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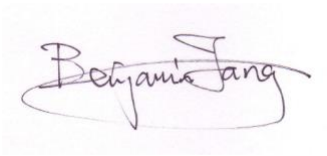
To Higher Degree by Research Administration Centre:

This report is concerning Velma Herwanto, a PhD candidate currently enrolled at the University of Sydney (student ID 460284686). I attest that changes to her thesis have been made to my satisfaction.

Yours sincerely,



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IMMUNOMETABOLISM OF CIRCULATING LEUKOCYTES IN PATIENTS WITH INFECTION AND SEPSIS

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy

FACULTY OF MEDICINE AND HEALTH

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PREFACE

Declaration

This thesis is submitted to the University of Sydney in fulfilment of requirements for the degree of Doctor of Philosophy. The work presented in this thesis is original except as acknowledged in the text. I, Velma Herwanto, hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Signature: Date: 29 July 2020

AUTHORSHIP ATTRIBUTION STATEMENT

In addition to the statements above, in cases where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author.

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Supervisor Name: A/Prof Benjamin Tang

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Ad maiorem Dei gloriam.

PUBLICATIONS AND PRESENTATIONS

Manuscripts

1. Herwanto V, Nalos M, McLean AS, Tang B. Immune dysfunction in sepsis: diagnosis and treatment options. ICU Management & Practice 2018;18(1):40-43 (included in Chapter 1). *I co-wrote the drafts of the MS.*

2. Tang BM, Herwanto V, McLean AS. Immune paralysis in sepsis: recent insights and future development. In: Annual Update in Intensive Care and Emergency Medicine 2018. 2018. p. 13-23 (included in Chapter 1). *I wrote the drafts of the MS.*

3. Herwanto V, Shetty A, Nalos M, Chakraborthy M, McLean A, Eslick GD, Tang B. Accuracy of quick Sequential Organ Failure Assessment score to predict sepsis mortality in 121 studies including 1,716,017 individuals: a systematic review and meta-analysis. Critical Care Explorations 2019;1:e0043 (refer to Appendix One). *I co-designed the study with BT, analysed the data and wrote the drafts of the MS.*

4. Herwanto V, Wang Y, Shojaei M, Khan A, Lai K, Shetty A, et al. Impaired peripheral blood mononuclear cell metabolism in patients at risk of developing sepsis: a cohort study. Submitted (included in Chapter 5). *I co-designed the study with YW, MS and BT, analysed the data and wrote the drafts of the MS.*

Posters and Presentations

1. Herwanto V, Wang Y, Shojaei M, Tang B, McLean AS. Reduced cellular respiration and ATP production in an in vitri model of sepsis. 38th International Symposium on Intensive Care and Emergency Medicine, Brussels, Belgium, March 2018. Poster presentation.

2. Herwanto V, Wang Y, Lai K, Shetty A, Shojaei M, Tang B, McLean AS, Booth DR. Metabolic profile of peripheral blood mononuclear cells in patients with low and high risk infections. Westmead Association Hospital Week Research Symposium, Westmead Hospital, Sydney, Australia, August 2018. Poster presentation.

3. Herwanto V, Wang Y, Shojaei M, Lai K, Shetty A, Tang B, McLean A, Booth DR. Metabolic profile of peripheral blood mononuclear cells in patients who are at risk of developing sepsis. The International Sepsis Forum's 12th Annual Symposium, Bangkok, Thailand, October 2018. Oral presentation, the Best International Abstract (refer to Appendix Two).

4. Herwanto V, Shetty A, Eslick GD, Tang B. Accuracy of qSOFA score to predict sepsis-related mortality in 99 studies consisting of 588,883 patients: a systematic review and meta-analysis. The International Sepsis Forum's 12th Annual Symposium, Bangkok, Thailand, October 2018. Poster presentation.

5. Herwanto V, Wang Y, Shojaei M, Shetty A, Lai K, Chew T, et al. Mitochondrial dysfunction and its related pathways in sepsis. Nepean Research Day, Sydney, Australia, September 2019. Oral presentation

6. Tang B, Wang Y, Herwanto V, Chew T. How to investigate host genomics in sepsis. Genomics, Sepsis & Intensive Care, Nepean Hospital, Sydney, Australia, October 2019. Oral presentation.

LIST OF ABBREVIATIONS

AAM	Alternatively activated macrophage
ADP	Adenosine diphosphate
APC	Antigen presenting cell
ATP	Adenosine triphosphate
Bcl-2	B-cell lymphoma 2
BSA	Bovine serum albumin
CAMs	Classically activated macrophage
CD	Cluster of differentiation
CI	Confidence interval
CRP	C-reactive protein
CTLA	Cytotoxic T lymphocyte-associated antigen
DC	Dendritic cell
DCFDA	2',7'-dichlorofluorescin diacetate
DEG	Differentially expressed gene
DMSO	Dimethyl sulfoxide
ECAR	Extracellular acidification rate
EDTA	Ethylenediaminetetracetic acid
ETC	Electron transport chain
FBS	Foetal bovine serum
FCCP	Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone
FDR	False discovery rate

GM-CSF	Granulocyte-macrophage colony-stimulating factor
GO	Gene ontology
HBSS/Ca/Mg	Hank's balanced salt solution supplemented with calcium and magnesium
HLA-DR	Human leukocyte antigen – DR isotype
ICU	Intensive care unit
IFN	Interferon
Ig	Immunoglobulin
IL-	Interleukin
IL-7R	Interleukin-7 receptor
LAG	Lymphocyte-activation gene
LPS	Lipopolysaccharide
MDSC	Myeloid-derived suppressor cell
MFI	Median fluorescence intensity
mRNA	Messenger ribonucleic acid
mROS	Mitochondrial reactive oxygen species
mTOR	Mammalian target of rapamycin
NETs	Neutrophil extracellular traps
NK cell	Natural killer cell
NO	Nitric oxide
OCR	Oxygen consumption rate
OXPHOS	Oxidative phosphorylation
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PCA	Principal component analysis

PD-1	Programmed death-1
PD-L1/2	Programmed death ligand-1/2
PI	Propidium iodide
PICS	Persistent inflammation, immunosuppression and catabolic syndrome
PMN	Polymorphonuclear
QC	Quality control
RNA	Ribonucleic acid
RNA-Seq	RNA sequencing
RNS	Reactive nitrogen species
RORγt	Retinoic acid receptor-related orphan receptor-γt
ROS	Reactive oxygen species
RPMI media	Roswell Park Memorial Institute media
SIRS	Systemic inflammatory response syndrome
SOFA score	Sequential organ failure assessment score
SRS	Sepsis response signature
T_H	T helper cell
T-bet	T-box transcription factor
Treg	Regulatory T cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
WGCNA	Weighted Gene Correlation Network Analysis

ABSTRACTS

Immune dysfunction is a major complication of sepsis. It increases susceptibility to nosocomial infection and contributes significantly to sepsis mortality. Immune dysfunction in sepsis has been associated with alterations in cellular metabolism which manifest as mitochondrial dysfunction and reduced cellular energy production. However, those alterations have been shown in established sepsis patients. Data are lacking in patients who are at early phase of infection who are yet to progress to sepsis. Our study aims to address this knowledge gap. Here, we present findings of a study that investigates metabolic alterations in the immune cells of infection patients. In particular, we compare the findings between those who develop sepsis with findings in those who did not develop sepsis (uncomplicated infection patients) to identify key pathologic mechanisms that underlie the progression from uncomplicated infection towards complicated infection (that is, sepsis).

First, in an *in vitro* model of sepsis, our preliminary experiment on peripheral blood mononuclear cells (PBMCs) indicated reduced mitochondrial respiration with increased intramitochondrial oxidative stress. Second, impaired mitochondrial respiration, with increased intramitochondrial oxidative stress, was observed in the PBMCs of patients with sepsis recruited from the emergency department. The level of oxidative stress significantly correlated with the severity of mitochondrial respiration impairment. Third, the findings of impaired mitochondrial respiration and increased intramitochondrial oxidative stress were also observed in patients with uncomplicated infection, albeit to a lesser intensity. Lastly,

further study on the PBMCs subsets, monocyte and T lymphocytes, corroborated the findings of metabolic alterations in sepsis as well as in uncomplicated infection patients.

Altogether, our study found that impaired mitochondrial respiration is detected in the immune cells of patients with uncomplicated infection, as it is in sepsis. Intramitochondrial oxidative stress is among several factors inducing the mitochondrial impairment, raising a possibility of its role as a potential target for preventing immune dysfunction.