.1 Gene and genome

An organism must be able to store and preserve its genetic information, express information along the process of life as well as transfer information to future generation. The important these steps are called as the central dogma of molecular biology (Figure 19)

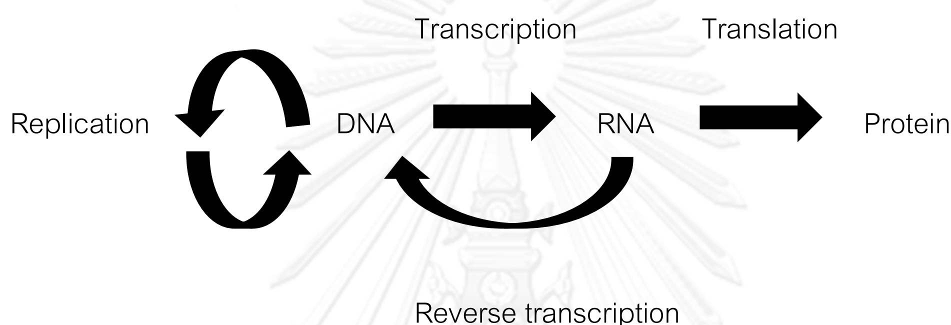


Figure 19 The central Dogma of Molecular Biology

Genetic information is stored in the base sequence of DNA molecules. Transcription is the first step of gene expression involves transfer of information in a double-stranded DNA molecule to a single-stranded RNA molecule. Only one strand of the DNA molecule called the template strand, is copied by RNA polymerase as it synthesis RNA in the 5’ to 3’ direction. Because RNA polymerase moves in the 3’ to 5’ direction along the template strand of DNA, therefore, the RNA product is antiparallel and complementary to the template. RNA polymerase recognizes start signals (promoters) and stop signals (terminators) for each of thousands of transcription units in the genome. RNA consists of three main types including ribosomal RNA (rRNA), transfer RNA (tRNA) and messenger RNA (mRNA). mRNA is only type of RNA that is translated the information in the RNA base sequence to the amino acid sequence of a protein. Each amino acid is specified by one or more nucleotide triplets (codons) in the DNA

(Figure 20). There are 64 codons; three codons are stop codons that terminate translation (nonsense codons). More than one codon can specify a single amino acid. All amino acids except methionine and tryptophan have more than one codon

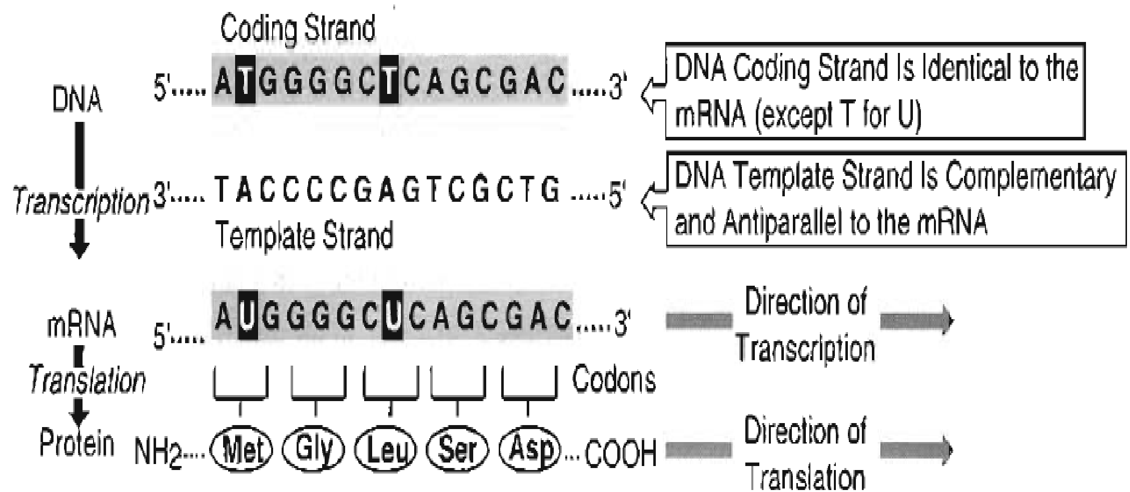


Figure 20 Flow of genetic information from DNA to protein

Nucleic acids (DNA and RNA) are assembled from nucleotides, which consist of three components including a nitrogenous base, a five carbon sugar (pentose) and phosphate. There are two types of nitrogen-containing bases including purines and pyrimidines. Purines contain two rings in their structure. The two purines commonly found in nucleic acids are adenine (A) and guanine (G). Both are found in DNA and RNA. On the contrary, pyrimidines have only one ring. Cytosine (C) is present in both DNA and RNA. Thymine (T) is usually found only in DNA, whereas uracil (U) is found only in RNA. If the pentose is ribose, the nucleic acid is RNA (ribonucleic acid), whereas the pentose is deoxyribose, the nucleic acid is DNA (deoxyribonucleic acid).

DNA consists of a double-stranded DNA molecule. The two strands are antiparallel (opposite in direction) and complementary. A (adenine) always pairs with T (thymine), and G (guanine) always pairs with C (cytosine). Thus, the base sequence on

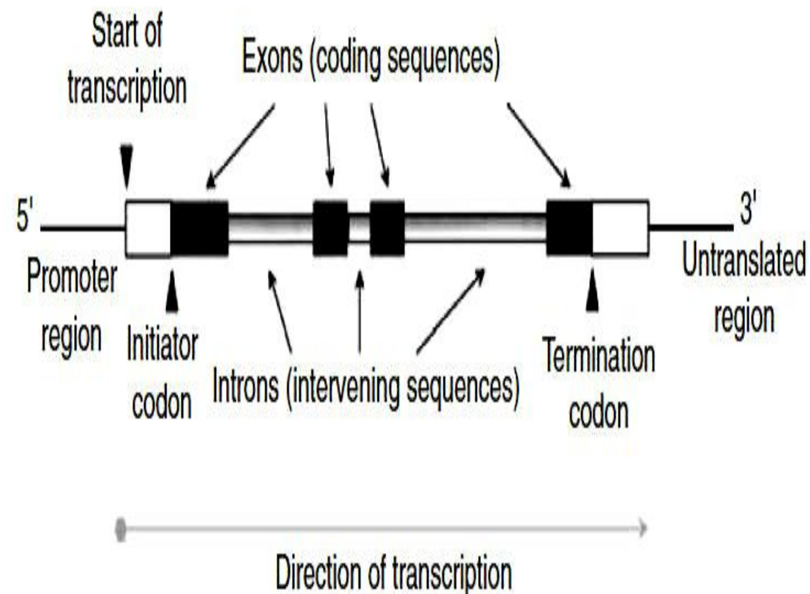
one strand defines the base sequence on the other strand. DNA within the human nucleus is organized into chromosomes. Normal human somatic cells are diploid with 46 chromosomes including 22 pairs of autosomes and 1 pair of sex chromosomes, either XY (male) or XX (female). Mature gametes have a haploid number of chromosomes (22 autosomes and either an X or Y sex chromosome). There are more than 3 billion base pairs in the human haploid chromosome. Chromosomes consist of chromatin that combined DNA, histones, and other DNA binding proteins. DNA replication is the process in which chromosome is duplicated before cell division. When cells divide, each daughter cell must receive one of the two identical copies of parental DNA.

9.2.2 Human genetic variability

Parental genotypes have important effect on an individual's genotype, however, the variations of human genome as genetic polymorphism also have been observed in the representing of 0.1% in general population which are markers of biologic diversity [142]. Some genotypic variations such as cytokine gene polymorphism are correlated with specific phenotypes relevant to several diseases including infectious and non-infectious causes[143,144].

Polymorphism might be demonstrated at any sites on human genome such as the promoter or 5'-flanking region, the exon(s) or the gene coding sequences, the intron(s) or the gene intervening sequences and the 3'-untranslated (3'-UTR) region which result in different functional effect (Figure 19) .

Polymorphism that involved the promoter region (5'-flanking region) of the gene can affect transcriptional activity that play a role on the functional relevance [145]. Polymorphism of exon may be silent or result in the changes in the gene expression and function. Polymorphism involving the intron may lead to a defect in RNA and mRNA processing. Finally, polymorphism in the 3'-UTR region may affect the mRNA half-life or ribosomal translation of mRNA [146].



Promoter region	Exon	Intron	Untranslated region
• Transcriptional activity	• Silent	• Defect in RNA processing	• mRNA half-life
	• Gene expression	• Defect in mRNA processing	• Ribosomal translation of mRNA
	• Gene function		

Figure 21 Structure of a human gene, sites of polymorphism, and functional relevance (143).

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

9.2.3 Type of human gene polymorphism

Human gene polymorphisms consist of three types [145] (Figure 22)

A. Single nucleotide polymorphism (SNP)

SNP is the most common type and usually consists of single nucleotide substitution.

B. Variable number of tandem repeats (VNTR) or minisatellite polymorphism

Minisatellite polymorphism results from the insertion in tandem of multiple copies of a nucleotide sequence of less than 100 base pairs between two restriction sites.

C. Microsatellite polymorphism

Microsatellites results from repeated short motif of one to five nucleotides several times.

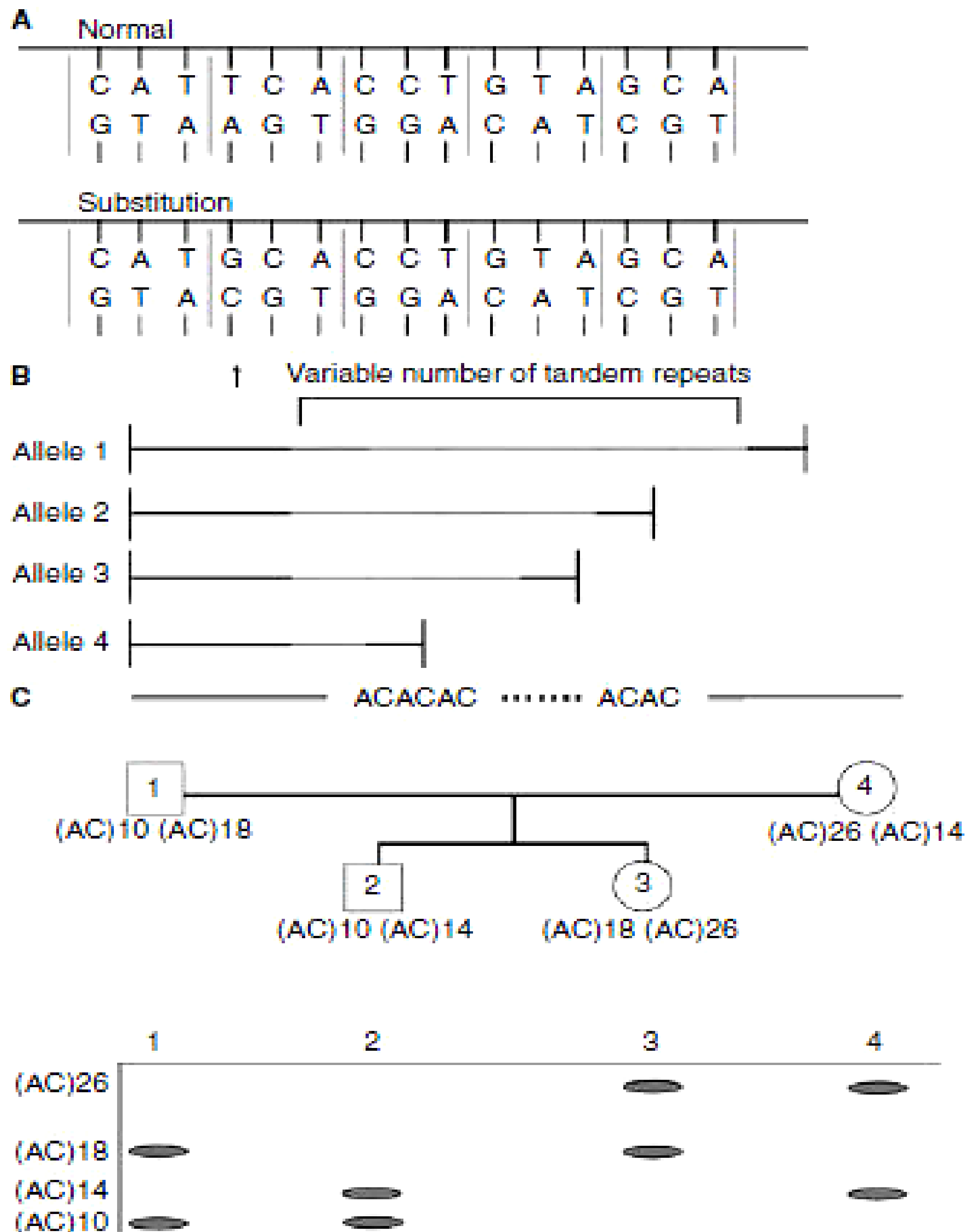


Figure 22. Types of gene polymorphism. (*A*) Single nucleotide polymorphism. (*B*) Variable number of tandem repeats or minisatellite loci polymorphism (DNA sequence 10 to 100 base pairs in length. (*C*) Microsatellite loci polymorphism (DNA sequence of repeating units of 2 to 4 nucleotides) (143)

9.2.4 Identifying human disease genes and susceptibility factors

A. Single-gene disorder or Monogenic Mendelian disorders

A gene is a hereditary factor, transmitted from parents to offspring, that influences traits among the offspring. Physically, a gene consists of a sequence of DNA bases that encode a specific protein. The physical location of a gene on a chromosome is termed a locus. Variation in the DNA sequence at a locus produces different forms of a gene, called alleles. Some alleles may result in a missing or abnormal protein, causing disease. When a specific site on a chromosome has multiple alleles in a population, it is termed a polymorphism ("multiple forms").

The specific DNA sequence at a locus is termed a genotype. In diploid somatic cells, a genotype may be homozygous (homozygote) at a given locus, indicating that the individual inherited the same allele from both parents. If he or she inherited different alleles from each parent, the genotype is heterozygous (heterozygote). The genotype is observed physically as a phenotype, which reflects the interaction of the genotype with the environment and with genes at other loci. If only one copy of an allele is required for its phenotypic expression, the allele is dominant (i.e., it is observable in the heterozygous state). If two copies of the allele are required for its expression (i.e., the disease phenotype is observable only in the homozygous state), it is recessive. The expression of the recessive allele is thus "hidden" in the heterozygote. The terms dominant and recessive provide a convenient classification of genetic diseases, as seen below. If two different alleles are both phenotypically expressed in a heterozygous genotype, the alleles are said to be codominant.

A mutation is an alteration in DNA sequence (thus, mutations produce new alleles). When mutations occur in cells giving rise to gametes, they can be transmitted to future generations. Missense mutations result in the substitution of a single amino acid in the polypeptide chain (e.g., sickle cell disease is caused by a missense mutation that produces a substitution of valine for glutamic acid in the /3-globin polypeptide). Nonsense mutations produce a stop codon, resulting in premature termination of translation and a truncated protein. Nucleotide bases may be inserted or deleted. When

the number of inserted or deleted bases is a multiple of three, the mutation is said to be in-frame. If not a multiple of three, the mutation is a frameshift, which alters all codons downstream of the mutation, typically producing a truncated or severely altered protein product. Mutations can occur in promoter and other regulatory regions or in genes for transcription factors that bind to these regions. This can decrease or increase the amount of gene product produced in the cell.

Mutations can also be classified according to their phenotypic effects. Mutations that cause a missing or decreased protein product are termed loss-of-function. Those that produce a protein product with a novel or abnormal function are termed gain-of-function. The recurrence risk is the probability that the offspring of a couple will express a genetic disease. For example, in the mating of a normal homozygote with a heterozygote that has a dominant disease-causing allele, the recurrence risk for each offspring is 1/2, or 50%. It is important to remember that each reproductive event is statistically independent of all previous events. Therefore, the recurrence risk remains the same regardless of the number of previously affected or unaffected offspring. Determining the mode of inheritance of a disease (e.g., autosomal dominant versus autosomal recessive) enables one to assign an appropriate recurrence risk for a family. There are several modes of inheritance including autosomal dominant, autosomal recessive, x-linked recessive, x-linked dominant and mitochondrial inheritance. Therefore, for identifying single gene disorder, the pedigrees analysis should be used.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

B. Population Genetics

Population genetics is the study of genetic variation in populations. Basic concepts of population genetics allow us to understand how and why the prevalence of various genetic diseases differs among populations. An essential step in understanding genetic variation is to measure it in populations. This is done by estimating genotype and gene frequencies.

b.1) Genotype Frequencies

For a given locus, the genotype frequency measures the proportion of each genotype in a population. For example, suppose that a population of 100 individuals has two possible alleles, labeled 1 and 2, there are three possible genotypes: 1-1, 1-2, and 2-2. We find that the genotypes are distributed as follows:

Genotype	Count
1-1	49
1-2	42
2-2	9
Total	100

The genotype frequency is then obtained by dividing the count *for* each genotype by the total number of individuals. Thus, the frequency of genotype 1-1 is 49/100 = 0.49 and the frequencies of genotypes 1-2 and 2-2 are 0.42 and 0.09, respectively.

b.2) Gene Frequencies

The gene frequency measures the proportion of chromosomes that contain a specific allele. Each individual with the 1-1 genotype has two copies of allele 1, and each heterozygote (1-2 genotype) has one copy of allele 1. Because each diploid somatic cell contains two copies of each autosome, our denominator is 200. Thus, the gene frequency of allele 1 in the population is: [(2 X 49) + 42] / 200 = 0.7.

The same approach can be used to estimate the frequency of allele 2, which is 0.3. A convenient shortcut is to remember that the gene frequencies for all of the alleles of a given locus must add up to 1. Therefore, we can obtain the frequency of allele 2 simply by subtracting the frequency of allele 1 (0.7) from 1.

b.3) Hardy-Weinberg Equilibrium

If a population is large, and if individuals mate at random with respect to their genotypes at a locus, the population should be in Hardy-Weinberg equilibrium, which means that there is a constant and predictable relationship between genotype frequencies and gene frequencies. This allows us to estimate genotype frequencies if we know gene frequencies, and vice versa. Suppose the gene frequency of allele 1, denoted p, is 0.4. If the locus has two alleles, then the frequency of allele2, denoted q, is $1- P = 1 - 0.4 = 0.6$.Knowing these frequencies, and assuming Hardy-Weinberg equilibrium, we can predict the frequencies of the three possible genotypes in our population. The assumption of random mating allows us to estimate the probability (frequency) that an individual has genotype 1-1 by simply multiplying the frequency of allele 1 by itself (i.e., this is the probability that the individual inherited a copy of allele 1 from his father *and* from his mother). Thus, the frequency of the 1-1 genotype is given by $p^2 = 0.4 \times 0.4 = 0.16$. Similarly the frequency of genotype 2-2 is given by $q^2 = 0.6 \times 0.6 = 0.36$.

The frequency of the heterozygous genotype, 1-2, is given by $2pq$. *Here,* we are multiplying the frequency of p and q and taking into account the fact that the individual could have obtained the heterozygous genotype in two ways: either the mother transmitted allele 1 and the father transmitted allele 2, *or* the mother transmitted allele 2 and the father transmitted allele 1. Because each of these events has a probability of pq, we multiply by 2 to account *for* the occurrence of either event (in essence the 2 reflects that fact that our cells are diploid, containing two copies of each autosome). To summarize the gene frequencies and the predicted frequencies of each genotype under Hardy-Weinberg equilibrium:

Frequency of allele 1 $(p) = 0.4$

Frequency of allele 2 $(q) = 0.6$

Frequency of genotype 1-1 = $p^2 = 0.4^2 = 0.16$

Frequency of genotype 1-2 = $2pq = 2 \times 0.4 \times 0.6 = 0.48$

Frequency of genotype 2-2 = $q^2 = 0.6^2 = 0.36$

Note that the genotype frequencies must add to 1:$p^2 + 2pq + q^2 = 1$

Although human populations are typically in Hardy-Weinberg equilibrium for most loci, deviations from equilibrium can be produced by nonrandom mating (i.e., in breeding;) or by the action of natural selection, genetic drift, or gene flow.

For identifying susceptibility genes in complex disease such as diabetes mellitus or cancer that is determined by genetic background, environment factors, and lifestyle, allelic association studies that compare a population of affected individuals with a control population could be performed as case-control studies. However, a population association can have many possible causes, not all of which are genetics. The several factors are proposed as follows:

: **Direct causation**: having allele A makes you susceptible to disease D. Possession of A may be neither necessary nor sufficient for somebody to develop D, but it increases the likelihood.

: **An epistatic effect**: people who have disease D might be more likely to survive and have children if they also have allele A.

: **Population stratification**: the population contains several genetically distinct subsets, and both the disease and allele A happen to be particularly frequent in one subset.

: **Type I error**: association studies normally test a large number of markers for association with a disease. Even without any true effect, 5% of results will be significant at the p=0.05 level and 1% at the p=0.01 level. These are false positive, or type I errors. The raw p-values need correcting for the number of questions asked.

: **Linkage disequilibrium**: the disease-associated allele A marks an ancestral chromosome segment that carries a sequence variant causing susceptibility to the disease. Most disease association studies aim to discover associations caused by linkage disequilibrium.it is then an additional step to identify the actual causative sequence variant as well as test functional evidence that it has a biological effect.

9.2.5 Genetic Approaches for Identifying Disease Genes

There are two primary strategies for mapping genes that cause or increase susceptibility to human disease including linkage analysis and allelic association studies. The indication and limitation of both methods are summarized in Table 10.

The HapMap Project completed in 2005, 30 provided a genomewide map of common single-base-pair variations (also known as single-nucleotide polymorphisms or SNPs) in persons from a variety of population groups. This project has shown that SNPs are very common throughout the human genome, are often correlated with their neighboring
SNPs, and occur, on average, approximately every 800 base pair. The HapMap Project has also provided a powerful tool (the so-called genomewide association study) that facilitates the identification of genetic associations with complex conditions. Over the past 5 years, genome-wide association studies have made it possible to measure the associations between mapped SNPs and the presence of common complex conditions in large patient cohorts, thereby revolutionizing the study of many traits and diseases. Most SNPs associated with common diseases explain a small proportion of the observed contribution of heredity to the risk of disease in many cases less than 5 to 10%, substantially limiting the use of these markers to predict risk. It thus comes as no surprise that as yet there are no evidence-based guidelines that recommend the use of SNP markers in assessing the risk of common diseases in clinical care. Considerable resources are being invested in discovering these unknown sources of heritable risk. Improved risk-analysis models incorporating genomic and nongenomic factors will emerge when an increased percentage of heritable risk can be measured. An important yield of genome-wide association studies is information about the role of specific proteins and biologic pathways in pathogenesis; these proteins and pathways are candidate targets for the development of preventive and therapeutic methods for disease management.

Table 10: Genetic Approaches for Identifying Disease Genes

Methods	Indications and advantages	Limitations
Linkage analysis	Analysis of monogenic traits	Difficult to collect large informative pedigrees
	Suitable for genome scan	Difficult to obtain sufficient statistical power for complex traits
	Control population not required	
	Useful for multifactorial disorder in isolated population	
Allele-sharing methods		
Affected sib and relative pair analyses	Suitable for identification of susceptibility genes in polygenic and multifactorial disorders	Difficult to collect sufficient number of subjects
	Suitable for genome scan	Difficult to obtain sufficient statistical power for complex traits
Sib pair analysis	Control population no required if allele frequencies are known	
	Statistical power can be increased by including parents and relatives	
Association studies		
Case-control studies Linkage disequilibrium Transmission distortion test	Suitable for identification of susceptibility genes in polygenic and multifactorial disorders	Required large sample size and matched control population
	Suitable for testing specific allelic variants of known candidate loci	False positive results in the absence of suitable control population
	Does not necessarily need relatives	

9.2.6 Methods Used for the Detection of Mutations

DNA sequence analysis is increasingly used as a diagnostic tool and has significantly effect on the diagnostic accuracy. It is helpful for determining carrier status and for prenatal testing in monogenic disorder. Numerous techniques are available for the detection of mutations (Table 11). In a very broad sense, one can distinguish between techniques that allow for screening the absence or presence of known mutations (screening mode) or techniques that definitively characterize mutations. Analyses of large alterations in the genome are possible using cytogenetics, fluorescent in situ hybridization (FISH) and Southern blotting.

More discrete sequence alterations rely heavily on the use of the PCR, which allows rapid gene amplification and analysis. Moreover, PCR makes it possible to perform genetic testing and mutation analysis with small amounts of DNA extracted from leukocytes or even from single cells, buccal cells, or hair roots. Screening for point mutations can be performed by numerous methods. Most are based on the recognition of mismatches between nucleic acid duplexes, electrophoretic separation of single or double-stranded DNA, or sequencing of DNA fragments amplified by PCR. DNA sequencing can be performed directly on PCR products or on fragments cloned into plasmid vectors amplified in bacterial host cells.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Reverse transcriptase PCR (RT-PCR) may be useful to detect absent or reduced levels of mRNA expression due to a mutated allele. Protein truncation tests (PTT) can be used to detect the broad array of mutations that result in premature termination of a polypeptide during its synthesis. The isolated cDNA is transcribed and translated in vitro, and the proteins are analyzed by gel electrophoresis. Comparison of electrophoretic mobility with the wild type protein allows detection of truncated mutants.

Table 11: Methods used for the detection of mutations

Method	Principle	Type of Mutation Detected
Commonly used techniques		
Cytogenic analysis	Unique visual appearance of various chromosomes	Numerical or structural abnormalities in chromosome
Fluorescent in situ hybridization (FISH)	Hybridization to chromosomes with fluorescently labeled probes	Numerical or structural abnormalities in chromosome
Southern blot	Hybridization with genomic probes or cDNA probe after digestion of high molecular DNA	Large deletion, insertion, rearrangement, expansions of triplet repeat, amplification
Polymerase chain reaction (PCR)	Amplification of DNA segment	Expansion of triplet repeats, variable number of tandem repeats (VNTR), gene rearrangements, translocations, prepare DNA for other mutation methods
Reverse transcriptase PCR (RT-PCR)	Reverse transcription, amplification of DNA segment → absence or reduction of mRNA transcription	Analyze expressed mRNA (cDNA) sequence, detect loss of expression
DNA sequencing	Direct sequencing of PCR products, sequencing of DNA segments cloned into plasmid vectors	Point mutation, small deletions, and insertions
Restriction fragment polymorphism (RFLP)	Detection of altered restriction pattern of genomic DNA (southern blot) or PCR products	Point mutation, small deletions, and insertions
Other techniques		
Single-strand conformational polymorphism (SSCP)	PCR of DNA segment. Mutations result in conformational change and altered mobility	Point mutation, small deletions, and insertions
Denaturing gradient gel electrophoresis (DGGE)	PCR of DNA segment. Mutations result in conformational change and altered mobility	Point mutation, small deletions, and insertions
RNAse cleavage	Cleavage of mismatch between mutated wild type sequence	Point mutation, small deletions, and insertions
Oligonucleotide specific hybridization (OSH)	Hybridization of PCR products to wild type or mutated oligonucleotides immobilized on chips or slides	Point mutation, small deletions, and insertions

The majority of traditional diagnostic methods are gel-based. Novel technologies for the analysis of mutations, genetic mapping, and mRNA expression

profiles are in rapid development. DNA chip technologies allow hybridization of DNA or RNA to hundreds of thousands of probes simultaneously. Microarrays are being used clinically for mutational analysis of several human disease genes, as well as for the identification of viral sequence variations. To gather with knowledge gain from human gene project, these technologies provide the foundation to expand from a focus on single gene to analyze at the scale of genome.

9.2.7 Genetic Polymorphism of Acute Inflammatory Response

Recently, there has been interest in exploring the role of genetic polymorphisms including cytokines and other immune modulators as potential determinants of susceptibility to or severity of acute illnesses including AKI. Selected list of polymorphism of immune response genes in humans are shown in Table 12. Positive associations between polymorphism of immune response genes and acute infectious and inflammatory disorders are shown in Table 13.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table 12. Selected list of polymorphism of immune response genes in humans [143,144]

Gene	Polymorphism			Gene expression or function
	Site	Class	Position	
Cytokines				
TNF-alpha	Promoter	SNP (G to A)	-238	Affected [147]
TNF-alpha	Promoter	SNP (G to A)	-308	Affected [148-151]
TNF-beta (LT-a)	Intron	SNP (G to A)	+250 (intron 1)	Affected[a] [152]
TNF-beta (LT-a)	Intron	SNP (G to A)	+1069 (intron 1)	Affected[a] [153]
IL-1alpha	Promoter	SNP (C to T)	-889	Affected [154]
IL-1alpha	Intron	46-bpVNTR	Intron 6	Unknown [155]
IL-1beta	Exon	SNP (C to T)	+3953 (exon 5)	Affected [156]
IL-1beta	Promoter	SNP (C to T)	-511	Affected [157]
IL-1Ra	Intron	86-bp VNTR	Intron 2	Affected [158]
IL-6	Promoter	SNP (G to C)	-174	Affected [159-161]
IL-6	Promoter	SNP (G to C)	-572	Affected [162]
IL-10	Promoter	SNP (G to A)	-1082	Affected [163,164]
IL-10	Promoter	SNP (C to T)	-819	Affected [147,164]
IL-10	Promoter	SNP (C to A)	-592	Affected [147]
Chemokines				
IL-8	Promoter	SNP (A to T)	-251	Affected [165,166]
IL-8	3'UTR	SNP (G to A)	+2767	Affected [167,168]
IL-8	Promoter	SNP (C to T)	-845	Affected [169]
MCP-1	Promoter	SNP (G to A)	-2518	Affected [170,171]

Table 12 (continued)				
Gene	Polymorphism			Gene expression or function
	Site	Class	Position	
Toll-like receptors (TLR)				
TLR2	Exon	SNP (C to T)	+2029	Affected [172]
TLR2	Exon	SNP (G to A)	+2251	Affected [173]
TLR4	Exon	SNP (A to G)	+896	Affected [174]
Heat shock proteins (HSP)				
HSP70-2	Exon	SNP (G to A)	+1267	Affected [175]
HSP70-2	Exon	SNP (G to A)	+1538	Unknown [176]
HSP70-Hom	Exon	SNP (C to T)	+2437	Unknown [176]
Oxidant stress-related enzymes				
HO-1	Promoter	MSR $(GT)_n$	-263, -185	Affected [177,178]
NADPH oxidase p22phox	Exon	SNP (C to T)	+242 (exon 4)	Affected [179]
NADPH oxidase p22phox	3'UTR	SNP (A to G)	+640	Unknown [179]
MPO	Promoter	SNP (G to A)	-463	Affected [180,181]
SOD3	Exon	SNP (C to G)	+637	Affected [182]
Catalase	Promoter	SNP (C to T)	-262	Affected [183]
GPX1	Exon	MSP $(GCG)_n$	Exon 1	Unaffected [184]
GPX1	Exon	SNP (C to T)	+593	Unaffected [185]
GST M1	Exon[b]	-	-	Affected [186,187]
iNOS	Promoter	SNP (C to T)	-1173	Affected [188]

Abbreviations are: SNP, single nucleotide substitution; VNTR, variable number of tandem repeats; MSR, microsatellite repeats; UTR, untranslated region; HSP, heat shock protein; MPO, myeloperoxidase; SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione-S transferase; iNOS, inducible nitric oxide synthase; TNF, tumor necrosis factor; IL, interleukin; HO, heme oxygenase; NADPH, nicotinamide adenine dinucleotide phosphate. [a] TNF-alpha production is affected, [b] Gene deletion prevalent in up to 50% of humans.

Table 13. Positive associations between polymorphism of immune response genes and acute infectious and inflammatory disorders [143,144]

Gene	Polymorphic allele	Acute illness
Cytokines		
TNF-alpha	-308 A-allele (TNFa2)	Sepsis/septic shock [69,70,189-191]
		Meningococcal disease [192]
		Cerebral malaria [193]
		Mucocutaneous leishmaniasis [194]
		Rate of body temperature normalization following cardiopulmonary bypass [195]
		Neonatal acute renal failure [72]
		Severe acute kidney-pancreas transplant rejection episodes [196]
		Increased risk of death in dialysis-requiring acute renal failure [47]
TNF-alpha	-238 A-allele	Malarial anemia [197]
TNF-beta[a]	+250 AA genotype	Septic shock in community-acquired pneumonia [198,199]
TNF-beta	+250 G-allele	Prolonged mechanical ventilation following cardiopulmonary bypass [71]
TNF-beta	+1069 (NcO1) allele 2/2	Septic shock following acute biliary pancreatitis [200]
		Higher circulating TNF-a levels following cardiopulmonary bypass [201]
		Increased mortality in severe sepsis [190]
		Susceptibility to severe posttraumatic sepsis [191]
IL-1alpha	-889 TT genotype	Osteomyelitis [202]
IL-1 beta	+3953 TT genotype	Osteomyelitis [202]
IL-1 beta	+3953 allele 2	Increased risk of ESRD in PR3-ANCA vasculitis [203]
IL-1 beta	-511 allele 2/2	Febrile seizure in children[204]
IL-1 beta	-511 allele 1/2	Survival in meningococcal disease [205]

จุฬาลงกรณ์มหาวิทยาลัย CHULALONGKORN UNIVERSITY

Table 13 (continued)

Gene	Polymorphic allele	Acute illness
Cytokine		
IL-1Ra	86 bp VNTR (allele 2)	Susceptibility to sepsis [206,207]
		Reduced Mantoux response to purified protein derivative of *Mycobacterium tuberculosis* [208]
		Increased risk of ESRD in PR3-ANCA vasculitis [203]
		Severity of Henoch-Schonlein purpura-associated nephritis [209]
		High soluble endothelial activation (vonWillebrand factor and E-selectin) markers in acute coronary syndromes[210]
IL-6	-174 GG genotype	Improved survival in sepsis [211]
IL-6	-174 C-allele	Neonatal acute renal failure [72]
IL-6	-572 C-allele	Higher serum C-reactive protein level [162]
IL-10	-1082 GA genotype	Meningococcal disease [212]
		Susceptibility to pulmonary tuberculosis [213]
IL-10	-1082 G-allele	Severity of illness in community-acquired pneumonia [214]
		Severe acute kidney-pancreas transplant rejection episodes [196]
		Decreased risk of death in dialysis-requiring acute renal failure [47]
IL-10	-592 A-allele	Increased mortality in sepsis [215]
IL-10	-592 AA genotype	Decreased risk of acute GVHD and death [216]
Chemokines		
IL-8	+2767 A-allele	Henoch-Schonlein purpura-associated nephritis [168]
IL-8	-251 A-allele	Enteroaggregative *Escherichia coli* diarrhea[165]
		Respiratory syncitial virus bronchiolitis[166]
IL-8	-845 C-allele	Severity of lupus nephritis[169]
MCP-1	-2518 G-allele	Premature kidney graft failure[217]

Table 13 (continued)		
Gene	Polymorphic allele	Acute illness
Toll-like receptors (TLR)		
TLR2	Arg753Gln	Gram-positive bacterial sepsis [173]
TLR2	Arg677Trp	Susceptibility to lepromatous leprosy [218]
TLR4	Asp299Gly, Thr399Ile	Gram-negative bacterial sepsis [219]
TLR4	Asp299Gly	Sepsis and septic shock[220,221]
Heat shock proteins (HSP)		
HSP70-2	+1267 GG genotype	Neonatal acute renal failure [175]
HSP70-2	+1267 AA genotype	Increased risk of septic shock [199]
HSP70-Hom	+1538 CT genotype	Multiple organ failure following trauma [176]
Oxidant stress-related enzymes		
HO-1	Long (GT)n repeats	Increased susceptibility to coronary artery disease in diabetic patients[178]
		Increased risk of restenosis following PTCA[222]
		Increased risk for developing abdominal aortic aneurysm[223]
		Increased susceptibility to pulmonary emphysema in cigarette smokers[224]
NADPH oxidase p22phox	+242 CC genotype	Increased oxidized high-density lipoprotein cholesterol in type 2 diabetes mellitus[225]
MPO	-463 GG-genotype	Increased prevalence of cardiovascular disease and higher circulating levels of pentosidine in ESRD[181]
GST	GST M1-0 genotype	Increased risk for lung cancer in heavy smokers[226]
GST	GST M1-B allele carrier	Decreased risk of delayed graft function[227]
iNOS	-1173 T-allele carrier	Decreased risk of malarial complications[188]

Abbreviations: ESRD, end-stage renal disease; PR3-ANCA, proteinase 3 antineutrophilic cytoplasmic antibody; PTCA, percutaneous transluminal coronary angioplasty; GVHD, graft versus host disease; GST, glutathione-S transferase; iNOS, inducible nitric oxide synthase; HO, heme oxygenase; MPO, myeloperoxidase; IL, interleukin; TNF, tumor necrosis factor; NADPH, nicotinamide adenine dinucleotide phosphate. [a]TNF-beta is lymphotoxin alpha (LT-alpha).

The gene coding for TNF-beta is located near the TNF-alpha gene. Single nucleotide polymorphism at position +1069 (G to A) in the first intron of the gene has been identified and defined as TNF-b1 (G) and TNF-b2 (A) variants [153]. TNF-b2 variant is associated with higher multiple organ failure scores and mortality in critical ill [190,191,200].

9.2.8 Genetic Polymorphism in AKI

In recent years, the role of genetic polymorphisms as potential determinants of adverse outcomes in patients with AKI has been explored [47-54]. First, an important source of ROS in AKI is NADPH oxidase (NOX), an enzyme which primarily produces superoxide [228]. This enzyme has multiple components, including 2 transmembrane subunits (gp91 phox and p22 phox), 3 cytosolic subunits (p40 phox, p47 phox, and p67 phox), and a GTP-binding protein (p21). The gene encoding the NADPH oxidase p22phox subunit polymorphisms as p22phox+242CCgenotype was associated with higher basal and stimulated superoxide generation when compared with p22phox+242 T-allele in human endothelial cells [229]. NADPH oxidase p22phox +242 T-allele was associated with dialysis requirement or hospital death among patients with AKI [50].

The p22 phox subunit is the final transporter in the chain transferring electrons from NADPH to molecular oxygen and is encoded by the subunit of the cytochrome b 245 (CYBA) genes, which is located on chromosome 16q24. Five polymorphisms of interest in the CYBA gene locus as promoter –1442 (G –A), intron 1 +383 (G- A), exon 4 +242 (C-T), and 3’-UTR +8897 (A-T), and +640 (A –G) were identified. The *CYBA* promoter –1442 (G –A) was associated with lower adjusted odds ratio whereas the *CYBA* exon 4 +242 (C-T), and +640 (A –G) had higher unadjusted odds ratio for the outcome of dialysis requirement or in-hospital death. Finally, the presence of the *CYBA* A-A-G-G haplotype [–1442 (G –A), 3’-UTR +8897 (A-T), exon 4 +242 (C-T), intron 1 +383 (G- A] was associated with dialysis requirement or in-hospital death [53].

Myeloperoxidase (MPO) is a lysosomal enzyme that plays a role in oxidative stress-mediated kidney injury. MPO polymorphisms rs22438289 (765 T to C), rs7208693 (157 G to T), rs2071409 (9890 A to C), and rs2759 (149 T to C) had 2-3-fold higher odds for composite outcomes of dialysis or in-hospital death or a composite of dialysis, assisted mechanical ventilation, or in-hospital death. The MPO T-G-A-T haplotype copy-number was associated with lower plasma MPO levels and lower adjusted odds for these composite outcomes [54].

Hypoxia-inducible factor-1alpha (HIF-1alpha) is a transcription factors that responds to tissue hypoxia including AKI. Polymorphism in the coding region of the HIF-1alpha gene at position +85 in exon (C-T substitution) promote transcription activity. T-allele carriers had significantly higher adjusted odds for dialysis requirement or in-hospital death; assisted mechanical ventilation or dialysis requirement; and the composite of assisted mechanical ventilation, dialysis requirement or in-hospital death in patients with AKI [51].

In addition, the catecholaminergic pathway is postulated as an important physical stress in acute kidney injury (AKI). Single nucleotide polymorphisms (SNPs) of phenylethanolamine N-methyltransferase (PNMT), the terminal enzyme of the catecholaminergic pathway were explored. The PNMT +1543 G allele was associated with development of AKI whereas PNMT -161 A allele was associated with lower mortality and hemodynamic shock. The PNMT +1543 G allele was also associated with oliguria [52].

Interestingly, the high TNF-alpha producer and low IL-6 producer genetic variants were associated with an increased risk for AKI in low-birth-weight infants [72]. Among patients with AKI who require dialysis, TNF-alpha high producer genotype (-308 A-allele carrier) was associated with a higher risk of death after adjustment for the APACHE II score (HR 2.47; 95% CI 1.06-5.77; P = 0.04) [47]. Of note, the TNF-alpha high

producer genotype was associated with increased odds for a higher the index of coexistent disease (ICED) score (an index of comorbidity), lower Karnofsky Index (a measure of functional status), and lower serum albumin compared with patients with the low producer genotype for this cytokine[73].

9.2.9 The TNF Locus

The TNF-alpha and TNF-beta genes are located on the short arm of chromosome 6. Genetic variations in transcriptional sites of the tumor necrosis factor (TNF) gene are associated with TNF synthesis. Several polymorphisms have been identified in the TNF gene promoter that affected on TNF levels were observed[60,61].

Wilson et al. identified a biallelic G to A transition polymorphism located at position -308 in the TNF promoter, which defined as TNF1 (-308G) and TNF2 (-308A) alleles[60]. The less common TNF-alpha gene at position –308 (G to A) [TNF2] have been associated with high promoter activity via promoted spontaneous and stimulated TNF-alpha production both in vitro and in vivo[62-66].

TNF2 carriage has been associated with adverse clinical outcomes including mortality among acute infectious diseases and critically ill patients [69,70,189,192,193,198]. In one such study, the TNF-alpha high producer genotype (TNF-a2 allele) was strongly associated with an increased susceptibility to septic shock and mortality [90]. Furthermore, TNF2 homozygous has higher circulating TNF levels than TNF1 homozygous [230] as well as was associated with higher mortality among patients with severe sepsis [190],[191]. Carriers of the TNF-alpha -308 A-allele had higher TNF-alpha production, higher APACHE II score and a higher mortality risk in patients with dialysis-requiring AKI [47].

In the present study, we aimed to explore the association of a functional polymorphism in the promoter region (position -308) of the *TNFA* gene (rs1800629) with kidney disease severity, including glomerular filtration markers and urinary tubular injury markers in a cohort of hospitalized adults with AKI.

CHAPTER III
MATERIALS AND METHODS

1. Study design

1) First part

A systematic review and meta-analysis of large cohort studies were performed to estimate the world incidence of AKI, its stages of severity, and describe geographic variations according to countries, regions and their economies.

2) Second part

A retrospective cohort study was performed to explore the association of a functional polymorphism in the promoter region (position -308) of the *TNFA* gene (rs1800629) with kidney disease severity, including glomerular filtration markers and urinary tubular injury markers in a cohort of hospitalized adults with AKI.

2. Sample size calculation

1) First part

The literatures were searched in MEDLINE (2004-Aug 23, 2012) to identify eligible studies using the Medical Subject Headings (MeSH) search terms "acute renal failure," "acute kidney failure," "acute renal insufficiency," "acute kidney insufficiency," "acute tubular necrosis," "acute kidney injury," or "acute renal injury". The year 2004 was selected as it corresponded to the year in which the RIFLE criteria were first published. The search was limited to human studies without language restrictions. EMBASE using similar search terms, and manually reviewed the bibliography of retrieved articles were used for additional relevant studies.

2) Second part

Following KDIGO guideline, AKI is defined as the increasing in SCr to $\geq$1.5 times baseline. Increase The mean baseline serum creatinine in our pilot study is 1.0

mg/dL. Therefore, the difference in serum creatinine between low producer and high producer at 0.5 mg/dL was set. Common standard deviation of serum creatinine in low and high producers was derived from our pilot study in 50 AKI patients. The sample size was calculated as following

$$N/group = 2\ [(Z_{\alpha/2}+Z_{\beta})\ \sigma\ /\Delta]^{2}$$

$Z_{\alpha/2} = Z_{0.05/2} = 1.96$

$Z_{\beta} = Z_{0.20} = 0.84$

σ = common SD of outcome variance in low and high producer=1.4

Δ = difference in mean between 2 groups =0.5

$$N/group = 2 \times [1.96+0.84) \times 1.4/\ 0.5]^{2}$$

$$= 122.9$$

The ratio of low producer to high producer was around 1:1 in the previous study. Therefore, we needed the sample size of at least 246 patients.

3. Study population

1) First part

Retrospective and prospective cohort studies (including post-hoc analyses derived from clinical trials) of adults and children that reported on the incidence of AKI were included. If more than one publication appeared on the same study, data from the most inclusive report were used. To improve generalizability, we only included studies of adults (age ≥18 years) and children with a minimum sample size of 500 and 50 subjects, respectively. Pairs of two authors initially screened the titles and abstracts of all the electronic citations, and then retrieved and re-screened full-text articles.

2) Second part

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Hospitalized patients with AKI were recruited from 2 acute care hospitals (Boston, Massachusetts, USA) between November 2003 and January 2007. All eligible patients were 18 years or older and received in-hospital nephrology consultation for AKI. Acute kidney injury was defined as a rise in serum creatinine by 0.5, 1.0, or 1.5 mg/dl from a baseline level of ≤ 1.9, 2.0-4.9, or ≥ 5.0 mg/dL, respectively [2]. This definition was adopted prior to the development of the AKI network consensus definition [8]. Exclusion criteria were age < 18 years, pregnancy, chronic dialysis, organ transplantation within the prior year, and urinary obstruction. Institutional review board approval was granted, and informed consent was obtained for each subject.

4. Data collection

1) First part

Due to the unanticipated large number of included articles, the data were extracted by pairs of authors. Disagreements were resolved through consensus and arbitration by a third author. Data extraction included country of origin, year of publication, study design, sample size, patient characteristics (age and sex), and clinical setting (e.g., cardiac surgery, nephrotoxins including radiocontrast exposure, critical care, trauma, heart failure, hematology/oncology, community-acquired, and hospital-acquired [unspecified]). The definition of AKI, and number of patients who developed AKI were also recorded. Countries were grouped within continents and world zones in accordance with the geo-scheme devised by the United Nations Statistics Division. Countries' economies were assessed according to four ranges of gross national income per capita derived from the World Bank's classification of income of economies: low (≤US$1,005), lower middle (US$1,006-$3,975), upper middle (US$3,976-$12,275), and high (≥US$12,276) income countries. Countries were also classified according to national total expenditure on health (representing the sum of general government and private health expenditures in a given year, calculated in national currency units in current prices) as a percentage of gross domestic product or GDP (representing the value of all final goods and services produced within a nation in a

given year), using the World Health Organization's world health statistics. In term of latitude, studies were classified as originating from countries located north or south of the equator.

We harmonized the AKI definitions adopted in the individual studies first by classifying them according to the RIFLE or AKIN criteria, other serum-creatinine based definitions, and administrative codes for AKI derived from the *International Classification of Diseases, Ninth or Tenth Revision, Clinical Modification* (ICD-9-CM or ICD-10-CM) methodology. We then reclassified studies that adopted the RIFLE (including the pediatric or pRIFLE) or AKIN serum creatinine-based criteria to define AKI and its severity as equivalent to the latest AKI definition and staging system proposed by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines for AKI. These studies were grouped and termed as having employed a KDIGO-equivalent AKI definition. The remaining studies that defined AKI according to other biochemical/urine output/dialysis requirement-based criteria or administrative diagnosis codes for AKI were analyzed separately. The quality of the cohort studies was assessed independently by pairs of 2 authors, using the Newcastle-Ottawa Scale, which allocates a maximum of 9 points for quality of the selection, comparability, and outcome of study populations. Study quality scores were defined arbitrarily as poor (0-3), fair (4-6), or good (7-9).

2) Second part

Medical records were reviewed prospectively to retrieve data on each subject, including demographic characteristics, coexisting conditions, hospitalization course and outcomes. Sepsis was ascertained using the systemic inflammatory response syndrome criteria [231]. Two severity-of-illness scores were calculated, the Acute Physiology and Chronic Health Evaluation (APACHE) II score [232] and the Multiple Organ Failure (MOF) score [233]. Pre-existing chronic kidney disease was defined on the basis of a baseline estimated glomerular filtration rate of less than 60 ml/min/1.73 m^2, which was calculated using the Modification of Diet in Renal Disease study equation [234].

5. DNA Extraction and Genotyping Analyses

At enrollment, EDTA-anticoagulated whole blood (10 mL) was collected, aliquoted into cryotubes and stored at -80°C. Genomic DNA was extracted from leukocytes using a spin column method (QIAamp DNA Blood mini kit, Qiagen Inc., Valencia, CA). A commercially-available polymerase chain reaction (PCR) technique (PCR product size = 125 base pairs) (One Lambda Inc., Canoga Park, CA) was used to analyze single nucleotide allelic variations in the promoter region of *TNFA* gene at position –308, using sequence-specific oligonucleotide primers as follow:

Generic primer (antisense): 5'-tctcggtttcttctccatcg-3'

Primer G (sense): 5'-ataggttttgaggggcatgg-3'(TNF1 allele)

Primer A (sense): 5-aataggttttgaggggcatga-3'(TNF2 allele)

DNA is amplified in a 10 ~ µL reaction. Final concentrations of reagents are: 1x AS Reaction buffer (AB Technologies) 200µM each dNTPs (AB Technologies), 1.5 mM $MgCl_2$ (AB Technologies), 8.5% (w/v) sucrose , 0.25 units Thermoprime ^{PLUS} DNA Polymerase (AB Technologies), 5 µM Specific primer mix, 1 µM Internal control primer mix ~100 ng DNA

The protocol for the PCR machine is as follows:

95°C 1 min

95°C 15 s 10 cycles

65°C 50 s 10 cycles

72°C 40 s 10 cycles

95°C 20 s 20 cycles

T°C 50 s 20 cycles

72°C 50 s 20 cycles

where T is the primer-specific annealing temperature of 59°C.[235].

In brief, a Perkin-Elmer 9600 thermocycler (Perkin-Elmer-Cetus, Norwalk, CT) was used to amplify the promoter regions by PCR. An internal control primer pair was included in every PCR reaction to exclude non-specific DNA amplification. Amplified

DNA fragments were separated 1% by agarose gel electrophoresis and stained with ethidium bromide, and the bands were visualized under ultraviolet light (Figure 23).

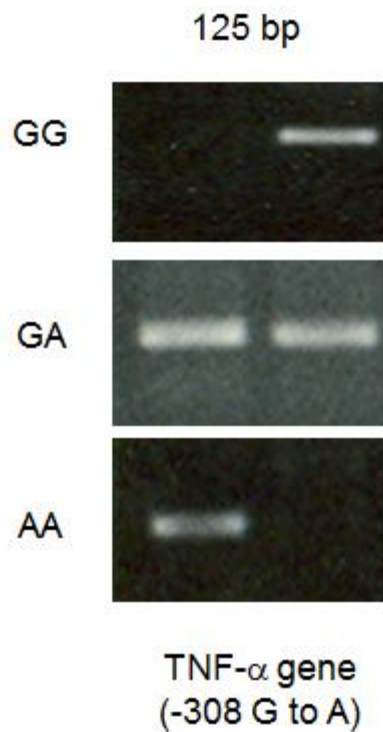


Figure 23 Representative DNA gels of the different TNF-α –308 G to A (233)

Two blinded investigators classified the *TNFA* genotypes as low (GG), intermediate (GA), and high (AA) producer genotypes, based on *in vitro* transfection studies using constructs of the minor allele, and human studies [236].

6. Serum Measurement of Glomerular Filtration Markers

Creatinine was measured by a modified Jaffé method, using a Beckman DXC 800 analyzer (Beckman Coulter, Brea, CA). Plasma cystatin C was measured by immunonephelometry using the BN II System (Siemens Healthcare Diagnostics, Deerfield, IL).

7. Urinary Measurement of Tubular Injury Markers

Urinary N-acetyl-β-D-glucosaminidase (NAG) activity was measured by a colorimetric assay (Boehringer Mannheim, Mannheim, Germany). As part of a previously

reported study, urinary KIM-1 was measured in a subset of patients (*n* = 204) by a microsphere-based Luminex assay[136]. The inter- and intra-assay coefficient of variation was <10.0%. Alpha-GST and pi-GST were measured by sandwich ELISA (Argutus Medical Ltd., Dublin, Ireland). The inter- and intra-assay coefficient of variation for alpha-GST was 9.1% and 8.0%, and for pi-GST, 7.5% and 2.0%, respectively. All measurements were performed in duplicate. All urinary biomarker levels were normalized to urinary creatinine, and expressed as mU/mg (for NAG) or ng/mg (for KIM-1, alpha-GST and pi-GST).

8. Statistical Analysis

1) First part

Inter-rater agreement for the final selection of the articles was evaluated by calculating the weighted Cohen's kappa coefficient using the package 'psych' in the R system software version 2.14.0. Random-effects model meta-analyses were conducted to generate pooled incidence rates of AKI, stages of severity, including stage 1-3 AKI and dialysis requirement.

All pooled estimates are provided with 95% confidence interval (CI). Heterogeneity was assessed using the I^2 index and the Q test P value. The I^2 index describes the percentage of total variation across studies due to true heterogeneity rather than chance, with a value of ≥75% indicating medium-to-high heterogeneity. To examine global patterns of AKI, we conducted random-effects subgroup and meta-regression analyses of AKI rates by geographic world regions, patterns of country economies and latitude. All meta-analyses were performed using Comprehensive Meta-Analysis, version 2.0 (Biostat, www.meta-analysis.com).

2) Second part

The genotype frequencies were tested for Hardy–Weinberg equilibrium using a standard Chi-square test for any deviation of the observed frequencies. Comparisons between genotype groups were made by the analysis of variance (ANOVA) test and the

Kruskal-Wallis test for continuous variables, and by Chi-square or Fisher's exact test for categorical variables. Continuous variables are reported as mean (standard deviation) or median (with 25th and 75th percentile) according to their distribution. Categorical variables are reported as count (percentage).

Multiple linear regression analyses were used to evaluate the association of the *TNFA* gene polymorphism with filtration (serum creatinine and cystatin C) and tubular injury markers (NAG, KIM-1), using dominant (i.e., one or two copies of the minor allele), recessive (i.e., two copies of the minor allele), and additive (per 1-allele copy increase) genetic models. NAG and KIM-1 were log-transformed because of their skewed distribution. To account for the high proportion of levels below the detection limit for alpha-GST and pi-GST, to bit regression with left censoring and the log-normal distribution was used [237]. All the analyses were adjusted for sex, race, age, baseline eGFR, sepsis, and dialysis requirement. Results from the regression models are provided as parameter estimate or relative ratio with 95% confidence interval (CI). All statistical analyses were performed using the SAS software (version 9.2, SAS Institute, Cary, NC). Differences were considered statistically significant at a P-value of less than 0.05.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

CHAPTER IV
RESULTS

1. First part

1.1. Study Characteristics and Quality Assessment

A total of 2,496 potentially relevant citations were identified and screened; 414 articles were retrieved for detailed evaluation of which 312 fulfilled eligibility criteria, representing close to 50 million subjects from more than 40 countries across the globe. The inter-rater agreement weighted kappa coefficient for the final selection of the articles was 0.82 (95% CI 0.76, 0.89). In brief, there were 194 retrospective cohort studies, 109 prospective cohort studies, 9 post-*hoc* cohorts derived from clinical trials, 154 subset of studies that used a KDIGO-equivalent AKI definition. All studies were published in the English language and publication spanned 8 years. There were 269 studies of adults, 42 studies of children, and 1 study combining adults and children. Studies of adults varied from 500 to 12,500,459 subjects, and studies of children from 64 to 60,160 subjects. The mean age of adults ranged from 23 to 80 years, and of children from 0 to 13 years.

Studies involved patients in various clinical settings, the largest, unspecified hospital-acquired AKI (28,887,330 subjects), followed by AKI post-cardiac surgery (15,100,244 subjects), and community-acquired AKI (3,048,354 subjects).

In terms of geography, most (49.0%) studies originated from America (142 studies from North America and 11 studies from South America), followed by Europe (98 studies), Asia (44 studies), Australia & New Zealand (10 studies), and Africa (2 studies). The top 3 world zones where studies were conducted were North America (142 studies), Northern Europe (27 studies), and Eastern Asia (26 studies).

Most (82.7%) studies originated from high-income countries (258 studies) followed by countries of upper middle income (40 studies). Most studies also originated

from countries that spent either 5-10% (136 studies) >10% (150 studies) of GDP on total health expenditure.

In terms of latitude, 92.3% of the studies originated from countries located north to the equator (288 studies). 194 studies (62.2%) were considered of good quality, 116 studies (37.3%) of fair quality, and 2 studies (0.6%) of poor quality.

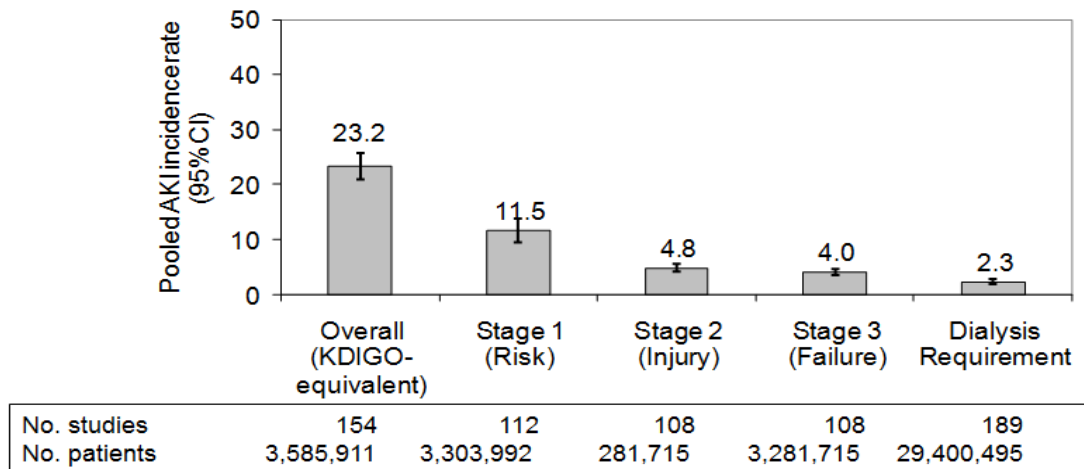
1.2. Pooled Incidence Rate of AKI

Using all 312 studies and irrespective of the AKI definition, the pooled incidence rate of AKI was 10.7% (95% CI 9.6, 11.9). When restricted to the 154 studies (130 of adults and 24 of children) that used a KDIGO-equivalent AKI definition, the pooled incidence rate of AKI was 23.2% (95% CI 21.0, 25.7).Lower pooled incidence rates were observed in studies using other clinical criteria to define AKI, including biochemical, urine output, or dialysis requirement-based criteria (5.0%; 95% CI 3.4-7.1; 130 studies) as well as administrative coding-based criteria (2.9%; 95% CI 2.3-3.7; 28 studies).

By meta-analysis, the pooled incidence rate of stage-1 AKI was 11.5% (112 studies), stage-2 AKI 4.8% (108 studies), and stage-3 AKI 4.0% (108 studies). The pooled incidence of dialysis requirement was 2.3% (189 studies) (Figure 24).

We restricted the remainder of our analyses to studies classified according to the KDIGO-equivalent AKI definition, to obtain more meaningful estimates of the disease across a wide spectrum of patient and country-level characteristics.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY



	Overall (KDIGO-equivalent)	Stage 1 (Risk)	Stage 2 (Injury)	Stage 3 (Failure)	Dialysis Requirement
No. studies	154	112	108	108	189
No. patients	3,585,911	3,303,992	281,715	3,281,715	29,400,495

Figure 24 Pooled incidence rate of AKI in studies that used KDIGO-equivalent serum creatinine-based AKI definition and staging system, or dialysis requirement.

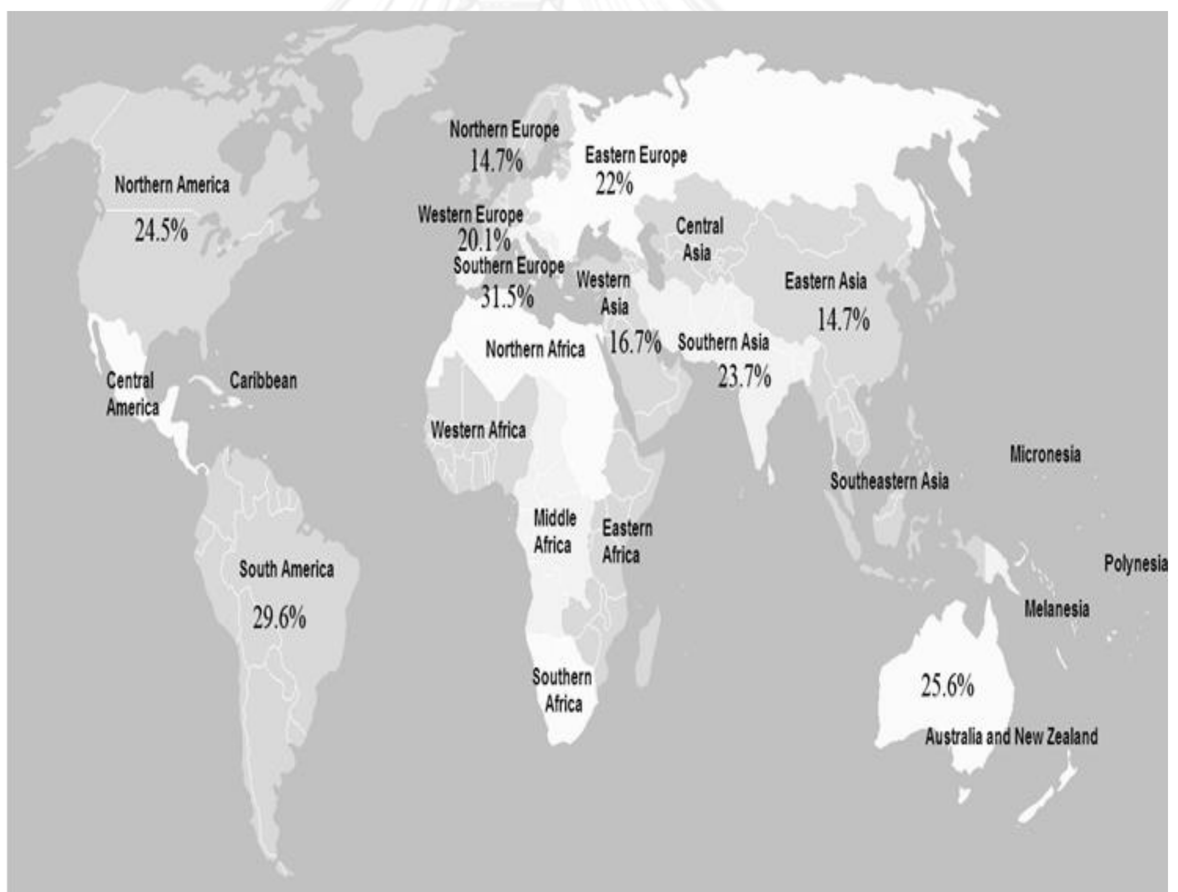


Figure 25: Pooled incidence rate of AKI by world zones in studies that used a KDIGO-equivalent serum creatinine-based AKI definition.

In brief, the pooled incidence rate of AKI in studies of adults and children was 21.6% (95% CI 19.3, 24.1) and 33.7% (95% CI 26.9, 41.3), respectively. The highest pooled incidence rate of AKI was observed in critical care settings (31.7%; 95% CI 28.6, 35.0), followed by cardiac surgery (24.3%; 95% CI 20.4, 28.8). When examined according to geographic regions of the world and patterns of country economies and latitude, the pooled incidence rate of AKI appeared higher in South *vs.* North America (29.6% *vs.* 24.5%), Southern *vs.* Northern Europe (31.5% *vs.* 14.7%), and South *vs.* Western or Eastern Asia (23.7% *vs.* 16.7% *vs.* 14.7%). The pooled incidence rate was also higher in Australia & New Zealand (25.6%; 95% CI 22.3, 29.3). (Figure 25)

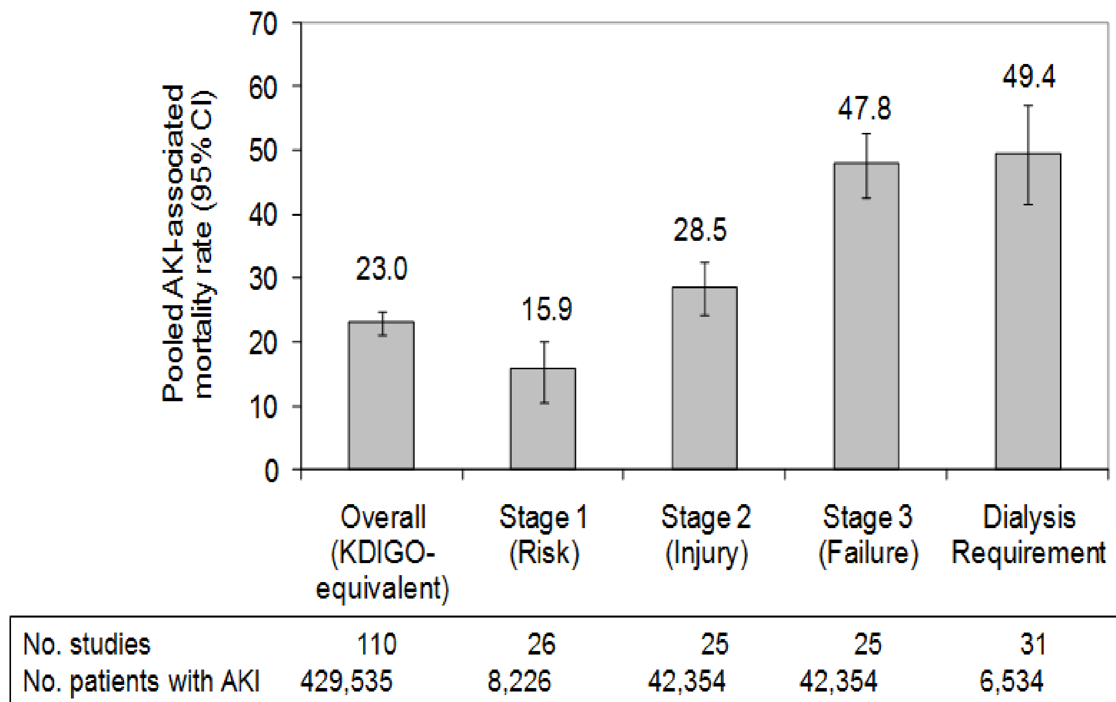
The pooled incidence rate of AKI from studies of countries located south of the equator appeared higher than that reported from countries located north of the equator (27.0% [95% CI 24.2, 30.] *vs.* 22.6% [95% CI 20.2, 25.2]).The pooled incidence rate of AKI appeared higher in studies from countries that spent >10% of GDP on total health expenditure compared with countries that spent ≤5% (25.2% [95% CI 22.3, 28.3] *vs.* 14.5% [95% CI 7.2, 26.9]), with a nonsignificant trend observed by meta-regression (P=0.097). The incidence rate of AKI declined over the span of 8 years (P=0.016).

1.3. AKI- Associated Mortality Rate

By meta-analysis, among a total of 110 studies (99 of adults and 11 of children) that used a KDIGO-equivalent AKI definition and assessed mortality, the pooled AKI-associated all-cause mortality rate was 23.0% (95% CI 21.3, 24.8), and increased with higher stages of severity (Figure 26).

In adults, the pooled mortality rate was 23.9% (95% CI 22.1, 25.7) and in children 13.8% (95% CI 8.8, 21.0). By meta-regression, AKI-associated mortality rate declined over the span of 8 years (P=0.02), and was inversely related to the percentage of country GDP spent on total health expenditure (P<0.001), and country gross national income per capita (P<0.001). At <3, 3-6, and >6 months of follow-up, the pooled AKI-associated mortality rate was 22.1%, 31.5%, and 27.7%, respectively.

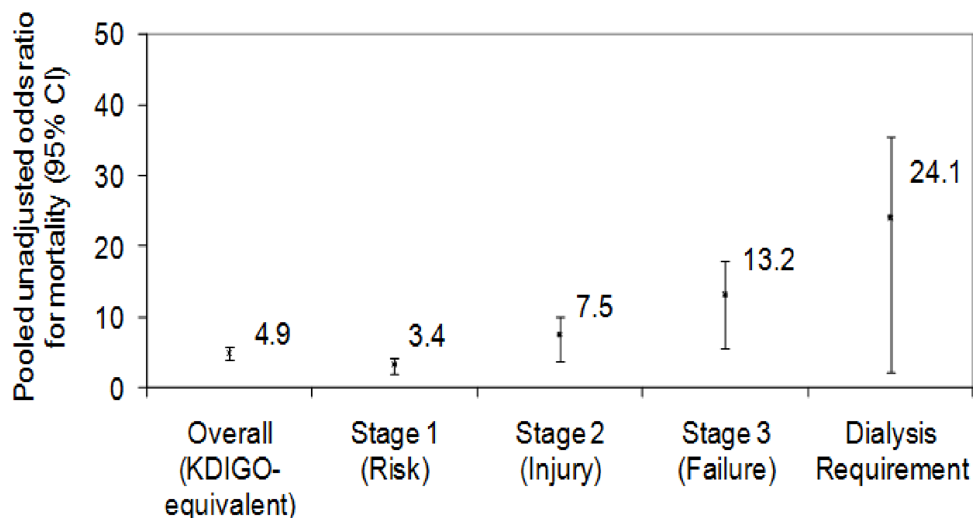
จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University



	Overall (KDIGO-equivalent)	Stage 1 (Risk)	Stage 2 (Injury)	Stage 3 (Failure)	Dialysis Requirement
No. studies	110	26	25	25	31
No. patients with AKI	429,535	8,226	42,354	42,354	6,534

Figure 26. Pooled AKI-associated mortality rate in studies that used a KDIGO-equivalent serum creatinine-based AKI definition and staging system, or dialysis requirement.

As shown in Figure 27 , among the 92 studies that provided a comparative non-AKI group, the pooled unadjusted odds ratio for all-cause mortality in patients with AKI was 4.94 (95% CI 4.13, 5.92) relative to patients without AKI. The pooled odds ratio for stage 1-3 AKI was 3.37 (95% CI 2.43, 4.68), 7.52 (95% CI 5.03, 11.27), and 13.19 (8.39, 20.76), respectively. For patients who required dialysis, the pooled odds ratio for mortality was the highest at 24.08 (95% CI 12.62, 45.95). Finally, the pooled odds ratio declined with longer duration of follow up. Indeed, the pooled odds ratio for mortality in patients with AKI was 5.58, 2.25, and 2.74, at <3, 3-6, and >6 months, respectively.



	Overall (KDIGO-equivalent)	Stage 1 (Risk)	Stage 2 (Injury)	Stage 3 (Failure)	Dialysis Requirement
No. studies	92	21	20	20	20
No. patients with AKI	405,616	90,048	40,631	38,914	4,427
No. patients without AKI	1,765,574	127,070	1,120,523	1,120,523	127,969

Figure 27. Pooled unadjusted odds ratio for all-cause mortality in patients with AKI relative to patients without AKI in studies that used a KDIGO-equivalent serum creatinine-based AKI definition and staging system, or dialysis requirement.

2. Second part

2.1. Characteristics of the cohort Stratified by *TNFA* genotypes

Genotyping was performed on a total of 262 subjects. The test for Hardy-Weinberg equilibrium showed deviation from expected genotype frequencies (Chi-square = 5.46;

P = 0.02, Figure 28).

The mean age of the cohort was 66 years, 53% were men, 91% were of white ethnicity, 73% were in the intensive care unit, and 43% had sepsis. The mean baseline eGFR was 53 mL/min/1.73 m^2, and mean APACHE II score was 20. At enrollment, 53% of patients had stage-3 AKI, and 20% had oliguria. The characteristics of the cohort stratified by the *TNFA* rs1800629 genotypes are shown in Table 14.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Marker Summary									
						Test for HWE			
Locus	Number of Indiv	Number of Alleles	Polymorph Info Content	Heterozygosity	Allelic Diversity	Chi-Square	DF	Pr > ChiSq	Prob Exact
Marker1	262	2	0.2511	0.2519	0.2944	5.4618	1	0.0194	0.0350

Allele Frequencies						
Locus	Allele	Count	Frequency	Standard Error	95% Confidence Limits	
Marker1	A	94	0.1794	0.0179	0.1469	0.2156
	G	430	0.8206	0.0179	0.7844	0.8531

Genotype Frequencies							
Locus	Genotype	Count	Frequency	HWD Coeff	Standard Error	95% Confidence Limits	
Marker1	A/A	14	0.0534	0.0213	0.0107	0.0018	0.0435
	A/G	66	0.2519	0.0213	0.0107	0.0018	0.0435
	G/G	182	0.6947	0.0213	0.0107	0.0018	0.0435

CHULALONGKORN UNIVERSITY

Figure 28 Hardy-Weinberg equilibrium test

In brief, demographic characteristics, co-existing conditions and disease severity measures did not differ significantly among the genotype groups, except for a higher number of failed organs, as defined by the MOF score, in the *TNFA* rs1800629 minor A-allele (GA/AA genotype) group (Figure 29, $P = 0.01$).

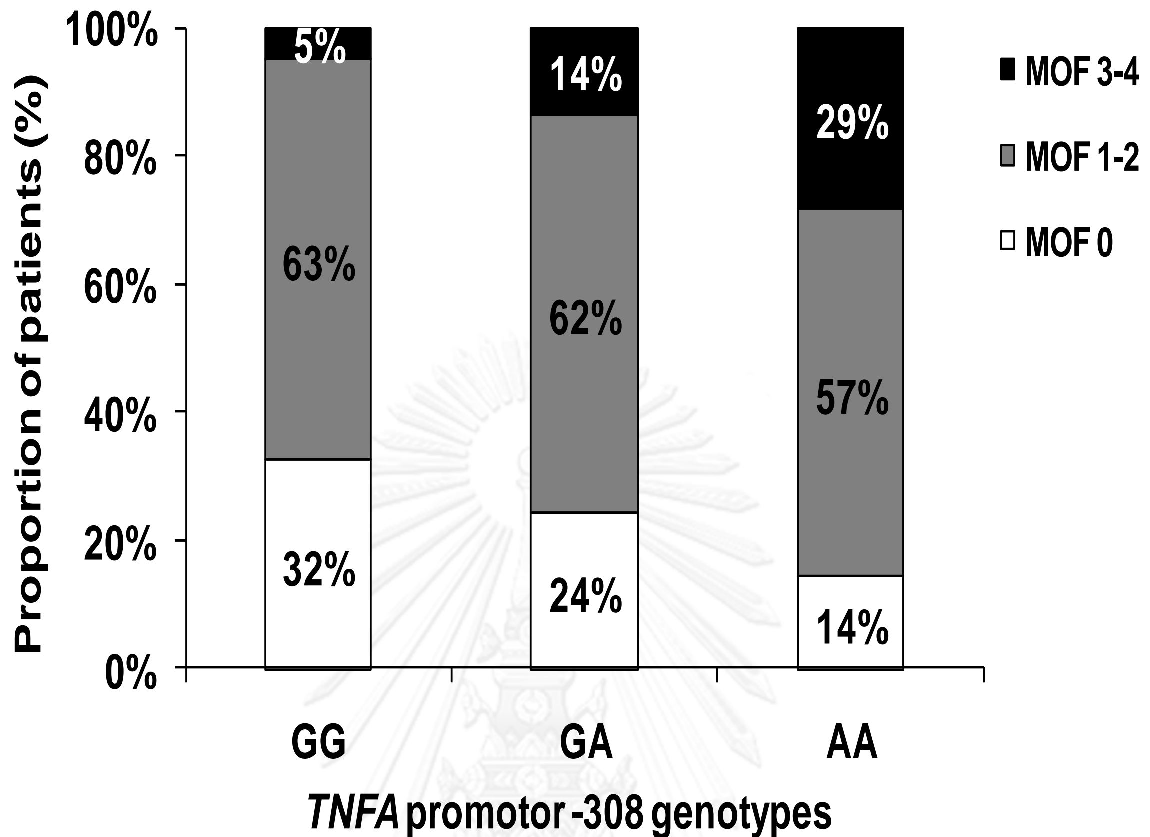


Figure 29. Multiple organ failure (MOF) score stratified by the *TNFA* rs1800629 gene polymorphism. P = 0.012 by x^2-test.

As shown in Table 14, with regard to the filtration markers, when compared with the GG-genotype, carriers of the *TNFA* rs1800629 GA and AA genotype had significantly higher peak (P=0.004), and discharge (P=0.004) serum creatinine level, whereas tended to have higher enrollment serum creatinine level (P=0.08).The carriers of the *TNFA* rs1800629 AA-genotype had significantly higher enrollment serum cystatin C level (P=0.04). In term of urinary markers, compared to the GG genotype group, carriers of the *TNFA* rs1800629 GA and AA genotype also had significantly higher urinary KIM-1 level (P=0.03), and urinary pi-GST level (P=0.03).

Table 14. Characteristics of the cohort according to the *TNFA* rs1800629 genotypes

Variable	GG (*n*=182)	GA (*n*=66)	AA (*n*=14)	P-value
Age, years	65 ± 16	66 ± 15	70 ± 12	0.485
Men	92 (50.6%)	38 (57.6%)	8 (57.1%)	0.583
White ethnicity	161 (88.5%)	62 (93.9%)	14 (100%)	0.198
BMI, kg/m^2	30 ± 9	30 ± 7	33 ± 7	0.432
Coexisting conditions				
Diabetes mellitus	79 (43.4%)	30 (45.5%)	7 (50.0%)	0.870
Heart failure	30 (16.5%)	9 (13.6%)	3 (21.4%)	0.736
Sepsis	76 (41.8%)	30 (45.5%)	6 (42.9%)	0.874
Baseline eGFR< 60 ml/min/1.73 m^2	118 (65.9%)	44 (69.8%)	12 (85.7%)	0.291
AKI stage				
Stage 1	85 (46.7%)	23 (35.4%)	4 (28.6%)	0.268
Stage 2	6 (3.3%)	5 (7.7%)	1 (7.1%)	
Stage 3	91 (50.0%)	37 (56.9%)	9 (64.3%)	
Cause of AKI				
Ischemic	64(35.4%)	23 (34.9%)	7 (50.0%)	0.794
Nephrotoxic	30 (16.6%)	11 (16.7%)	0 (0.0%)	
Sepsis	19 (10.5%)	4 (6.1%)	1 (7.1%)	
Atheroembolic	9 (5.0%)	4 (6.1%)	1 (7.1%)	
Other/ unknown/ multifactorial	59 (32.6%)	24 (36.4%)	5 (35.7%)	
ICU admission	133 (73.1%)	48 (72.7%)	11 (78.6%)	0.898
Assisted mechanical ventilation	46 (25.3%)	13 (19.7%)	3 (21.4%)	0.646
APACHE II score	20 ± 7	19 ± 5	21 ± 8	0.428
Enrollment serum cystatin C, mg/L	3.0 ± 1.2	3.1 ± 1.1	4.0 ± 1.7	**0.039**
Serum creatinine, mg/dL				
Enrollment	3.4 ± 1.6	3.9 ± 2.0	4.0 ± 1.6	0.084
Peak	4.0 ± 1.9	5.2 ± 3.9	4.8 ± 1.9	**0.004**
Discharge	2.3 ± 1.4	3.1 ± 2.3	2.0 ± 0.9	**0.004**
Urinary tubular injury marker				
KIM-1 , ng/mg	2.9 (1.2, 7.4)	4.9 (2.4, 9.3)	6.7 (3.3, 9.3)	**0.034**
NAG, mU/mg	39.9 (17.0, 87.6)	29.6 (12.1, 76.3)	32.7 (15.7, 114.9)	0.333
alpha-GST, ng/mg	15.6 (6.0, 35.7)	10.7 (3.7, 29.8)	19.3 (7.9, 50.1)	0.458
pi-GST, ng/mg	274.0 (97.0, 826.9)	274.5 (61.8, 1062.5)	1397.3(1164.7,2440.9)	**0.031**
Oliguria, %	33 (18.4%)	14 (21.9%)	4 (28.6%)	0.589
Dialysis requirement	68 (37.4%)	27 (40.9%)	8 (57.1%)	0.329
In-hospital death	39 (21.4%)	13 (19.7%)	5 (35.7%)	0.411

Continuous variables are presented as mean (standard deviation) or median (25th and 75th percentile), and categorical variables as percentage

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

2.2. Association of *TNFA* genotypes with filtration and tubular injury markers

The results of the multivariable dominant and additive genetic models are displayed in Tables 15 and 16. There were some significant associations between the *TNFA* rs1800629 minor A-allele and both filtration and tubular injury markers.

There were association between the *TNFA* rs1800629 AA genotype group and recessive model and higher enrollment serum cystatin C in the unadjusted model, after adjustment for sex, race, age, baseline eGFR, sepsis, which became non-significant after adding dialysis requirement in the model. Indeed, after adjustment for sex, race, age, baseline eGFR, sepsis, and dialysis requirement, GA genotype and dominant model had a higher enrollment serum creatinine, a higher peak serum creatinine, and a higher serum creatinine at hospital discharge when compared with the GG genotype. There was a weak association between the *TNFA* rs1800629 AA genotype group and higher enrollment serum cystatin C (P = 0.046) in the unadjusted additive model, which became non-significant in the multivariable analysis. There was a weak association between the *TNFA* rs1800629 AA genotype group and higher enrollment serum creatinine in the unadjusted additive model, which became non-significant after adding dialysis requirement in the model.

In the additive models, after adjustment for sex, race, age, baseline eGFR, sepsis, and dialysis requirement, compared with the GG genotype, the *TNFA* rs1800629 minor A-allele group had a higher peak serum creatinine.

Similarly, in the dominant model, after adjustment for sex, race, age, baseline eGFR, sepsis, and dialysis requirement, compared with the GG genotype, the *TNFA* rs1800629 minor A-allele group had a higher urinary KIM-1 (P = 0.008). A similar association was observed in the multivariable genotype GA and additive models.

In the dominant models, after adjustment for sex, race, age, and sepsis, compared with the GG genotype, the *TNFA* rs1800629 minor A-allele group had a higher MOF score of 0.26 (95% CI 0.03, 0.49; P=0.024). This association persisted in the additive model (0.26; 95% CI 0.07, 0.44; P=0.006). Finally, the *TNFA* rs1800629 minor A-allele did not have association with dialysis requirement in neither dominant nor additive

model (OR= 1.34, 95%CI 0.76-2.37, P=0.31, OR= 1.32, 95%CI 0.84-2.08, P=0.23, respectively) as well as in-hospital mortality after adjusted sex, race and APACHE score(OR= 1.32, 95%CI 0.63-2.75, P=0.47, OR= 1.30, 95%CI 0.73-2.30, P=0.37, respectively).

Table 15. Association of the *TNFA* rs1800629 polymorphism with filtration markers.

Genetic model	Enrollment serum cystatin C estimate, mg/L (95% CI)	P-value	Enrollment serum creatinine, mg/dL (95% CI)	P-value	Peak serum creatinine estimate, mg/dL (95% CI)	P-value	Discharge serum creatinine estimate, mg/dL (95% CI)	P-value
Genotype AA (vs GG)								
Unadjusted	0.99 (0.23, 1.75)	0.011	0.61 (-0.34, 1.55)	0.207	0.75 (-0.63, 2.13)	0.287	-0.28 (-1.19, 0.63)	0.546
Adjusted age, sex, race and baseline eGFR	0.82 (0.08, 1.55)	0.031	0.50 (-0.37, 1.37)	0.255	0.72 (-0.62, 2.05)	0.292	-0.41 (-1.23, 0.41)	0.325
Adjusted age, sex, race, baseline eGFR, and sepsis	0.81 (0.07, 1.54)	0.032	0.51 (-0.36, 1.38)	0.247	0.72 (-0.61, 2.06)	0.288	-0.40 (-1.22, 0.42)	0.336
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.64 (-0.06,1.33)	0.073	0.33 (-0.49, 1.15)	0.432	0.38 (-0.84, 1.59)	0.545	-0.52 (-1.31, 0.28)	0.203
Genotype GA (vs GG)								
Unadjusted	0.09 (-0.28, 0.46)	0.620	0.50 (0.01, 0.99)	0.045	1.22 (0.51, 1.94)	0.001	0.76 (0.29, 1.24)	0.002
Adjusted age, sex, race and baseline eGFR	0.11 (-0.26, 0.47)	0.558	0.47 (0.02, 0.93)	0.042	1.17 (0.47, 1.87)	0.001	0.62 (0.19, 1.06)	0.005
Adjusted age, sex, race, baseline eGFR, and sepsis	0.10 (-0.26, 0.47)	0.578	0.48 (0.03, 0.94)	0.039	1.18 (0.47, 1.88)	0.001	0.64 (0.20, 1.07)	0.004
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.09 (-0.25, 0.44)	0.597	0.48 (0.05, 0.91)	0.029	1.17 (0.53, 1.81)	<0.001	0.63 (0.21, 1.05)	0.003

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

(Table 15 continued)

Genetic model	Enrollment serum cystatin C estimate, mg/L (95% CI)	P-value	Enrollment serum creatinine, mg/dL (95% CI)	P- value	Peak serum creatinine estimate, mg/dL (95% CI)	P-value	Discharge serum creatinine estimate, mg/dL (95% CI)	P-value
Dominant model (A-allele *vs.* GG)								
Unadjusted	0.23 (-0.12, 0.58)	0.193	0.52 (0.06, 0.98)	0.026	1.14 (0.47, 1.81)	0.001	0.58 (0.14, 1.03)	0.010
Adjusted age, sex, race and baseline eGFR	0.22 (-0.12, 0.57)	0.209	0.48 (0.05, 0.91)	0.028	1.09 (0.43, 1.75)	0.001	0.44 (0.03, 0.85)	0.034
Adjusted age, sex, race, baseline eGFR, and sepsis	0.22 (-0.13, 0.56)	0.221	0.49 (0.06, 0.92)	0.025	1.10 (0.44, 1.75)	0.001	0.45 (0.04, 0.86)	0.030
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.18 (-0.15, 0.50)	0.281	0.45 (0.05, 0.86)	0.028	1.03 (0.43, 1.63)	0.001	0.43 (0.03,0.83)	0.034
Recessive model (AA vs G allele)								
Unadjusted	0.97 (0.22, 1.72)	0.012	0.47 (-0.47, 1.42)	0.323	0.42 (-0.98, 1.82)	0.552	-0.48 (-1.40, 0.44)	0.301
Adjusted age, sex, race, and baseline eGFR	0.79 (0.05, 1.52)	0.036	0.37 (-0.50, 1.24)	0.400	0.39 (-0.96, 1.73)	0.570	-0.58 (-1.41, 0.24)	0.163
Adjusted age, sex, race, baseline eGFR, and sepsis	0.78 (0.05, 1.51)	0.037	0.38 (-0.49, 1.24)	0.394	0.39 (-0.96, 1.74)	0.568	-0.58 (-1.40, 0.24)	0.166
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.61 (-0.08, 1.30)	0.083	0.19 (-0.63, 1.01)	0.644	0.05 (-1.19, 1.28)	0.942	-0.70 (-1.50, 0.11)	0.089

(Table 15 continued)

Genetic model	Enrollment serum cystatin C estimate, mg/L (95% CI)	P-value	Enrollment serum creatinine, mg/dL (95% CI)	P- value	Peak serum creatinine estimate, mg/dL (95% CI)	P-value	Discharge serum creatinine estimate, mg/dL (95% CI)	P-value
Additive model (per 1 A-allele copy increase)								
Unadjusted	0.29 (0.01, 0.57)	0.046	0.40 (0.04, 0.76)	0.032	0.78 (0.25, 1.32)	0.004	0.29 (-0.06, 0.65)	0.104
Adjusted age, sex, race, and baseline eGFR	0.26 (-0.02, 0.53)	0.070	0.36 (0.02, 0.69)	0.039	0.74 (0.22, 1.26)	0.006	0.18 (-0.14, 0.51)	0.263
Adjusted age, sex, race, baseline eGFR, and sepsis	0.25 (-0.03, 0.53)	0.075	0.36 (0.02, 0.70)	0.036	0.74 (0.22, 1.27)	0.005	0.19 (-0.13, 0.51)	0.244
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.20 (-0.06, 0.46)	0.127	0.31 (-0.01,0.63)	0.055	0.65 (0.17, 1.13)	0.008	0.16 (-0.15, 0.48)	0.316

Sample size on enrollment cystatin C (*n*=233), enrollment serum creatinine (*n*=262), peak serum creatinine (*n*=262), and discharge serum creatinine (*n*=262).

Table 16. Association of the *TNFA* rs1800629 polymorphism with tubular injury markers.

Genetic model	Urinary NAG relative ratio (95% CI)	P value	Urinary alpha-GST relative ratio (95% CI)	P value	Urinary pi-GST relative ratio (95% CI)	P value	Urinary KIM-1 relative ratio (95% CI)	P value
Genotype AA (vs GG)								
Unadjusted	1.15 (0.52, 2.55)	0.568	1.20 (0.23, 6.22)	0.370	2.74 (0.56, 13.40)	0.458	2.34 (0.91, 6.01)	0.080
Adjusted age, sex, race and baseline eGFR	1.20 (0.58, 2.48)	0.397	1.17 (0.23, 6.04)	0.526	3.49 (0.73, 16.70)	0.291	2.44 (0.98, 6.03)	0.054
Adjusted age, sex, race, baseline eGFR, and sepsis	1.17 (0.57, 2.40)	0.348	1.18 (0.23, 6.13)	0.528	3.40 (0.71, 16.25)	0.306	2.41 (0.98, 5.96)	0.056
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	1.01 (0.51, 2.02)	0.353	1.22 (0.23, 6.35)	0.517	3.11 (0.66, 14.77)	0.357	2.33 (0.95, 5.76)	0.065
Genotype GA (vs GG)								
Unadjusted	0.83 (0.57, 1.22)	0.568	0.56 (0.25, 1.29)	0.370	1.04 (0.48, 2.25)	0.458	1.67 (1.08, 2.59)	0.024
Adjusted age, sex, race and baseline eGFR	0.81 (0.57, 1.15)	0.397	0.63 (0.27, 1.46)	0.526	1.04 (0.47, 2.27)	0.291	1.68 (1.09, 2.57)	0.017
Adjusted age, sex, race, baseline eGFR, and sepsis	0.79 (0.56, 1.12)	0.348	0.63 (0.27, 1.47)	0.528	1.03 (0.47, 2.25)	0.306	1.67 (1.09, 2.56)	0.018
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	1.01 (0.51, 2.02)	0.353	0.63 (0.27, 1.46)	0.517	1.03 (0.47, 2.24)	0.357	1.65 (1.08 ,2.52)	0.022

(Table 16 continued)

Genetic model	Urinary NAG relative ratio (95% CI)	P value	Urinary alpha-GST relative ratio (95% CI)	P value	Urinary pi-GST relative ratio (95% CI)	P value	Urinary KIM-1 relative ratio (95% CI)	P value
Dominant model (A-allele *vs.* GG)								
Unadjusted	0.87 (0.61, 1.25)	0.459	0.64 (0.29, 1.39)	0.259	1.21 (0.58, 2.51)	0.618	1.75 (1.16, 2.65)	0.008
Adjusted age, sex, race, and baseline eGFR,	0.86 (0.61, 1.20)	0.365	0.70 (0.32, 1.54)	0.372	1.26 (0.60, 2.64)	0.544	1.77 (1.18, 2.65)	0.006
Adjusted age, sex, race, baseline eGFR, and sepsis	0.84 (0.60, 1.17)	0.299	0.70 (0.32, 1.55)	0.378	1.24 (0.59, 2.61)	0.565	1.76 (1.18, 2.64)	0.006
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.81 (0.59, 1.12)	0.205	0.70 (0.32, 1.55)	0.381	1.23 (0.59, 2.56)	0.587	1.73 (1.16, 2.59)	0.008
Recessive model (AA vs G allele)								
Unadjusted	1.21 (0.55, 2.67)	0.634	1.40 (0.27, 7.19)	0.688	2.72 (0.56, 13.09)	0.213	2.02 (0.78, 5.22)	0.144
Adjusted age, sex, race, and baseline eGFR	1.27 (0.62, 2.63)	0.511	1.33 (0.26, 6.81)	0.729	3.45 (0.73, 16.25)	0.117	2.10 (0.85, 5.22)	0.110
Adjusted age, sex, race, baseline eGFR, and sepsis	1.25 (0.61, 2.56)	0.537	1.35 (0.26, 6.91)	0.718	3.37 (0.72, 15.85)	0.124	2.08 (0.84, 5.16)	0.112
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	1.09 (0.55, 2.17)	0.811	1.39 (0.27, 7.16)	0.691	3.09 (0.66, 14.40)	0.152	2.01 (0.81, 4.98)	0.129

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

(Table 16 continued)

Genetic model	Urinary NAG relative ratio (95% CI)	P value	Urinary alpha-GST relative ratio (95% CI)	P value	Urinary pi-GST relative ratio (95% CI)	P value	Urinary KIM-1 relative ratio (95% CI)	P value
Additive model (per 1 A-allele copy increase)								
Unadjusted	0.94 (0.70, 1.26)	0.669	0.79 (0.42, 1.47)	0.452	1.30 (0.72, 2.35)	0.384	1.61 (1.14, 2.26)	0.007
Adjusted age, sex, race, and baseline eGFR,	0.93 (0.71, 1.23)	0.625	0.83 (0.44, 1.56)	0.562	1.39 (0.77, 2.52)	0.278	1.62 (1.16, 2.26)	0.005
Adjusted age, sex, race, baseline eGFR, and sepsis	0.92 (0.70, 1.20)	0.542	0.83 (0.44, 1.57)	0.572	1.38 (0.76, 2.50)	0.294	1.61 (1.16, 2.25)	0.005
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.88 (0.68, 1.14)	0.347	0.84 (0.44, 1.58)	0.584	1.35 (0.74, 2.43)	0.325	1.59 (1.14, 2.21)	0.006

Sample size on urinary NAG (*n*=234), alpha-GST (*n*=243), pi-GST (*n*=246), and KIM-1 (*n*=204).

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER V
DISCUSSION

The first part of thesis was performed by using meta-analysis to estimate the incidence of AKI around the world, and describe geographic variations according to countries, regions and their economies. A total of 312 large cohort studies representing close to 50 million patients published since 2004, the year in which the RIFLE criteria were first published were identified. Following a process of harmonization, AKI was reclassified in 154 studies in accordance with the KDIGO AKI definition and staging system, thus allowing for more harmonious estimates. Worldwide, using the KDIGO definition, 21.6% or 1 in 5 adults, and 33.7% or 1 in 3 children experienced AKI. Higher rates of AKI were observed in critical care settings and following cardiac surgery, identifying these high-risk populations in urgent need for interventions that let us made the second part of thesis to answer this question. This is clearly an issue in regions located south of the equator, where AKI tends to develop in rural communities in response to infections such as gastroenteritis, malaria, leptospirosis, and hemolytic-uremic syndrome. These findings highlight an important knowledge gap in the published AKI literature. A higher rate of AKI in children due in part to a higher representation of studies of critically ill children (38%) was observed. In adults, the pooled mortality rate was 23.9%, and in children 13.8%. In studies where stages of AKI could be ascertained, patients with stage 1-3 AKI experienced a 3- to 7-fold higher odds for death compared to those without AKI.

Another important finding was the disproportionate representation of certain countries and clinical settings in the scientific literature. Nearly half of the studies were from North America, which represents only 5% of the world population. This contrasted with only 2 studies from Africa, home to 15% of the world population. Most studies originated from high-income countries (82.7%), and countries that spent ≥5% of GDP on total health expenditure (91.7%). While AKI rates were higher in studies from countries that spent a greater percentage of GDP on total health expenditure, AKI-

associated mortality rate was inversely associated with percentage of country GDP spent on total health expenditure and gross national income per capita, and suggesting improved healthcare delivery. We found a decline in AKI rates and associated mortality over the span of 8 years that might result from the early detection by using risk assessment including genetic risk and new biomarkers such as urine and serum.

This is the first and largest meta-analysis that has systematically examined the incidence of AKI around the globe. By imposing a large study sample size, limiting the search strategy to a more contemporaneous period representing an era of development of consensus definitions for AKI, and harmonizing the definition of AKI for consistency with the latest classification and staging system, we were able to generate more valid and generalizable pooled point estimates.

There are several important limitations, however. The pooled rates were not standardized or normalized to at-risk periods. Most studies originated from high-income countries, involving hospitalized and often critically-ill patients. Assumptions were required to harmonize definitions of AKI according to one classification and staging system to generate pooled estimates, possibly introducing biases. The under-representation of studies of community-acquired AKI represents a biased sample as we imposed a large sample size for study inclusion, which likely excluded smaller reports of community-acquired AKI originating from developing countries. Although sources of study heterogeneity were explored through a broad range of subgroup and meta-regression analyses, heterogeneity remained significant across all examined subgroups. In addition, by linking study-level aggregated data to country-level geographic and economic characteristics, our analyses are susceptible to ecological inference fallacy. Higher AKI rates in studies from developed countries with greater percentage of GDP allocated to healthcare expenditure may be due to ascertainment bias as a result of wider availability of laboratory testing and publication bias, as more studies are published in such regions of the world. We were also unable to assess long-term kidney-related endpoints. In a recent meta-analysis of 13 cohort studies, the pooled incidence

rate of chronic kidney disease and kidney failure in patients with AKI was 25.8 and 8.6 per 100 person-years, respectively [238].

These meta-analysis carries implications for the scientific community and future development of public health policies related to AKI. Point estimates for the incidence of AKI, its stages of severity and associated mortality, including patterns of variations according to clinical settings, world geographic regions and economies should be taken into consideration during the planning, design, and execution of trials for AKI. A recent review identifies an abundance of underpowered small single-center trials in AKI [239], which may influence prematurely clinical practice. This lack of large definitive trials should galvanize the development of multi-national inter-disciplinary AKI trial networks, encouraging close collaborations of the academic, private, and governmental sectors to help reduce the global burden of AKI. There is also a need for more studies on AKI in less developed and lower income countries, and in community settings, where the implementation of low-cost strategies (e.g., oral hydration for treatment of gastroenteritis) might have a large impact on preventing AKI.

In first conclusion, there is a need for greater innovation in the prevention and treatment of AKI, a condition that affects worldwide 1 in 5 hospitalized patients. This analysis provides a platform to facilitate discussions among healthcare professionals, the public, and policy makers to raise awareness about AKI and its associated healthcare burden. These discussions should encourage providers to design better hospital-based healthcare delivery systems that focus on the prevention, early detection, and treatment of AKI, improve patient safety, and ultimately, preserve kidney health and well-being while mitigating the long-term costly burden of chronic kidney disease.

In the second part of thesis, a cohort of hospitalized adults with AKI, the association between a functional polymorphism (at position -308; rs1800629) located in the promoter region of the *TNFA* gene, which is a pivotal pro-inflammatory cytokine, and kidney disease severity, including levels of glomerular filtration and tubular injury markers were explored. Carriers of the *TNFA* rs1800629 minor A-allele (GA and AA) had

higher levels of filtration markers, including higher serum creatinine and cystatin C, and higher urinary tubular injury markers, including KIM-1 and pi-GST were demonstrated. Carriers of the *TNFA* rs1800629 minor A-allele (GA and AA) also experienced more organ system dysfunction, as evidenced by a higher MOF score.

Although systemic and renal hemodynamic insults are important in the pathogenesis of AKI, increasing evidence also supports a critical role for inflammatory mechanisms, following both ischemic and nephrotoxic injury [240],[241]. Experimental studies indicate that TNF-alpha plays a central role in nephrotoxin- and endotoxin-induced inflammatory responses in the kidney, leading to AKI [107 242]. The role of anti-inflammatory strategies in limiting tissue injury is emerging. For example, the administration of IL-10 inhibits the expression of pro-inflammatory cytokines in experimental model of ischemic and nephrotoxic injury and improves renal histology [106,243]. Finally, IL-10 gene transfer significantly attenuates glomerular lesions and ameliorates kidney function in experimental crescentic glomerulonephritis [244]. Taken together, these results argue that cytokines play an important role in the pathogenesis of AKI.

Even though polymorphisms affecting key pro- and anti-inflammatory cytokines might modulate the susceptibility to cytokine-mediated renal injury, the use of genetic epidemiology for the study of cytokine genes has received little attention in AKI [47,245]. Although several polymorphisms of cytokine genes might be functionally relevant in human disease [68], we chose to evaluate the genetic variants of TNF-alpha.

Given its importance in orchestrating the cytokine storm during inflammatory responses, one might anticipate that polymorphisms altering the expression of TNF-alpha might alter cytokine-mediated injury in conditions such as AKI. Several polymorphisms in the gene encoding TNF-alpha have been described. Among them, the G to A single nucleotide substitution within the promoter region at position –308 is the most widely studied. The TNF-alpha –308 A-allele, also referred to as the TNF-alpha 2 allele, has been associated with high promoter activity[147,246,247]. Moreover, the TNF-alpha 2 allele has been found to correlate with enhanced spontaneous and stimulated TNF-alpha production both *in vitro*[148,149] and *in vivo* [150,151].

A previous study had evaluated the relationship of the *TNFA* rs1800629 polymorphism with adverse outcomes in several acute clinical settings[248], including acute myocardial infarction, acute pancreatitis, and sepsis[249-251]. In a study of 603 patients, carriers of the rs1800629 *TNFA* minor A-allele (GA and AA) had significantly higher levels of biomarkers of cardiac injury, including troponin I, creatine kinase-MB, and lactate dehydrogenase[249]. These findings are consistent with our results demonstrating in a cohort of patients with AKI, an independent association between carriers of the *TNFA* rs1800629 minor A-allele (GA and AA) and higher levels of filtration (serum creatinine and cystatin C) and tubular injury (KIM-1 and pi-GST) markers.

In this thesis, carriers of the *TNFA* rs1800629 minor A-allele (GA and AA) also had higher enrollment serum creatinine and peak creatinine in multivariate models were demonstrated. Of note, the enrollment, peak, discharge creatinine as well as cystatin C were taken while the patient was on any form of renal replacement therapy that might be interfered overall outcomes. 103 patients (39%) who required renal replacement therapy, 36 initiated dialysis prior to study enrollment (median of 1 day prior to study enrollment), which might confound the association of the *TNFA* rs1800629 polymorphism with serum creatinine and cystatin C. To address the confounding effect of renal replacement therapy on the outcome of serum creatinine and cystatin C, the additional multivariable regression analyses where adjusted model for age, sex, race, baseline eGFR, sepsis, and dialysis requirements were used. The estimated effects were not markedly attenuated, after adding this covariate to the models.

KIM-1 is a biomarker of renal tubular cell dedifferentiation and injury, and is expressed when the injured renal proximal tubule assumes a dedifferentiated phenotype [135]. KIM-1 is strongly up-regulated in proximal tubular epithelial cells during various states characterized by epithelial cell dedifferentiation including ischemia, toxic renal injury, polycystic kidney disease and renal cell carcinoma [135,252-255]. The soluble form of KIM-1 is a useful biomarker for the early detection of AKI [256], and has recently been used to predict adverse clinical outcomes [257]. To our knowledge, this is the first study examining genetic determinants of urinary markers of kidney injury. We can only

speculate as to whether genetic-dependent TNF-alpha transcription at the renal parenchymal tissue level might influence the inflammatory renal tubular cellular responses to ischemic or nephrotoxic injury, such as the up-regulation of KIM-1, and the extent of serum creatinine rise.

However, the reliance on a single time point biomarker measurement obtained following enrollment and the absence of subsequent measurements in the first 24-hour period to ascertain biomarker performance might be an important concern. KIM-1 seems to act as tropinin I that undergoes slow decreasing after injury insults when compared with NAG and alpha-GST [38,45,141]. We can only presume as to whether the performance of these markers would be improved or worsened if measured at subsequent time points from the kidney insult.

In our study, we demonstrated that carriers of the *TNFA* rs1800629 minor A-allele (GA and AA) also had higher serum creatinine at hospital discharge. We can only speculate as to whether carriers of this genetic marker are at an increased long-term risk of developing chronic kidney disease. In a cohort of 231 patients with chronic kidney failure and 180 healthy matched control subjects, the *TNFA* rs1800629 minor A-allele (GA and AA) was a strong risk modifier for development of kidney failure [258]. In kidney transplant recipients, a similar association has been observed whereby the *TNFA* rs1800629 minor A-allele (GA and AA) was associated with an increased risk of chronic allograft nephropathy [259].

We observed an association between the *TNFA* rs1800629 minor A-allele (GA and AA) and organ system dysfunction, as defined by the MOF score. A similar association has previously been observed in patients with acute pancreatitis [250], sepsis [69], and community-acquired pneumonia [251]. In total of 280 community-acquired pneumonia patients carrying at least one AA (tumor necrosis factor [TNF] high secretor genotype had an 18.0% risk of septic shock versus 6.8% (p=0.006). GG homozygotes (TNF low secretors) at both loci had only a 2.9% risk of septic shock [251]. A recent meta-analysis demonstrated an association between the *TNFA* rs1800629 minor A-allele carrier(GA and AA) and development of sepsis [260]. The association between the *TNFA*

rs1800629 minor A-allele (GA and AA) and a higher MOF score is consistent with a similar association observed in a cohort of patients with chronic kidney disease and a higher burden of co-morbidities [73]. Although prior studies of patients with sepsis or dialysis-requiring AKI demonstrated an association between the *TNFA* rs1800629 minor A-allele carrier (GA and AA) and an adjusted increased risk of death [47,69] , we were unable to demonstrate a similar association in the present study.

The role of down-regulation of TNF-alpha has been proposed. Glucocorticoids, pentoxifylline, and thalidomide inhibit TNF-alpha synthesis at different levels of the biosynthetic pathway. Nuclear factor-kB (NF-kB) is a cytoplasmic protein that plays an essential role pivotal in TNF-alpha expression [261]. Glucocorticoids can inhibit TNF-alpha synthesis by interfering with NF-kB activity [262,263] as well as reducing post-transcription protein translation [264].

The xanthine derivative pentoxifylline, a non-specific phosphodiesterase (PDE) inhibitor, reduces TNF-alpha mRNA expression in monocytes [265,266] via generation of cyclic adenosine monophosphate (cAMP) [265], which inhibits NF-kB-mediated transcription[267]. In addition, the PDE isoenzyme type 4 is predominant in monocytes [268]. Rolipram is a specific PDE type-4 inhibitor which is a 500-fold more potent inhibitor of TNF-alpha synthesis in human monocytes compared with pentoxifylline [269].

Of note, thalidomide is a synthetic derivative of glutamic acid which also selectively inhibits *in vitro* synthesis of TNF-alpha by enhancing mRNA degradation [270,271] whereas IL-10 reduces transcription and synthesis of TNF-alpha [272], and its administration to healthy volunteers inhibits the endotoxin induced rise in body temperature and release of TNF-alpha and other pro-inflammatory cytokines[273] .

Both monoclonal antibodies to TNF-alpha as well as soluble TNF receptors can also neutralize this cytokine. Inflixmab and Adalilumab are two examples of monoclonal antibodies against TNF-alpha whereas Etanercept is a TNF-alpha type II receptor-IgG1 fusion protein which binds specifically to TNF-alpha, resulting in its biologically inactive form [274]. Finally, somatostatin is also capable of down-regulating cell surface TNF receptor expression in human macrophages, resulting in less responsive to

TNF-alpha [275]. These promising biological drugs await further investigation, as immune modulation therapies for acute inflammatory states such as AKI.

To our knowledge, this is the first study that examines the association between a functionally relevant polymorphism in the *TNFA* gene and AKI disease severity, as measured by levels of filtration and tubular injury markers. The heterogeneity of the cohort was offset by the selective inclusion of subjects with more advanced AKI requiring formal nephrology consultation. Our cohort was relatively small for the purpose of genetic studies, but was 91% white, reducing the potential impact of race and ethnicity on genotype prevalence. However, there are several study limitations that are worthy of mention.

Genetic polymorphism association studies do not establish causality and the results must be interpreted with caution due to the relatively small sample size with chance accounting for the results, especially when biologic plausibility has not been determined or is unknown. Nevertheless, these studies help generate new hypotheses. There is a clear need for a better understanding of genetically linked polymorphisms, whereby the polymorphism of a particular gene might just be a marker for another, yet to be identified, disease-causing sequence variant [276]. This more comprehensive approach requires the use of haplotype and linkage analyses and a good understanding of neighboring candidate genes located on the same chromosome. Furthermore, most published studies describing the frequency of cytokine gene polymorphisms in the general population are biased due to a lack of consideration of ethnicity and geographical boundary [277]. In the present U.S.-based study, there were no discernable cytokine genotype differences according to ethnicity, lending more credibility to the findings.

The *TNFA* rs1800629 polymorphism did not fulfill the Hardy Weinberg equilibrium and this might be due in part to the relatively modest sample size of our study or natural selection. We did not have a second cohort to validate our findings in a replication cohort. In our cohort, we had a higher prevalence rate of pre-existing CKD, as defined by eGFR of less than 60 mL/min/1.73 m^2 due to the selection criteria used to

identify eligible patients with AKI, mainly the criteria by Hou S et al, which require higher absolute rises in serum creatinine according to the baseline serum creatinine value. All our multivariable analyses, however, are adjusted for the baseline eGFR, which did no markedly attenuate the effect sizes. Although we found an association with AKI-related surrogate endpoints, we were unable to demonstrate an association with hard clinical endpoints. Finally, survival bias is a possible concern when genetic markers are being evaluated as predictors of disease severity and adverse outcomes.

In conclusion, the present study explores the complex nature of how a functionally relevant genetic variant in the *TNFA* gene might influence the severity of kidney injury in patients with AKI, and supports the hypothesis that the study of polymorphisms of host genes as genetic risk markers in the setting of acute illnesses such as AKI has merit. Additional studies are needed to establish the mechanisms underlying the influence of the identified *TNFA* gene polymorphism on severity of AKI traits.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

REFERENCES

1. Mehta RL, Chertow GM. Acute renal failure definitions and classification: time for change? *J Am Soc Nephrol.* Aug 2003;14(8):2178-2187.
2. Hou SH, Bushinsky DA, Wish JB, Cohen JJ, Harrington JT. Hospital-acquired renal insufficiency: a prospective study. *Am J Med.* Feb 1983;74(2):243-248.
3. Ronco C, Kellum JA, Mehta R. Acute dialysis quality initiative (ADQI). *Nephrol Dial Transplant.* Aug 2001;16(8):1555-1558.
4. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care.* Aug 2004;8(4):R204-212.
5. Ostermann M, Chang RW. Acute kidney injury in the intensive care unit according to RIFLE. *Crit Care Med.* Aug 2007;35(8):1837-1843; quiz 1852.
6. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int.* Mar 2008;73(5):538-546.
7. Pickering JW, Endre ZH. GFR shot by RIFLE: errors in staging acute kidney injury. *Lancet.* Apr 18 2009;373(9672):1318-1319.
8. Mehta RL, Kellum JA, Shah SV, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care.* 2007;11(2):R31.
9. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol.* Nov 2005;16(11):3365-3370.
10. Liangos O, Wald R, O'Bell JW, Price L, Pereira BJ, Jaber BL. Epidemiology and outcomes of acute renal failure in hospitalized patients: a national survey. *Clin J Am Soc Nephrol.* Jan 2006;1(1):43-51.
11. Metnitz PG, Krenn CG, Steltzer H, et al. Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. *Crit Care Med.* Sep 2002;30(9):2051-2058.
12. Wald R, Quinn RR, Luo J, et al. Chronic dialysis and death among survivors of acute kidney injury requiring dialysis. *JAMA.* Sep 16 2009;302(11):1179-1185.
13. Himmelfarb J, Ikizler TA. Acute kidney injury: changing lexicography, definitions, and epidemiology. *Kidney Int.* May 2007;71(10):971-976.

14. Ostermann M, Chang RW. Challenges of defining acute kidney injury. *QJM : monthly journal of the Association of Physicians.* Mar 2011;104(3):237-243.

15. Forster AJ, Kyeremanteng K, Hooper J, Shojania KG, van Walraven C. The impact of adverse events in the intensive care unit on hospital mortality and length of stay. *BMC health services research.* 2008;8:259.

16. Uchino S, Kellum JA, Bellomo R, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA.* Aug 17 2005;294(7):813-818.

17. Kellum JA, Hoste EA. Acute kidney injury: epidemiology and assessment. *Scandinavian journal of clinical and laboratory investigation. Supplementum.* 2008;241:6-11.

18. Zeng X, McMahon GM, Brunelli SM, Bates DW, Waikar SS. Incidence, Outcomes, and Comparisons across Definitions of AKI in Hospitalized Individuals. *Clin J Am Soc Nephrol.* Oct 31 2013.

19. Waikar SS, Betensky RA, Bonventre JV. Creatinine as the gold standard for kidney injury biomarker studies? *Nephrol Dial Transplant.* Nov 2009;24(11):3263-3265.

20. Herget-Rosenthal S, Marggraf G, Husing J, et al. Early detection of acute renal failure by serum cystatin C. *Kidney Int.* Sep 2004;66(3):1115-1122.

21. Tolkoff-Rubin NE, Rubin RH, Bonventre JV. Noninvasive renal diagnostic studies. *Clin Lab Med.* Sep 1988;8(3):507-526.

22. Borregaard N, Sehested M, Nielsen BS, Sengelov H, Kjeldsen L. Biosynthesis of granule proteins in normal human bone marrow cells. Gelatinase is a marker of terminal neutrophil differentiation. *Blood.* Feb 1 1995;85(3):812-817.

23. Mori K, Lee HT, Rapoport D, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest.* Mar 2005;115(3):610-621.

24. Hvidberg V, Jacobsen C, Strong RK, Cowland JB, Moestrup SK, Borregaard N. The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake. *FEBS Lett.* Jan 31 2005;579(3):773-777.

25. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis.* Dec 2009;54(6):1012-1024.

Chulalongkorn University

26. Ichimura T, Asseldonk EJ, Humphreys BD, Gunaratnam L, Duffield JS, Bonventre JV. Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. *J Clin Invest.* May 2008;118(5):1657-1668.

27. Bailly V, Zhang Z, Meier W, Cate R, Sanicola M, Bonventre JV. Shedding of kidney injury molecule-1, a putative adhesion protein involved in renal regeneration. *J Biol Chem.* Oct 18 2002;277(42):39739-39748.

28. Bonventre JV. Kidney injury molecule-1 (KIM-1): a urinary biomarker and much more. *Nephrol Dial Transplant.* Nov 2009;24(11):3265-3268.

29. Maatman RG, van de Westerlo EM, van Kuppevelt TH, Veerkamp JH. Molecular identification of the liver- and the heart-type fatty acid-binding proteins in human and rat kidney. Use of the reverse transcriptase polymerase chain reaction. *Biochem J.* Nov 15 1992;288 (Pt 1):285-290.

30. Kamijo-Ikemori A, Sugaya T, Kimura K. Urinary fatty acid binding protein in renal disease. *Clin Chim Acta.* Dec 2006;374(1-2):1-7.

31. Kamijo A, Sugaya T, Hikawa A, et al. Urinary excretion of fatty acid-binding protein reflects stress overload on the proximal tubules. *Am J Pathol.* Oct 2004;165(4):1243-1255.

32. Negishi K, Noiri E, Doi K, et al. Monitoring of urinary L-type fatty acid-binding protein predicts histological severity of acute kidney injury. *Am J Pathol.* Apr 2009;174(4):1154-1159.

33. Doi K, Noiri E, Maeda-Mamiya R, et al. Urinary L-type fatty acid-binding protein as a new biomarker of sepsis complicated with acute kidney injury. *Crit Care Med.* Oct 2010;38(10):2037-2042.

34. Susantitaphong P, Siribamrungwong M, Doi K, Noiri E, Terrin N, Jaber BL. Performance of Urinary Liver-Type Fatty Acid-Binding Protein in Acute Kidney Injury: A Meta-analysis. *Am J Kidney Dis.* Mar 2013;61(3):430-439.

35. Endre ZH, Pickering JW, Walker RJ, et al. Improved performance of urinary biomarkers of acute kidney injury in the critically ill by stratification for injury duration and baseline renal function. *Kidney Int.* May 2011;79(10):1119-1130.

36. Siew ED, Ikizler TA, Gebretsadik T, et al. Elevated urinary IL-18 levels at the time of ICU admission predict adverse clinical outcomes. *Clin J Am Soc Nephrol.* Aug 2010;5(8):1497-1505.

37. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. *J Am Soc Nephrol.* Oct 2005;16(10):3046-3052.

38. Liangos O, Tighiouart H, Perianayagam MC, et al. Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass. *Biomarkers.* Sep 2009;14(6):423-431.

39. Price RG. The role of NAG (N-acetyl-beta-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. *Clin Nephrol.* 1992;38 Suppl 1:S14-19.

40. Wellwood JM, Ellis BG, Price RG, Hammond K, Thompson AE, Jones NF. Urinary N-acetyl- beta-D-glucosaminidase activities in patients with renal disease. *Br Med J.* Aug 16 1975;3(5980):408-411.

41. Kang HK, Kim DK, Lee BH, et al. Urinary N-acetyl-beta-D-glucosaminidase and malondialdehyde as a markers of renal damage in burned patients. *J Korean Med Sci.* Oct 2001;16(5):598-602.

42. Ascione R, Lloyd CT, Underwood MJ, Gomes WJ, Angelini GD. On-pump versus off-pump coronary revascularization: evaluation of renal function. *Ann Thorac Surg.* Aug 1999;68(2):493-498.

43. Westhuyzen J, Endre ZH, Reece G, Reith DM, Saltissi D, Morgan TJ. Measurement of tubular enzymuria facilitates early detection of acute renal impairment in the intensive care unit. *Nephrol Dial Transplant.* Mar 2003;18(3):543-551.

44. Harrison DJ, Kharbanda R, Cunningham DS, McLellan LI, Hayes JD. Distribution of glutathione S-transferase isoenzymes in human kidney: basis for possible markers of renal injury. *J Clin Pathol.* Jun 1989;42(6):624-628.

45. Susantitaphong P PM, Tighiouart H, Kouznetsov D, Liangos O, Jaber BL. Urinary α- and π-Glutathione S-Transferases for Early Detection of Acute Kidney Injury Following Cardiopulmonary Bypass. *Biomarker.* 2012;In press.

46. Seabra VF, Perianayagam MC, Tighiouart H, Liangos O, dos Santos OF, Jaber BL. Urinary alpha-GST and pi-GST for prediction of dialysis requirement or in-hospital death in established acute kidney injury. *Biomarkers.* Dec 2011;16(8):709-717.

47. Jaber BL, Rao M, Guo D, et al. Cytokine gene promoter polymorphisms and mortality in acute renal failure. *Cytokine.* Mar 7 2004;25(5):212-219.

48. Liangos O, Balakrishnan VS, Pereira BJ, Jaber BL. Cytokine single nucleotide polymorphism. Role in acute renal failure. *Contrib Nephrol.* 2004;144:63-75.

49. Haase-Fielitz A, Haase M, Bellomo R, Dragun D. Genetic polymorphisms in sepsis- and cardiopulmonary bypass-associated acute kidney injury. *Contrib Nephrol.* 2007;156:75-91.

50. Perianayagam MC, Liangos O, Kolyada AY, et al. NADPH oxidase p22phox and catalase gene variants are associated with biomarkers of oxidative stress and adverse outcomes in acute renal failure. *J Am Soc Nephrol.* Jan 2007;18(1):255-263.

51. Kolyada AY, Tighiouart H, Perianayagam MC, Liangos O, Madias NE, Jaber BL. A genetic variant of hypoxia-inducible factor-1alpha is associated with adverse outcomes in acute kidney injury. *Kidney Int.* Jun 2009;75(12):1322-1329.

52. Alam A, O'Connor DT, Perianayagam MC, et al. Phenylethanolamine N-methyltransferase gene polymorphisms and adverse outcomes in acute kidney injury. *Nephron Clin Pract.* 2010;114(4):c253-259.

53. Perianayagam MC, Tighiouart H, Nievergelt CM, O'Connor DT, Liangos O, Jaber BL. CYBA Gene Polymorphisms and Adverse Outcomes in Acute Kidney Injury: A Prospective Cohort Study. *Nephron Extra.* Jan 2011;1(1):112-123.

54. Perianayagam MC, Tighiouart H, Liangos O, et al. Polymorphisms in the myeloperoxidase gene locus are associated with acute kidney injury-related outcomes. *Kidney Int.* Jun 27 2012.

55. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol.* Aug 2003;14(8):2199-2210.

56. Kinsey GR, Li L, Okusa MD. Inflammation in acute kidney injury. *Nephron Exp Nephrol.* 2008;109(4):e102-107.

57. Akcay A, Nguyen Q, Edelstein CL. Mediators of inflammation in acute kidney injury. *Mediators Inflamm.* 2009;2009:137072.

58. Lee DW, Faubel S, Edelstein CL. Cytokines in acute kidney injury (AKI). *Clin Nephrol.* Sep 2011;76(3):165-173.

59. White LE, Hassoun HT. Inflammatory Mechanisms of Organ Crosstalk during Ischemic Acute Kidney Injury. *Int J Nephrol.* 2012;2012:505197.

60. Wilson AG, di Giovine FS, Blakemore AI, Duff GW. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet.* Aug 1992;1(5):353.

61. Uglialoro AM, Turbay D, Pesavento PA, et al. Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-alpha gene promoter. *Tissue Antigens.* Oct 1998;52(4):359-367.

62. Jacob CO, Fronek Z, Lewis GD, Koo M, Hansen JA, McDevitt HO. Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor alpha: relevance to genetic predisposition to

systemic lupus erythematosus. *Proc Natl Acad Sci U S A.* Feb 1990;87(3):1233-1237.

63. Abraham LJ, French MA, Dawkins RL. Polymorphic MHC ancestral haplotypes affect the activity of tumour necrosis factor-alpha. *Clin Exp Immunol.* Apr 1993;92(1):14-18.

64. Pociot F, Briant L, Jongeneel CV, et al. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-alpha and TNF-beta by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *Eur J Immunol.* Jan 1993;23(1):224-231.

65. Warzocha K, Ribeiro P, Bienvenu J, et al. Genetic polymorphisms in the tumor necrosis factor locus influence non-Hodgkin's lymphoma outcome. *Blood.* May 15 1998;91(10):3574-3581.

66. Louis E, Franchimont D, Piron A, et al. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol.* Sep 1998;113(3):401-406.

67. Kadiroglu AK, Sit D, Atay AE, Kayabasi H, Altintas A, Yilmaz ME. The evaluation of effects of demographic features, biochemical parameters, and cytokines on clinical outcomes in patients with acute renal failure. *Ren Fail.* 2007;29(4):503-508.

68. Jaber BL, Liangos O, Pereira BJ, Balakrishnan VS. Polymorphism of immunomodulatory cytokine genes: implications in acute renal failure. *Blood Purif.* 2004;22(1):101-111.

69. Mira JP, Cariou A, Grall F, et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. *JAMA.* Aug 11 1999;282(6):561-568.

70. Tang GJ, Huang SL, Yien HW, et al. Tumor necrosis factor gene polymorphism and septic shock in surgical infection. *Crit Care Med.* Aug 2000;28(8):2733-2736.

71. Yende S, Quasney MW, Tolley E, Zhang Q, Wunderink RG. Association of tumor necrosis factor gene polymorphisms and prolonged mechanical ventilation after coronary artery bypass surgery. *Crit Care Med.* Jan 2003;31(1):133-140.

72. Treszl A, Toth-Heyn P, Kocsis I, et al. Interleukin genetic variants and the risk of renal failure in infants with infection. *Pediatr Nephrol.* Sep 2002;17(9):713-717.

73. Balakrishnan VS, Guo D, Rao M, et al. Cytokine gene polymorphisms in hemodialysis patients: association with comorbidity, functionality, and serum albumin. *Kidney Int.* Apr 2004;65(4):1449-1460.

74. Ali T, Khan I, Simpson W, et al. Incidence and outcomes in acute kidney injury: a comprehensive population-based study. *J Am Soc Nephrol.* Apr 2007;18(4):1292-1298.

75. Fang Y, Ding X, Zhong Y, et al. Acute kidney injury in a Chinese hospitalized population. *Blood Purif.* 2010;30(2):120-126.

76. Thakar CV, Christianson A, Freyberg R, Almenoff P, Render ML. Incidence and outcomes of acute kidney injury in intensive care units: a Veterans Administration study. *Crit Care Med.* Sep 2009;37(9):2552-2558.

77. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med.* Feb 2006;34(2):344-353.

78. Hsu RK, McCulloch CE, Ku E, Dudley RA, Hsu CY. Regional variation in the incidence of dialysis-requiring AKI in the United States. *Clin J Am Soc Nephrol.* Sep 2013;8(9):1476-1481.

79. Cerda J, Bagga A, Kher V, Chakravarthi RM. The contrasting characteristics of acute kidney injury in developed and developing countries. *Nature clinical practice. Nephrology.* Mar 2008;4(3):138-153.

80. Bellomo R, Kellum JA, Ronco C. Acute kidney injury. *Lancet.* Aug 25 2012;380(9843):756-766.

81. Coca SG, Yusuf B, Shlipak MG, Garg AX, Parikh CR. Long-term risk of mortality and other adverse outcomes after acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis.* Jun 2009;53(6):961-973.

82. Chawla LS, Kimmel PL. Acute kidney injury and chronic kidney disease: an integrated clinical syndrome. *Kidney Int.* Sep 2012;82(5):516-524.

83. Fernando Liaño JP. *Atlas of diseases of the kidney*. Philadelphia: Current Medicine; 1999.

84. Flo TH, Halaas O, Torp S, et al. Differential expression of Toll-like receptor 2 in human cells. *J Leukoc Biol.* Mar 2001;69(3):474-481.

85. Dybdahl B, Wahba A, Lien E, et al. Inflammatory response after open heart surgery: release of heat-shock protein 70 and signaling through toll-like receptor-4. *Circulation.* Feb 12 2002;105(6):685-690.

86. Thadhani R, Pascual M, Bonventre JV. Acute renal failure. *N Engl J Med.* May 30 1996;334(22):1448-1460.

87. Sheridan AM, Bonventre JV. Cell biology and molecular mechanisms of injury in ischemic acute renal failure. *Curr Opin Nephrol Hypertens.* Jul 2000;9(4):427-434.

88. Kwon O, Phillips CL, Molitoris BA. Ischemia induces alterations in actin filaments in renal vascular smooth muscle cells. *Am J Physiol Renal Physiol.* Jun 2002;282(6):F1012-1019.

89. Kourembanas S, McQuillan LP, Leung GK, Faller DV. Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. *J Clin Invest.* Jul 1993;92(1):99-104.

90. Hunley TE, Kon V. Endothelin in ischemic acute renal failure. *Curr Opin Nephrol Hypertens.* Jul 1997;6(4):394-400.

91. Denton MD, Chertow GM, Brady HR. "Renal-dose" dopamine for the treatment of acute renal failure: scientific rationale, experimental studies and clinical trials. *Kidney Int.* Jul 1996;50(1):4-14.

92. Lewis J, Salem MM, Chertow GM, et al. Atrial natriuretic factor in oliguric acute renal failure. Anaritide Acute Renal Failure Study Group. *Am J Kidney Dis.* Oct 2000;36(4):767-774.

93. Suwa T, Hogg JC, Klut ME, Hards J, van Eeden SF. Interleukin-6 changes deformability of neutrophils and induces their sequestration in the lung. *Am J Respir Crit Care Med.* Mar 2001;163(4):970-976.

94. Segerer S, Nelson PJ, Schlondorff D. Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. *J Am Soc Nephrol.* Jan 2000;11(1):152-176.

95. Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. *N Engl J Med.* Feb 12 1998;338(7):436-445.

96. Donnahoo KK, Meldrum DR, Shenkar R, Chung CS, Abraham E, Harken AH. Early renal ischemia, with or without reperfusion, activates NFkappaB and increases TNF-alpha bioactivity in the kidney. *J Urol.* Apr 2000;163(4):1328-1332.

97. Chakravorty SJ, Cockwell P, Girdlestone J, Brooks CJ, Savage CO. Fractalkine expression on human renal tubular epithelial cells: potential role in mononuclear cell adhesion. *Clin Exp Immunol.* Jul 2002;129(1):150-159.

98. Safirstein R, Megyesi J, Saggi SJ, et al. Expression of cytokine-like genes JE and KC is increased during renal ischemia. *Am J Physiol.* Dec 1991;261(6 Pt 2):F1095-1101.

99. Kapper S, Beck G, Riedel S, et al. Modulation of chemokine production and expression of adhesion molecules in renal tubular epithelial and endothelial cells by catecholamines. *Transplantation.* Jul 27 2002;74(2):253-260.

100. JABER BL PB. Inflammatory mediators in sepsis: Rationale for extracorporeal therapies. *Am J Kidney Dis.* 1996;28:S35–S49.

101. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med.* Feb 11 1999;340(6):448-454.

102. Koch T. Origin and mediators involved in sepsis and the systemic inflammatory response syndrome. *Kidney Int Suppl.* Feb 1998;64:S66-69.

103. van der Poll T, Jansen J, Levi M, ten Cate H, ten Cate JW, van Deventer SJ. Regulation of interleukin 10 release by tumor necrosis factor in humans and chimpanzees. *J Exp Med.* Nov 1 1994;180(5):1985-1988.

104. Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol.* 1998;16:27-55.

105. Pereira BJ. Cytokine production in patients on dialysis. *Blood Purif.* 1995;13(3-4):135-146.

106. Deng J, Kohda Y, Chiao H, et al. Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. *Kidney Int.* Dec 2001;60(6):2118-2128.

107. Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin Invest.* Sep 2002;110(6):835-842.

108. Burne-Taney MJ, Kofler J, Yokota N, Weisfeldt M, Traystman RJ, Rabb H. Acute renal failure after whole body ischemia is characterized by inflammation and T cell-mediated injury. *Am J Physiol Renal Physiol.* Jul 2003;285(1):F87-94.

109. Kelly KJ, Williams WW, Jr., Colvin RB, Bonventre JV. Antibody to intercellular adhesion molecule 1 protects the kidney against ischemic injury. *Proc Natl Acad Sci U S A.* Jan 18 1994;91(2):812-816.

110. Kelly KJ, Meehan SM, Colvin RB, Williams WW, Bonventre JV. Protection from toxicant-mediated renal injury in the rat with anti-CD54 antibody. *Kidney Int.* Sep 1999;56(3):922-931.

111. Gormley SM, McBride WT, Armstrong MA, et al. Plasma and urinary cytokine homeostasis and renal dysfunction during cardiac surgery. *Anesthesiology.* Nov 2000;93(5):1210-1216; discussion 1215A.

112. Gormley SM, McBride WT, Armstrong MA, et al. Plasma and urinary cytokine homeostasis and renal function during cardiac surgery without cardiopulmonary bypass. *Cytokine.* Jan 21 2002;17(2):61-65.

Chulalongkorn University

113. Simmons EM, Himmelfarb J, Sezer MT, et al. Plasma cytokine levels predict mortality in patients with acute renal failure. *Kidney Int.* Apr 2004;65(4):1357-1365.

114. Iglesias J, Marik PE, Levine JS. Elevated serum levels of the type I and type II receptors for tumor necrosis factor-alpha as predictive factors for ARF in patients with septic shock. *Am J Kidney Dis.* Jan 2003;41(1):62-75.

115. Loisa P, Rinne T, Laine S, Hurme M, Kaukinen S. Anti-inflammatory cytokine response and the development of multiple organ failure in severe sepsis. *Acta Anaesthesiol Scand.* Mar 2003;47(3):319-325.

116. Azumi H, Inoue N, Takeshita S, et al. Expression of NADH/NADPH oxidase p22phox in human coronary arteries. *Circulation.* Oct 5 1999;100(14):1494-1498.

117. Macdonald J, Galley HF, Webster NR. Oxidative stress and gene expression in sepsis. *Br J Anaesth.* Feb 2003;90(2):221-232.

118. Ursini F, Maiorino M, Brigelius-Flohe R, et al. Diversity of glutathione peroxidases. *Methods Enzymol.* 1995;252:38-53.

119. Flohe L. Glutathione peroxidase. *Basic Life Sci.* 1988;49:663-668.

120. Fukai T, Folz RJ, Landmesser U, Harrison DG. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc Res.* Aug 1 2002;55(2):239-249.

121. Baliga R, Ueda N, Walker PD, Shah SV. Oxidant mechanisms in toxic acute renal failure. *Drug Metab Rev.* Nov 1999;31(4):971-997.

122. Alonso A, Lau J, Jaber BL, Weintraub A, Sarnak MJ. Prevention of radiocontrast nephropathy with N-acetylcysteine in patients with chronic kidney disease: a meta-analysis of randomized, controlled trials. *Am J Kidney Dis.* Jan 2004;43(1):1-9.

123. Waddington SN, Mosley K, Cattell V. Induced nitric oxide (NO) synthesis in heterologous nephrotoxic nephritis; effects of selective inhibition in neutrophil-dependent glomerulonephritis. *Clin Exp Immunol.* Nov 1999;118(2):309-314.

124. Ricci Z, Ronco C. Pulmonary/renal interaction. *Curr Opin Crit Care.* Feb 2010;16(1):13-18.

125. Kramer AA, Postler G, Salhab KF, Mendez C, Carey LC, Rabb H. Renal ischemia/reperfusion leads to macrophage-mediated increase in pulmonary vascular permeability. *Kidney Int.* Jun 1999;55(6):2362-2367.

126. Rabb H, Wang Z, Nemoto T, Hotchkiss J, Yokota N, Soleimani M. Acute renal failure leads to dysregulation of lung salt and water channels. *Kidney Int.* Feb 2003;63(2):600-606.

127. Hassoun HT, Grigoryev DN, Lie ML, et al. Ischemic acute kidney injury induces a distant organ functional and genomic response distinguishable from bilateral nephrectomy. *Am J Physiol Renal Physiol.* Jul 2007;293(1):F30-40.

128. Deng J, Hu X, Yuen PS, Star RA. Alpha-melanocyte-stimulating hormone inhibits lung injury after renal ischemia/reperfusion. *Am J Respir Crit Care Med.* Mar 15 2004;169(6):749-756.

129. Klein CL, Hoke TS, Fang WF, Altmann CJ, Douglas IS, Faubel S. Interleukin-6 mediates lung injury following ischemic acute kidney injury or bilateral nephrectomy. *Kidney Int.* Oct 2008;74(7):901-909.

130. Schwabe RF, Schnabl B, Kweon YO, Brenner DA. CD40 activates NF-kappa B and c-Jun N-terminal kinase and enhances chemokine secretion on activated human hepatic stellate cells. *J Immunol.* Jun 1 2001;166(11):6812-6819.

131. Golab F, Kadkhodaee M, Zahmatkesh M, et al. Ischemic and non-ischemic acute kidney injury cause hepatic damage. *Kidney Int.* Apr 2009;75(8):783-792.

132. Stevens LA, Coresh J, Schmid CH, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis.* Mar 2008;51(3):395-406.

133. Wald R, Liangos O, Perianayagam MC, et al. Plasma cystatin C and acute kidney injury after cardiopulmonary bypass. *Clin J Am Soc Nephrol.* Aug 2010;5(8):1373-1379.

134. Perianayagam MC, Seabra VF, Tighiouart H, Liangos O, Jaber BL. Serum cystatin C for prediction of dialysis requirement or death in acute kidney injury: a comparative study. *Am J Kidney Dis.* Dec 2009;54(6):1025-1033.

135. Ichimura T, Bonventre JV, Bailly V, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *J Biol Chem.* Feb 13 1998;273(7):4135-4142.

136. Liangos O, Perianayagam MC, Vaidya VS, et al. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol.* Mar 2007;18(3):904-912.

137. McMahon BA, Koyner JL, Murray PT. Urinary glutathione S-transferases in the pathogenesis and diagnostic evaluation of acute kidney injury following cardiac surgery: a critical review. *Curr Opin Crit Care.* Oct 7 2010.

138. Dieterle F SF. *Biomarkers of acute kidney injury*. New York: John Wiley and Sons, Inc; 2010.

139. Boldt J, Brenner T, Lang J, Kumle B, Isgro F. Kidney-specific proteins in elderly patients undergoing cardiac surgery with cardiopulmonary bypass. *Anesth Analg.* Dec 2003;97(6):1582-1589.

140. Eijkenboom JJ, van Eijk LT, Pickkers P, Peters WH, Wetzels JF, van der Hoeven HG. Small increases in the urinary excretion of glutathione S-transferase A1 and P1 after cardiac surgery are not associated with clinically relevant renal injury. *Intensive Care Med.* May 2005;31(5):664-667.

141. Koyner JL, Vaidya VS, Bennett MR, et al. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol.* Dec 2010;5(12):2154-2165.

142. Guttmacher AE, Collins FS. Genomic medicine--a primer. *N Engl J Med.* Nov 7 2002;347(19):1512-1520.

143. Bidwell J, Keen L, Gallagher G, et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 1. *Genes Immun.* Apr 2001;2(2):61-70.

144. Haukim N, Bidwell JL, Smith AJ, et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 2. *Genes Immun.* Sep 2002;3(6):313-330.

145. MW T. *GENETIC VARIATION IN INDIVIDUALS: Mutation and Polymorphism*. Philadelphia: WB Saunders Company; 2001.

146. van Deventer SJ. Cytokine and cytokine receptor polymorphisms in infectious disease. *Intensive Care Med.* 2000;26 Suppl 1:S98-102.

147. Morse H, Olomolaiye O, Wood N, Keen L, Bidwell J. Induced heteroduplex genotyping of TNF-alpha, IL-1beta, IL-6 and IL-10 polymorphisms associated with transcriptional regulation. *Cytokine.* 1999;11:789-795.

148. Wilson AG, Symons JA, McDowell TL, McDewitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor [alpha] promoter on transcriptional activation. *Proc Natl Acad Sci.* 1997;94:3195-3199.

149. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-[alpha] promoter polymorphism effects transcription. *Mol Immunol.* 1997;34:391-399.

150. Warzocha K, Ribeiro P, Bienvenu J. Genetic polymorphisms in the tumor necrosis factor locus influence non-Hodgkin's lymphoma outcome. *Blood.* 1998;91:3574-3581.

151. Louis E, Franchimont D, Piron A. Tumor necrosis factor gene polymorphism influences TNF-alpha production in lipopolysaccharide-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol.* 1998;113:401-406.

152. Pociot F, Molvig J, Wogensen L, et al. A tumour necrosis factor beta gene polymorphism in relation to monokine secretion and insulin-dependent diabetes mellitus. *Scand J Immunol.* Jan 1991;33(1):37-49.

153. Abraham LJ, Du DC, Zahedi K, Dawkins RL, Whitehead AS. Haplotypic polymorphisms of the TNFB gene. *Immunogenetics.* 1991;33(1):50-53.

154. Dominici R, Cattaneo M, Malferrari G, et al. Cloning and functional analysis of the allelic polymorphism in the transcription regulatory region of interleukin-1 alpha. *Immunogenetics.* May 2002;54(2):82-86.

155. Bailly S, di Giovine FS, Duff GW. Polymorphic tandem repeat region in interleukin-1 alpha intron 6. *Hum Genet.* Mar 1993;91(1):85-86.

156. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest.* Jun 1992;22(6):396-402.

157. Read RC, Cannings C, Naylor SC, et al. Variation within genes encoding interleukin-1 and the interleukin-1 receptor antagonist influence the severity of meningococcal disease. *Ann Intern Med.* Apr 1 2003;138(7):534-541.

158. Tountas NA, Casini-Raggi V, Yang H, et al. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. *Gastroenterology.* Oct 1999;117(4):806-813.

159. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest.* Oct 1 1998;102(7):1369-1376.

160. Fernandez-Real JM, Broch M, Vendrell J, Richart C, Ricart W. Interleukin-6 gene polymorphism and lipid abnormalities in healthy subjects. *J Clin Endocrinol Metab.* Mar 2000;85(3):1334-1339.

161. Heesen M, Obertacke U, Schade FU, Bloemeke B, Majetschak M. The interleukin-6 G(-174)C polymorphism and the ex vivo interleukin-6 response to endotoxin in severely injured blunt trauma patients. *Eur Cytokine Netw.* Jan-Mar 2002;13(1):72-77.

162. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. *J Clin Endocrinol Metab.* Jan 2003;88(1):255-259.

163. Westendorp RG, Langermans JA, Huizinga TW, et al. Genetic influence on cytokine production and fatal meningococcal disease. *Lancet.* Jan 18 1997;349(9046):170-173.

164. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum.* Jun 1999;42(6):1101-1108.

165. Jiang ZD, Okhuysen PC, Guo DC, et al. Genetic susceptibility to enteroaggregative Escherichia coli diarrhea: polymorphism in the interleukin-8 promotor region. *J Infect Dis.* Aug 15 2003;188(4):506-511.

166. Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax.* Dec 2000;55(12):1023-1027.

167. Yu Y, Chadee K. The 3'-untranslated region of human interleukin-8 mRNA suppresses IL-8 gene expression. *Immunology.* Apr 2001;102(4):498-505.

168. Amoli MM, Thomson W, Hajeer AH, et al. Interleukin 8 gene polymorphism is associated with increased risk of nephritis in cutaneous vasculitis. *J Rheumatol.* Nov 2002;29(11):2367-2370.

169. Rovin BH, Lu L, Zhang X. A novel interleukin-8 polymorphism is associated with severe systemic lupus erythematosus nephritis. *Kidney Int.* Jul 2002;62(1):261-265.

170. Rovin BH, Lu L, Saxena R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. *Biochem Biophys Res Commun.* Jun 7 1999;259(2):344-348.

171. Kim HL, Lee DS, Yang SH, et al. The polymorphism of monocyte chemoattractant protein-1 is associated with the renal disease of SLE. *Am J Kidney Dis.* Dec 2002;40(6):1146-1152.

172. Kang TJ, Lee SB, Chae GT. A polymorphism in the toll-like receptor 2 is associated with IL-12 production from monocyte in lepromatous leprosy. *Cytokine.* Oct 21 2002;20(2):56-62.

173. Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect Immun.* Nov 2000;68(11):6398-6401.

174. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet.* Jun 2000;25(2):187-191.

175. Fekete A, Treszl A, Toth-Heyn P, et al. Association between heat shock protein 72 gene polymorphism and acute renal failure in premature neonates. *Pediatr Res.* Oct 2003;54(4):452-455.

176. Schroder O, Schulte KM, Ostermann P, Roher HD, Ekkernkamp A, Laun RA. Heat shock protein 70 genotypes HSPA1B and HSPA1L influence cytokine concentrations and interfere with outcome after major injury. *Crit Care Med.* Jan 2003;31(1):73-79.

177. Lavrovsky Y, Schwartzman ML, Levere RD, Kappas A, Abraham NG. Identification of binding sites for transcription factors NF-kappa B and AP-2 in the promoter region of the human heme oxygenase 1 gene. *Proc Natl Acad Sci U S A.* Jun 21 1994;91(13):5987-5991.

178. Chen YH, Lin SJ, Lin MW, et al. Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. *Hum Genet.* Jul 2002;111(1):1-8.

179. Inoue N, Kawashima S, Kanazawa K, Yamada S, Akita H, Yokoyama M. Polymorphism of the NADH/NADPH oxidase p22 phox gene in patients with coronary artery disease. *Circulation.* Jan 20 1998;97(2):135-137.

180. Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M, Reynolds WF. An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. *J Biol Chem.* Jun 14 1996;271(24):14412-14420.

181. Pecoits-Filho R, Stenvinkel P, Marchlewska A, et al. A functional variant of the myeloperoxidase gene is associated with cardiovascular disease in end-stage renal disease patients. *Kidney Int Suppl.* May 2003(84):S172-176.

182. Sandstrom J, Nilsson P, Karlsson K, Marklund SL. 10-fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparin-binding domain. *J Biol Chem.* Jul 22 1994;269(29):19163-19166.

183. Forsberg L, Lyrenas L, de Faire U, Morgenstern R. A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is

correlated to blood catalase levels. *Free Radic Biol Med.* Mar 1 2001;30(5):500-505.

184. Shen Q, Townes PL, Padden C, Newburger PE. An in-frame trinucleotide repeat in the coding region of the human cellular glutathione peroxidase (GPX1) gene: in vivo polymorphism and in vitro instability. *Genomics.* Sep 1 1994;23(1):292-294.

185. Forsberg L, de Faire U, Marklund SL, Andersson PM, Stegmayr B, Morgenstern R. Phenotype determination of a common Pro-Leu polymorphism in human glutathione peroxidase 1. *Blood Cells Mol Dis.* Oct 2000;26(5):423-426.

186. Widersten M, Pearson WR, Engstrom A, Mannervik B. Heterologous expression of the allelic variant mu-class glutathione transferases mu and psi. *Biochem J.* Jun 1 1991;276 (Pt 2):519-524.

187. Board PG. Biochemical genetics of glutathione-S-transferase in man. *Am J Hum Genet.* Jan 1981;33(1):36-43.

188. Hobbs MR, Udhayakumar V, Levesque MC, et al. A new NOS2 promoter polymorphism associated with increased nitric oxide production and protection from severe malaria in Tanzanian and Kenyan children. *Lancet.* Nov 9 2002;360(9344):1468-1475.

189. Weitkamp JH, Stuber F, Bartmann P. Pilot study assessing TNF gene polymorphism as a prognostic marker for disease progression in neonates with sepsis. *Infection.* Mar-Apr 2000;28(2):92-96.

190. Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-alpha concentrations and outcome of patients with severe sepsis. *Crit Care Med.* Mar 1996;24(3):381-384.

191. Majetschak M, Flohe S, Obertacke U, et al. Relation of a TNF gene polymorphism to severe sepsis in trauma patients. *Ann Surg.* Aug 1999;230(2):207-214.

192. Nadel S, Newport MJ, Booy R, Levin M. Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. *J Infect Dis.* Oct 1996;174(4):878-880.

193. McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. *Nature.* Oct 6 1994;371(6497):508-510.

Chulalongkorn University

194. Cabrera M, Shaw MA, Sharples C, et al. Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. *J Exp Med.* Nov 1 1995;182(5):1259-1264.

195. SHAIKH K YS, WATERER GW. Effect of tumor necrosis factor gene polymorphisms on restoration of body temperature after coronary artery bypass graft surgery. *Chest.* 2000;118:109 S.

196. Pelletier R, Pravica V, Perrey C, et al. Evidence for a genetic predisposition towards acute rejection after kidney and simultaneous kidney-pancreas transplantation. *Transplantation.* Aug 27 2000;70(4):674-680.

197. McGuire W, Knight JC, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Severe malarial anemia and cerebral malaria are associated with different tumor necrosis factor promoter alleles. *J Infect Dis.* Jan 1999;179(1):287-290.

198. Wunderink RG, Waterer GW, Cantor RM, Quasney MW. Tumor necrosis factor gene polymorphisms and the variable presentation and outcome of community-acquired pneumonia. *Chest.* Mar 2002;121(3 Suppl):87S.

199. Waterer GW, ElBahlawan L, Quasney MW, Zhang Q, Kessler LA, Wunderink RG. Heat shock protein 70-2+1267 AA homozygotes have an increased risk of septic shock in adults with community-acquired pneumonia. *Crit Care Med.* May 2003;31(5):1367-1372.

200. Zhang D, Li J, Jiang ZW, Yu B, Tang X. Association of two polymorphisms of tumor necrosis factor gene with acute severe pancreatitis. *J Surg Res.* Jun 15 2003;112(2):138-143.

201. Schroeder S, Borger N, Wrigge H, et al. A tumor necrosis factor gene polymorphism influences the inflammatory response after cardiac operation. *Ann Thorac Surg.* Feb 2003;75(2):534-537.

202. Asensi V, Alvarez V, Valle E, et al. IL-1 alpha (-889) promoter polymorphism is a risk factor for osteomyelitis. *Am J Med Genet A.* Jun 1 2003;119A(2):132-136.

203. Borgmann S, Endisch G, Hacker UT, Song BS, Fricke H. Proinflammatory genotype of interleukin-1 and interleukin-1 receptor antagonist is associated with ESRD in proteinase 3-ANCA vasculitis patients. *Am J Kidney Dis.* May 2003;41(5):933-942.

204. Virta M, Hurme M, Helminen M. Increased frequency of interleukin-1beta (-511) allele 2 in febrile seizures. *Pediatr Neurol.* Mar 2002;26(3):192-195.

205. Read RC, Camp NJ, di Giovine FS, et al. An interleukin-1 genotype is associated with fatal outcome of meningococcal disease. *J Infect Dis.* Nov 2000;182(5):1557-1560.

206. Fang XM, Schroder S, Hoeft A, Stuber F. Comparison of two polymorphisms of the interleukin-1 gene family: interleukin-1 receptor antagonist polymorphism contributes to susceptibility to severe sepsis. *Crit Care Med.* Jul 1999;27(7):1330-1334.

207. Ma P, Chen D, Pan J, Du B. Genomic polymorphism within interleukin-1 family cytokines influences the outcome of septic patients. *Crit Care Med.* May 2002;30(5):1046-1050.

208. Wilkinson RJ, Patel P, Llewelyn M, et al. Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1beta on tuberculosis. *J Exp Med.* Jun 21 1999;189(12):1863-1874.

209. Amoli MM, Thomson W, Hajeer AH, et al. Interleukin 1 receptor antagonist gene polymorphism is associated with severe renal involvement and renal sequelae in Henoch-Schonlein purpura. *J Rheumatol.* Jul 2002;29(7):1404-1407.

210. Ray KK, Camp NJ, Bennett CE, Francis SE, Crossman DC. Genetic variation at the interleukin-1 locus is a determinant of changes in soluble endothelial factors in patients with acute coronary syndromes. *Clin Sci (Lond).* Sep 2002;103(3):303-310.

211. Schluter B, Raufhake C, Erren M, et al. Effect of the interleukin-6 promoter polymorphism (-174 G/C) on the incidence and outcome of sepsis. *Crit Care Med.* Jan 2002;30(1):32-37.

212. van der Pol WL, Huizinga TW, Vidarsson G, et al. Relevance of Fcgamma receptor and interleukin-10 polymorphisms for meningococcal disease. *J Infect Dis.* Dec 15 2001;184(12):1548-1555.

213. Delgado JC, Baena A, Thim S, Goldfeld AE. Ethnic-specific genetic associations with pulmonary tuberculosis. *J Infect Dis.* Nov 15 2002;186(10):1463-1468.

214. Gallagher PM, Lowe G, Fitzgerald T, et al. Association of IL-10 polymorphism with severity of illness in community acquired pneumonia. *Thorax.* Feb 2003;58(2):154-156.

215. Lowe PR, Galley HF, Abdel-Fattah A, Webster NR. Influence of interleukin-10 polymorphisms on interleukin-10 expression and survival in critically ill patients. *Crit Care Med.* Jan 2003;31(1):34-38.

216. Lin MT, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med.* Dec 4 2003;349(23):2201-2210.

217. Kruger B, Schroppel B, Ashkan R, et al. A Monocyte chemoattractant protein-1 (MCP-1) polymorphism and outcome after renal transplantation. *J Am Soc Nephrol.* Oct 2002;13(10):2585-2589.

218. Bochud PY, Hawn TR, Aderem A. Cutting edge: a Toll-like receptor 2 polymorphism that is associated with lepromatous leprosy is unable to mediate mycobacterial signaling. *J Immunol.* Apr 1 2003;170(7):3451-3454.

219. Agnese DM, Calvano JE, Hahm SJ, et al. Human toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections. *J Infect Dis.* Nov 15 2002;186(10):1522-1525.

220. Child NJ, Yang IA, Pulletz MC, et al. Polymorphisms in Toll-like receptor 4 and the systemic inflammatory response syndrome. *Biochem Soc Trans.* Jun 2003;31(Pt 3):652-653.

221. Lorenz E, Mira JP, Frees KL, Schwartz DA. Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. *Arch Intern Med.* May 13 2002;162(9):1028-1032.

222. Exner M, Schillinger M, Minar E, et al. Heme oxygenase-1 gene promoter microsatellite polymorphism is associated with restenosis after percutaneous transluminal angioplasty. *J Endovasc Ther.* Oct 2001;8(5):433-440.

223. Schillinger M, Exner M, Mlekusch W, et al. Heme oxygenase-1 gene promoter polymorphism is associated with abdominal aortic aneurysm. *Thromb Res.* Apr 15 2002;106(2):131-136.

224. Yamada N, Yamaya M, Okinaga S, et al. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet.* Jan 2000;66(1):187-195.

225. Nakano T, Matsunaga S, Nagata A, Maruyama T. NAD(P)H oxidase p22phox Gene C242T polymorphism and lipoprotein oxidation. *Clin Chim Acta.* Sep 2003;335(1-2):101-107.

226. Seidegard J, Pero RW, Markowitz MM, Roush G, Miller DG, Beattie EJ. Isoenzyme(s) of glutathione transferase (class Mu) as a marker for the susceptibility to lung cancer: a follow up study. *Carcinogenesis.* Jan 1990;11(1):33-36.

227. St Peter SD, Imber CJ, Jones DC, et al. Genetic determinants of delayed graft function after kidney transplantation. *Transplantation.* Sep 27 2002;74(6):809-813.

228. Greene EL, Paller MS. Oxygen free radicals in acute renal failure. *Miner Electrolyte Metab.* 1991;17(2):124-132.

229. Guzik TJ, West NE, Black E, et al. Functional effect of the C242T polymorphism in the NAD(P)H oxidase p22phox gene on vascular superoxide production in atherosclerosis. *Circulation.* Oct 10 2000;102(15):1744-1747.

230. Bouma G, Crusius JB, Oudkerk Pool M, et al. Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol.* Apr 1996;43(4):456-463.

231. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med.* Apr 2003;31(4):1250-1256.

232. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med.* Oct 1985;13(10):818-829.

233. Knaus WA, Wagner DP. Multiple systems organ failure: epidemiology and prognosis. *Crit Care Clin.* Apr 1989;5(2):221-232.

234. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med.* Mar 16 1999;130(6):461-470.

235. Perrey C, Turner SJ, Pravica V, Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms. *Transpl Immunol.* Jun 1999;7(2):127-128.

236. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci U S A.* Apr 1 1997;94(7):3195-3199.

237. Tobin J. Estimation for relationships with limited dependent variables. *Econometrica* 1958;26:24-36.

238. Coca SG, Singanamala S, Parikh CR. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. *Kidney Int.* Mar 2012;81(5):442-448.

239. Faubel S, Chawla LS, Chertow GM, Goldstein SL, Jaber BL, Liu KD. Ongoing clinical trials in AKI. *Clin J Am Soc Nephrol.* May 2012;7(5):861-873.

240. Bonventre J, Weinberg J. Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol.* 2003;14:2199-2210.

241. Ramesh G, Reeves WB. Inflammatory cytokines in acute renal failure. *Kidney Int Suppl.* Oct 2004(91):S56-61.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

242. Cunningham PN, Dyanov HM, Park P, Wang J, Newell KA, Quigg RJ. Acute renal failure in endotoxemia is caused by TNF acting directly on TNF receptor-1 in kidney. *J Immunol.* 2002;168(11):5817-5823.

243. Kitching A, Katerelos M, Mudge S, Tipping P, Power D, Holdsworth S. Interleukin-10 inhibits experimental mesangial proliferative glomerulonephritis. *Clin Exp Immunol.* 2002;128:36-43.

244. El-Shemi AG, Fujinaka H, Matsuki A, et al. Suppression of experimental crescentic glomerulonephritis by interleukin-10 gene transfer. *Kidney Int.* Apr 2004;65(4):1280-1289.

245. Jaber B, Pereira B, Bonventre J, Balakrishnan V. Polymorphism of host response genes: Implications in the pathogenesis and treatment of acute renal failure. *Kidney Int.* 2005;67:14-33.

246. Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LB, Duff GW. An allelic polymorphism within the human tumor necrosis factor-alpha promoter rgion is strongly associated with HLA A1, B8, and DR3 alleles. *J Exp Med.* 1993;177:557-560.

247. Abraham LJ, Kroeger KM. Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J Leukoc Biol.* Oct 1999;66(4):562-566.

248. Jaber BL, Pereira BJ, Bonventre JV, Balakrishnan VS. Polymorphism of host response genes: implications in the pathogenesis and treatment of acute renal failure. *Kidney Int.* Jan 2005;67(1):14-33.

249. Antonicelli R, Olivieri F, Cavallone L, et al. Tumor necrosis factor-alpha gene -308G>A polymorphism is associated with ST-elevation myocardial infarction and with high plasma levels of biochemical ischemia markers. *Coron Artery Dis.* Dec 2005;16(8):489-493.

250. Bishehsari F, Sharma A, Stello K, et al. TNF-alpha gene (TNFA) variants increase risk for multi-organ dysfunction syndrome (MODS) in acute pancreatitis. *Pancreatology.* Mar 2012;12(2):113-118.

251. Waterer GW, Quasney MW, Cantor RM, Wunderink RG. Septic shock and respiratory failure in community-acquired pneumonia have different TNF polymorphism associations. *Am J Respir Crit Care Med.* Jun 2001;163(7):1599-1604.

252. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int.* 2002;62(1):237-244.

253. Kuehn EW, Park KM, Somlo S, Bonventre JV. Kidney injury molecule-1 expression in murine polycystic kidney disease. *Am J Physiol Renal Physiol.* Dec 2002;283(6):F1326-1336.

254. Ichimura T, Hung CC, Yang SA, Stevens JL, Bonventre JV. Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury. *Am J Physiol Renal Physiol.* Mar 2004;286(3):F552-563.

255. Han WK, Alinani A, Wu CL, et al. Human kidney injury molecule-1 is a tissue and urinary tumor marker of renal cell carcinoma. *J Am Soc Nephrol.* Apr 2005;16(4):1126-1134.

256. Liangos O, Han W, Wald R, et al. Urinary Kidney Injury Molecule-1 level is an early and sensitive marker of acute kidney injury following cardiopulmonary bypass (Abstract). *J Am Soc Nephrol.* 2006;17(403A).

257. Liangos O, Perianayagam MC, Vaidya VS, et al. Urinary N-Acetyl-{beta}-(D)-Glucosaminidase Activity and Kidney Injury Molecule-1 Level Are Associated with Adverse Outcomes in Acute Renal Failure. *J Am Soc Nephrol.* Jan 31 2007.

258. Manchanda PK, Kumar A, Kaul A, Mittal RD. Correlation between a gene polymorphism of tumor necrosis factor-alpha (G/A) and end-stage renal disease: a pilot study from north India. *Clin Chim Acta.* Aug 2006;370(1-2):152-157.

259. Nikolova PN, Ivanova MI, Mihailova SM, et al. Cytokine gene polymorphism in kidney transplantation--impact of TGF-beta 1, TNF-alpha and IL-6 on graft outcome. *Transpl Immunol.* Feb 2008;18(4):344-348.

260. Teuffel O, Ethier MC, Beyene J, Sung L. Association between tumor necrosis factor-alpha promoter -308 A/G polymorphism and susceptibility to sepsis and sepsis mortality: a systematic review and meta-analysis. *Crit Care Med.* Jan 2010;38(1):276-282.

261. Marx J. How the glucocorticoids suppress immunity. *Science.* Oct 13 1995;270(5234):232-233.

262. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science.* Oct 13 1995;270(5234):286-290.

263. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS, Jr. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science.* Oct 13 1995;270(5234):283-286.

264. Han J, Thompson P, Beutler B. Dexamethasone and pentoxifylline inhibit endotoxin-induced cachectin/tumor necrosis factor synthesis at separate points in the signaling pathway. *J Exp Med.* Jul 1 1990;172(1):391-394.

265. Strieter RM, Remick DG, Ward PA, et al. Cellular and molecular regulation of tumor necrosis factor-alpha production by pentoxifylline. *Biochem Biophys Res Commun.* Sep 30 1988;155(3):1230-1236.

266. Doherty GM, Jensen JC, Alexander HR, Buresh CM, Norton JA. Pentoxifylline suppression of tumor necrosis factor gene transcription. *Surgery.* Aug 1991;110(2):192-198.

267. Parry GC, Mackman N. Role of cyclic AMP response element-binding protein in cyclic AMP inhibition of NF-kappaB-mediated transcription. *J Immunol.* Dec 1 1997;159(11):5450-5456.

268. Hartmann G, Bidlingmaier C, Siegmund B, et al. Specific type IV phosphodiesterase inhibitor rolipram mitigates experimental colitis in mice. *J Pharmacol Exp Ther.* Jan 2000;292(1):22-30.

269. Semmler J, Wachtel H, Endres S. The specific type IV phosphodiesterase inhibitor rolipram suppresses tumor necrosis factor-alpha production by human mononuclear cells. *Int J Immunopharmacol.* Apr 1993;15(3):409-413.

270. Sampaio EP, Sarno EN, Galilly R, Cohn ZA, Kaplan G. Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. *J Exp Med.* Mar 1 1991;173(3):699-703.

271. Moreira AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA, Kaplan G. Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. *J Exp Med.* Jun 1 1993;177(6):1675-1680.

272. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol.* 2001;19:683-765.

273. Pajkrt D, Camoglio L, Tiel-van Buul MC, et al. Attenuation of proinflammatory response by recombinant human IL-10 in human endotoxemia: effect of timing of recombinant human IL-10 administration. *J Immunol.* Apr 15 1997;158(8):3971-3977.

274. Calabrese LH. Molecular differences in anticytokine therapies. *Clin Exp Rheumatol.* Mar-Apr 2003;21(2):241-248.

275. Bermudez LE, Wu M, Young LS. Effect of stress-related hormones on macrophage receptors and response to tumor necrosis factor. *Lymphokine Res.* Summer 1990;9(2):137-145.

276. Nabel E. Genomic Medicine: Cardiovascular Disease. *N Engl J Med.* 2003;349:60-72.

277. Ridker P, Stampfer M. Assessment of genetic markers for coronary thrombosis: promise and precaution. *Lancet.* 1999;353:687-688.

APPENDIX

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

VITA

Miss. Paweena Susantitaphong was born on July 17th, 1979 in Bangkok. She received her Bachelor degree with first honors in medicine from Faculty of Medicine, Chulalongkorn University in 2002. She has been trained in Internal Medicine at the Khon Kaen University in Thailand, and later has been specialized in Clinical Nephrology at the Chulalongkorn University. She received her Master's degree of medicine from Faculty of Medicine, Chulalongkorn University in 2008. Then she worked as an instructor in Division of Nephrology Chulalongkorn University for 1 year and she was also a visiting fellow at the Nephrology Section of the University Hospital Ghent in Belgium. She received International Society of Nephrology COMGAN Fellowship Award in 2011-2012. She recently completed a Clinical Research Fellowship in Nephrology at the St. Elizabeth's Medical Center, Tufts University School of Medicine in Boston, Massachusetts, USA and she has been an adjunct instructor at Tufts University School of Medicine, Boston, USA.

Her major research interests include acute kidney injury including early detection, biomarkers of kidney injury, genetic polymorphisms, early treatment strategies, and dialysis support. She is also interested in extracorporeal multi-organ support and dialysis, as well as chronic kidney disease-mineral and bone disease.

She has contributed more than 25 chapters in medical textbooks, and has published over 20 articles in various regional and international peer-reviewed journals. She is an ad-hoc reviewer for several scientific journals, including American Journal of Kidney Disease, Therapeutic Apheresis and Dialysis, Nephrology Dialysis Transplantation, Nephrology, Renal Failure, Nephrology, Advances in Chronic Kidney Disease. She has had several abstract and poster presentations both in Thailand and in the US, and is regularly invited as a speaker in national medical meetings and conferences.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University