

**A STUDY OF BIOMARKERS FOR DIAGNOSIS,
OUTCOME PREDICTION AND ANTIBIOTIC THERAPY
GUIDANCE IN SEPSIS**

BY

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ABSTRACT

Sepsis is common in the ICU worldwide and contributes to high mortality. However, timely diagnosis, outcome prediction and antibiotic monitoring in sepsis remains challenging. In Chapter Three, the diagnostic value of model-based insulin sensitivity (SI) for sepsis was studied in 38 non-diabetics on their ICU admission in a cross-sectional study. The findings indicated that baseline SI was significantly lower in sepsis ($n = 18$) versus non-sepsis ($n = 20$) (0.996 ± 1.269 versus $5.012 \pm 4.930 \times 10^{-4}$ L/mU/min, $P = 0.002$), with clinically valid diagnostic performance (AUC 0.814). In Chapter Four, similar methodology was applied to a mixed cohort of 86 diabetic and non-diabetic patients newly admitted to ICU. Although baseline SI was significantly lower in sepsis ($n = 41$) versus non-sepsis ($n = 45$) (0.560 ± 0.676 versus $1.097 \pm 1.473 \times 10^{-4}$ L/mU/min, $P = 0.037$), the biomarker failed to diagnose sepsis in this cohort. Hence, model-based SI may be a useful diagnostic test of sepsis when specifically applied to the non-diabetic ICU patients. In Chapter Five, the prognostic value of a combination of biomarkers in sepsis was explored in a prospective cohort study of 159 ICU patients. It was found that a prediction equation utilizing baseline total leukocytes count, procalcitonin, interleukin-6 and arylesterase activity of paraoxonase-1 predicted 30-day mortality with a remarkable performance (AUC 0.814). Therefore, a multi-marker approach using these biomarkers may be a useful predictor of mortality in sepsis. In Chapter Six, the utility of point-of-care procalcitonin (POCT) to guide duration of antibiotic in the ICU was examined in a randomized-controlled trial. Eighty patients were allocated to either the POCT-guided arm ($n = 40$) or control arm ($n = 40$). The mean duration of antibiotic was 6.3 ± 2.1 days in the POCT-guided arm versus 9.1 ± 4.7 days in control arm ($P = 0.001$), while there was no significant difference in 30-day mortality. Thus, POCT guidance reduced antibiotic duration without compromising mortality in our patients.

خلاصة البحث

يعتبر إنتان شائع في وحدة العناية المركزة في جميع أنحاء العالم ويساهم في ارتفاع معدل الوفيات. ومع ذلك، لا يزال التشخيص في الوقت المناسب والتنبؤ بالنتائج ورصد المضادات الحيوية في التسمم يمثل تحديًا. وفي الباب الثالث، تمت دراسة القيمة التشخيصية لحساسية الأنسولين (ايس.اي) المستندة إلى النموذج للإنتان في 38 مريض غير مصاب بالسكري عند قبولهم في وحدة العناية المركزة في دراسة مقطعية. وأشارت النتائج إلى أن خط الأساس SI كان أقل بشكل ملحوظ في الإنتان (ن = 18) مقابل غير الإنتان (ن = 20) (0.996 ± 1.269) مقابل $5.012 \pm 4.930 \times 10^{-4}$ L/mU/min، $(P = 0.002)$ ، مع أداء تشخيصي صالح سريريًا (AUC 0.814). وفي الباب الرابع، تم تطبيق منهجية مماثلة على مجموعة مختلطة من 86 مريضًا بالسكري وغير المصابين بالبول السكري تم قبولهم حديثًا في وحدة العناية المركزة. على الرغم من أن الخط الأساسي (ايس.اي) كان أقل بشكل ملحوظ في التسمم (ن = 41) مقابل التسمم (ن = 45) (0.560 ± 0.676) مقابل $1.097 \pm 1.473 \times 10^{-4}$ L/mU/min، $(P = 0.037)$ لتشخيص الإنتان في هذه المجموعة. وبالتالي، قد يكون SI المستندة إلى نموذج اختبار تشخيصي مفيد من تعفن الدم عند تطبيقها على وجه التحديد لمرضى ICU غير المصابين بالسكري. وفي الباب الخامس، تم استكشاف قيمة النذير لمجموعة من المؤشرات الحيوية في الإنتان في دراسة الأثراب المحتملين من 159 مريضًا وحدة العناية المركزة. وقد وجد أن معادلة التنبؤ باستخدام عدد خلايا الدم البيضاء وبروكالسيتونين وإنترلوكين 6 واريستراس نشاط من المتوقع للوفوروكسوناز 1 وتوقع وفاة 30 يوما مع أداء ملحوظ (AUC 0.814). ولذلك، قد يكون اتباع نهج متعدد العلامات باستخدام هذه المؤشرات الحيوية مؤشرا مفيدا للوفيات في الإنتان. وفي الباب السادس، تم فحص فائدة بروكالسيتونين نقطة الرعاية (بي.او.سي.تي) لتوجيه مدة المضادات الحيوية في وحدة العناية المركزة في تجربة عشوائية محكمة. وتم تخصيص ثمانين مريضًا إما إلى الذراع الموجهة (بي.او.سي.تي) (ن = 40) أو ذراع التحكم (ن = 40). وكان متوسط مدة المضادات الحيوية 2.1 ± 6.3 يوم في الذراع الموجهة (بي.او.سي.تي) مقابل 4.7 ± 9.1 أيام في ذراع التحكم ($P = 0.001$)، في حين لم يكن هناك فرق كبير في وفيات لمدة 30 يوما. وبالتالي، خفضت التوجيه (بي.او.سي.تي) مدة المضادات الحيوية دون المساس الوفيات في المرضى.

APPROVAL PAGE

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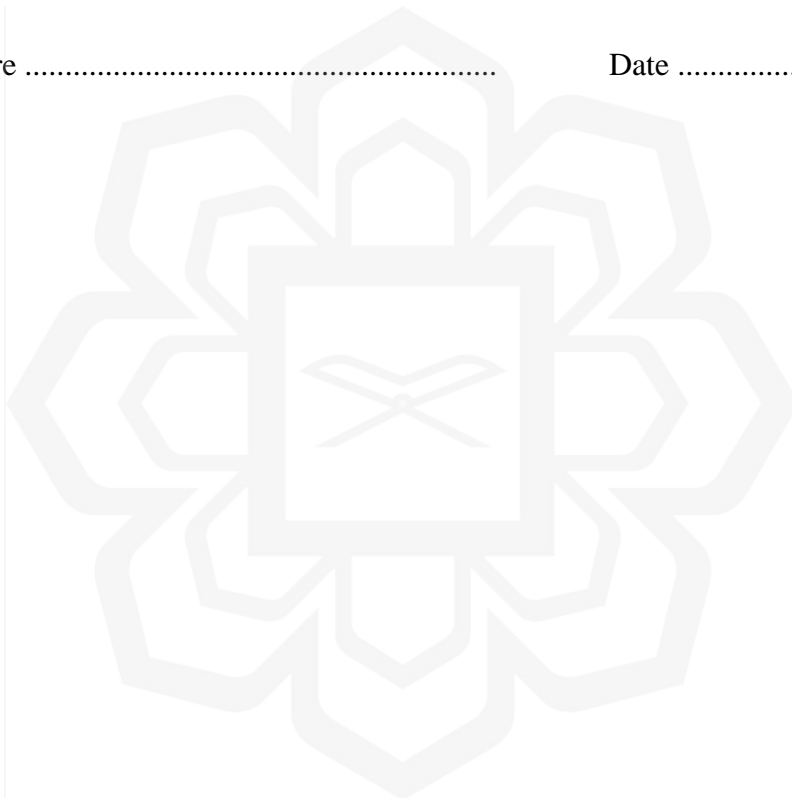
DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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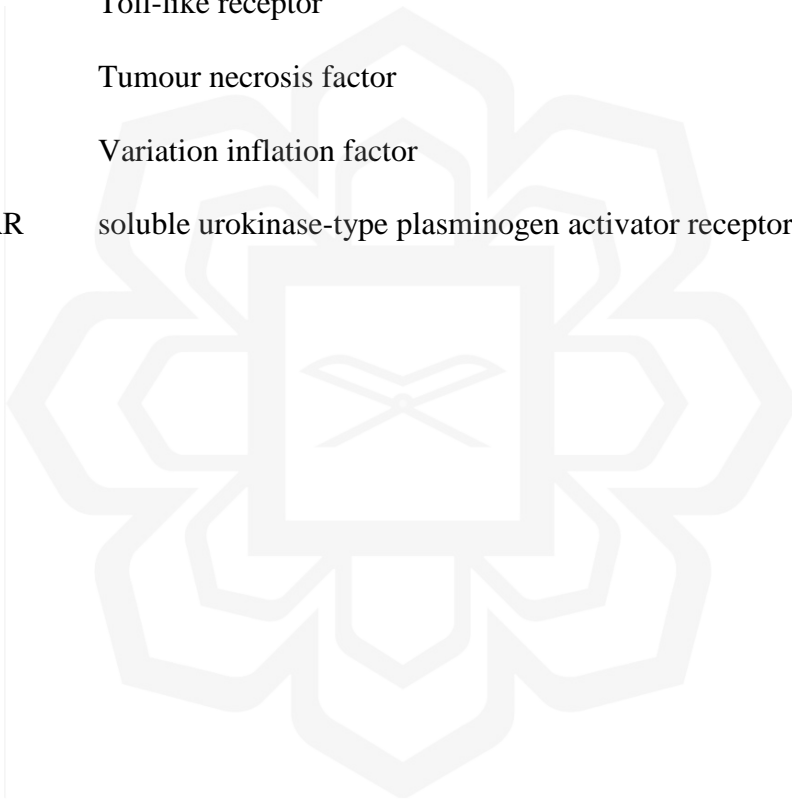
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LIST OF ABBREVIATIONS

ACCP	American College of Chest Physicians
AMR	Antimicrobial resistance
APACHE II	Acute Physiology and Chronic Health Evaluation II Score
AUC	Area under the curve
ARE	Arylesterase
BG	Blood glucose
cfNRI	Category-free net reclassification improvement
CI	Confidence interval
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
CV	Coefficient of variation
DM	Diabetes mellitus
ED	Emergency Department
EG	Endothelial glycocalyx
HTAA	Hospital Tengku Ampuan Afzan
HUSM	Hospital Universiti Sains Malaysia
ICING	Intensive Control of Insulin-Nutrition-Glucose
ICU	Intensive care unit
IDI	Integrated discrimination improvement
IUMMC	International Islamic University of Malaysia Medical Centre
IL	Interleukin
IP	1-specificity

IS	Integrated sensitivity
IREC	International Islamic University of Malaysia Research Ethics Committee
LMIC	Low- and middle-income countries
MDR	Multi-drug resistant
MYR	Malaysian Ringgit
NLR	Negative likelihood ratio
NMRR	National Medical Research Registry
NPV	Negative predictive value
NRI	Net reclassification improvement
OR	Odds ratio
POC	Point-of-care
POCT	Point-of-care procalcitonin
PON-1	Paraoxonase-1
PCT	Procalcitonin
PLR	Positive likelihood ratio
PPV	Positive predictive value
PRR	Pattern Recognition Receptor
RCT	Randomized-controlled trial
ROC	Receiver operating characteristics curve
RRT	Renal replacement therapy
SAPS II	Simplified Acute Physiology Score
SCCM	Society of Critical Care Medicine
SI	Insulin sensitivity

SD	Standard deviation
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment
SS	Sepsis Score
STAR	Stochastic Targeted Glycaemic Control
sTREM	soluble triggering receptor expressed on myeloid cells
TLC	Total leukocytes count
TLR	Toll-like receptor
TNF	Tumour necrosis factor
VIF	Variation inflation factor
v-SUPAR	soluble urokinase-type plasminogen activator receptor



CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Sepsis, a condition characterized by a dysregulated host response to infection, afflicts millions of people worldwide each year (Fleischmann et al., 2016). Multiple studies suggest that the incidence of sepsis is alarmingly increasing (Álvaro-Meca et al., 2018; Meyer et al., 2018). Furthermore, patients diagnosed with sepsis are estimated to have in-hospital mortality rate more than 10%, while in its most severe form i.e. septic shock, the mortality rate can reach more than 40% (Singer et al., 2016). Adverse outcome of sepsis is not limited to increase mortality risk. A recent study suggests that sepsis may worsen or result in new chronic diseases and leads to development of persistent cognitive and functional impairments among the survivors (Calsavara, Nobre, Barichello, & Teixeira, 2018). Not only sepsis is common and lethal, it is the single most expensive condition treated in hospitals (Paoli, Reynolds, Sinha, Gitlin, & Crouser, 2018). Additionally, sepsis is one of the most common reasons for intensive care unit (ICU) admission throughout the world and was the most common cause of death among critically ill patients in non-coronary ICUs (Perner et al., 2016). The burden of sepsis in low- and middle-income countries (LMICs) is even higher (Kwizera et al., 2018). In Malaysia, a country considered as upper middle-income, sepsis is among the leading cause of admission to the Ministry of Health ICUs. According to Malaysian Registry of Intensive Care, sepsis was the first leading cause of admission to the Ministry of Health ICUs in 2017, with mortality rate of 41.6% (Tai, Lim, Mohd Nor, Ismail & Wan Ismail, 2017). The magnitude of the problems of sepsis are summarized in Table 1.1.

Table 1.1 Notable information about sepsis

Notable information	Details
<i>High incidence</i>	An estimated of 31.5 million people are treated each year for sepsis (Fleischmann et al., 2016). The incidence is even higher in low- and middle-income countries.
<i>High mortality</i>	More than 10% for sepsis, and more than 40% for septic shock (Singer et al., 2016).
<i>High morbidity</i>	May worsen or result in new chronic diseases, and lead to development of persistent cognitive and functional impairments among the survivors (Calsavara et al., 2018).
<i>High cost to treat</i>	The most expensive condition treated in the United States (Paoli et al., 2018).
<i>Sepsis in ICU</i>	One of the most common reasons for ICU admission throughout the world and the most common cause of death among critically ill patients in non-coronary ICUs (Perner et al., 2016).

Note. ICU, intensive care unit

1.2 PROBLEM STATEMENT

Considering the magnitude of its problem, immediate treatment of sepsis is required, which first necessitates its timely and accurate diagnosis. Nevertheless, prompt diagnosis of sepsis in critical care has many challenges. Blood culture results are considered the most accepted tool to clinically diagnose infection, but this takes at least 24 to 48 hours to process (Lambregts, Bernards, van der Beek, Visser, & de Boer, 2019). Biomarker tests have been developed to facilitate early diagnosis of sepsis, but they still suffer some disadvantages. There are many sepsis biomarkers, but none has sufficient specificity or sensitivity to be routinely employed in clinical practice (Larsen & Petersen, 2017). Furthermore, a minimum lag time of typically two to three hours is still present (van Engelen, Wiersinga, Scicluna, & van der Poll, 2018), and biomarkers are generally expensive. Therefore, other markers must be

investigated to assist in making the timeliest, accurate, and cost-effective diagnosis of sepsis. Sepsis is known to have a negative effect on insulin sensitivity (SI). The SI profiles of a patient can be generated using a mathematical glucose-insulin system model i.e. model-based SI. However, to our knowledge, the performance of model-based SI as a diagnostic biomarker of sepsis has been under-explored.

The other mainstay in the management of sepsis is early recognition of which patients who are least likely to survive and thus benefit from aggressive treatment approaches. This outcome prediction in sepsis is currently done mostly via clinical scoring systems, such as the Sequential Organ Failure Assessment (SOFA) score (Vincent et al., 1996). However, clinical scoring systems were generated to assess severity of illness of general ICU patients and not primarily for sepsis patients. Concerning this limitation, biomarkers were proposed as useful tools for the prognostication of sepsis. A singular ideal biomarker has not yet been identified; an alternative approach is to shift research focus to a combination of several biomarkers to assess risk in sepsis. Nevertheless, the optimal multi-marker approach for outcome prediction in sepsis is yet to be determined.

Timely, appropriate and adequate antibiotic therapy is of paramount importance in sepsis. However, overly long course is undesirable because of side effects and increasing antimicrobial resistance (AMR) (Lomazzi, Moore, Johnson, Balasegaram, & Borisch, 2019). Therefore, specific biomarkers for resolution of sepsis might assist the ICU physicians in making decisions on antibiotic therapy on an individual basis. Several studies have shown that biomarker guidance using procalcitonin (PCT) can reduce the duration of antibiotic treatment, without compromising the safety outcome (Deliberato et al., 2013; Hohn et al., 2013; de Jong, 2016; Svoboda, Kantorová, Scheer, Radvanova, & Radvan, 2007). However, majority

of the studies were conducted in the setting of Western population. More importantly, all the studies utilised the standard laboratory method, which can be logistically and economically challenging with several hours or more of turnaround time. Point-of-care PCT (POCT) may overcome some of the problems related to the current existing technologies. To our knowledge, POCT detection as a tool to guide antibiotic discontinuation in the critically ill patients has not yet been evaluated in any clinical trials.

1.3 AIMS

The current thesis is divided into four sub-studies. The aim of the first study was to determine the capability of model-based SI to become a new biomarker for diagnosis of sepsis in the non-diabetic critically ill patients (Chapter Three). The second study was to assess the performance of the same biomarker but in an extended cohort of critically ill patients that included both the diabetic and non-diabetic patients (Chapter Four). The third study intended to investigate the prognostic value of a multi-marker approach for mortality prediction in critically ill patients with sepsis (Chapter Five). Finally, the last study was to evaluate the usefulness of POCT to guide the duration of antibiotic therapy in our local ICU (Chapter Six).

1.4 SIGNIFICANCE OF THE STUDY

The unmet clinical need of a reliable tool to rapidly identify sepsis could potentially be improved with evaluation of a new biomarker such as SI. This will have implications for clinicians in terms of timely diagnosis and potentially starting appropriate treatments. Early identification and treatment of sepsis with appropriate antibiotic has been shown to significantly reduce sepsis-related mortality (Liu et al.,

2017). The effective and early treatment of serious infections prevents progression to organ dysfunction or even septic shock and allows care to be provided at lower cost (Bochud, Bonten, Marchetti, & Calandra, 2004).

The difficulties in predicting the outcome of sepsis using the currently available tool can be aided by evaluation of a multi-marker panel. Outcome prediction tools in sepsis aim to assess the severity of the illness; and assign patients into different risk categories. This is of particular importance because patients at high risk may benefit from earlier clinical intervention, while low-risk patients may benefit from not undergoing unnecessary procedures. Thus, knowing where the patients reside on the spectrum of sepsis may lead to improved outcome.

Evaluation of POCT-guided antibiotic therapy will have implications for clinicians in terms of decision for antibiotic duration. Reduced duration of antibiotic administration has several advantages. First, it might contain the emergence of AMR in the ICU. Furthermore, reduced exposure to antibiotic therapy has been associated with a significant decrease in 28-day mortality, possibly the result of fewer side effects of antibiotic use (de Jong et al., 2016). Additionally, saving of antibiotic cost has been demonstrated in several studies (Deliberato et al., 2013; Schroeder et al., 2009). A lower medical cost is desirable to both hospital systems and the patients.

CHAPTER TWO

LITERATURE REVIEW

In Chapter One, it is apparent that managing sepsis in the ICU continues to pose challenges for the clinicians. The search for novel biomarkers that might better inform clinicians treating such patients are therefore sorely needed. This has been of great interest for research in sepsis and is also the focus of this thesis. Difficulty in identifying such markers is in part due to the complex heterogeneity of sepsis, resulting from the broad and vague definition of this condition based on numerous possible clinical signs and symptoms as well as an incomplete understanding of the underlying pathophysiology of this complex condition (Biron, Ayala, & Lomas-Neira, 2015). This chapter will begin by examining the definitions and pathophysiology of sepsis, then move to seeing the attempts that have been made so far in identifying biomarkers utility for sepsis management.

2.1 SEPSIS DEFINITIONS

The definition of sepsis has shifted over time. Prior to 1991, the physiological derangement characteristic of sepsis was referred by a variety of terms that were often used interchangeably, including “sepsis”, “septicaemia” and “septic syndrome”. In 1991, a conference was held by the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) to address the lack of consensus regarding the definition of sepsis and the difficulties this created in studies and treatment. This conference and its outcome are now referred to as Sepsis-1 (Bone et al., 1992). This was followed by Sepsis-2 (Levy et al., 2003) in 2001 and Sepsis-3 (Singer et al., 2016) in 2016.

2.1.1 Sepsis-1 Definitions

The ACCP and the SCCM convened in Chicago in 1991 and highlighted that sepsis was an ‘ongoing process’ (Bone et al., 1992). Systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock and multiple organ dysfunction syndrome began to be used in clinical practice. Sepsis was defined as the documentation of two or more SIRS criteria, in addition to known or suspected infection, while severe sepsis was defined as clinical sepsis accompanied by organ dysfunction, hypo-perfusion or hypotension. Septic shock is defined as a clinical display in which fluid-resistant hypotension is observed (Table 2.1).

2.1.2 Limitations of Sepsis-1 Definitions

Although the Sepsis-1 definitions consider the combination of infection and SIRS response as sepsis, a sepsis-like clinical picture may be observed without infection. The current significance of inflammation is non-specific and may manifest in many conditions. A good example of the sepsis-like statement is the hyperkinetic state after cardiac surgery without any infection which displays a very different prognosis and therapeutic approach from those of real sepsis. Moreover, sepsis is a complex interplay of pro- and anti-inflammatory responses and now evolves into two phases: hyper-inflammation and hypo-inflammation (Hotchkiss, Guillaume, & Didier, 2013). Therefore, the inflammation itself carries little meaning, because inflammation is a very non-specific response to any insult from minor trauma to complicated autoimmune disease.