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Ding, Zhiyi

2022

Document Version:
Publisher's PDF, also known as Version of record

Link to publication

Citation for published version (APA):
Ding, Z. (2022). *Neutrophil and endothelial cell-mediated inflammation in abdominal sepsis*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Malmö]. Lund University, Faculty of Medicine.

Total number of authors:
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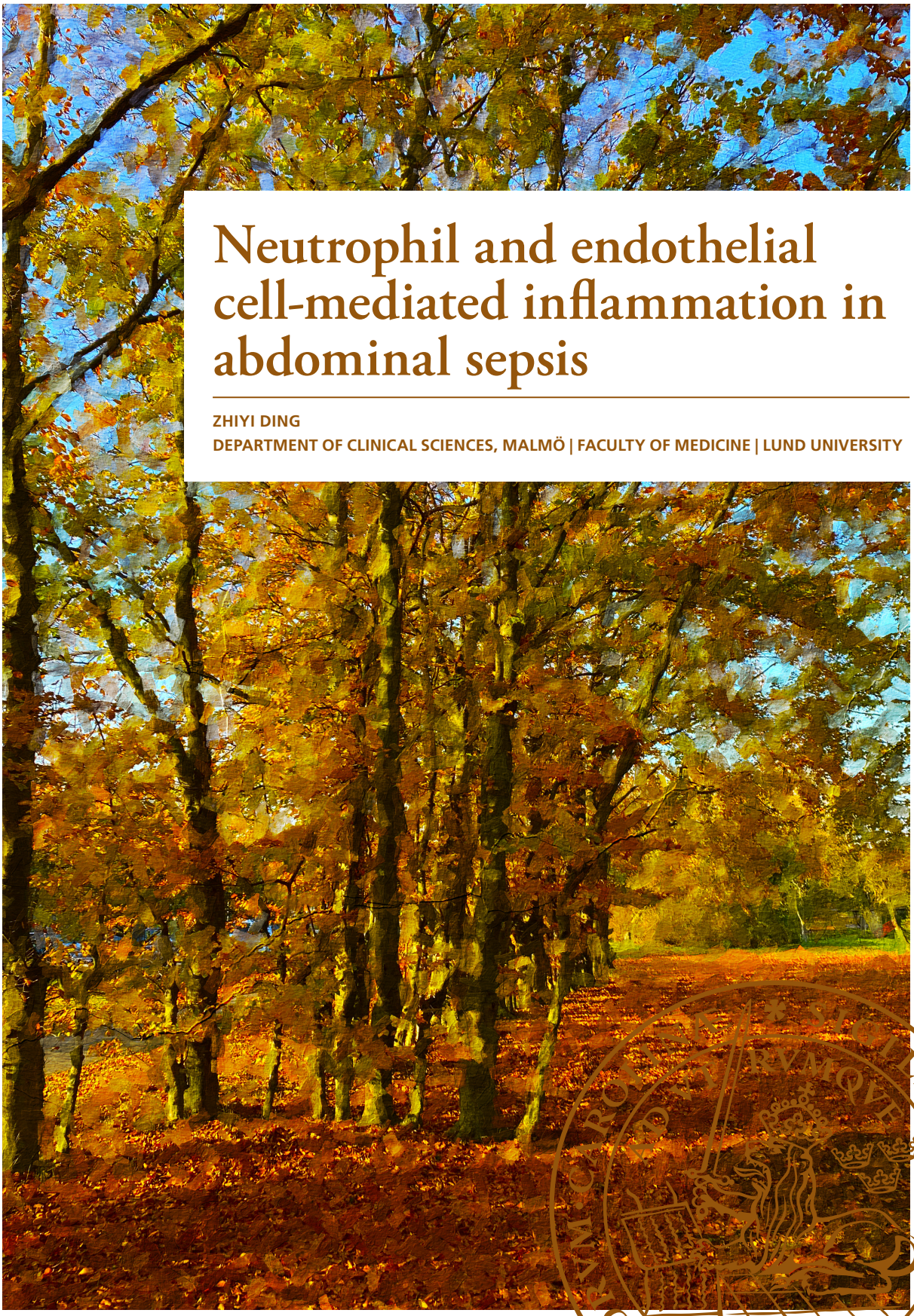

Neutrophil and endothelial cell-mediated inflammation in abdominal sepsis

ZHIYI DING
DEPARTMENT OF CLINICAL SCIENCES, MALMÖ | FACULTY OF MEDICINE | LUND UNIVERSITY

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Zhiyi Ding

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University
To be publicly defended via zoom on 28th of April, 2022 at 09:00
From Room 91-10-014, Department of Clinical Sciences
Jan Waldenströmgata35, Malmö, Sweden

Faculty opponent

Prof. Norbert Nemeth, MD, PhD, DSc

Department of Operative Techniques and Surgical Research
Faculty of Medicine, University of Debrecen
Debrecen, Hungary

Organization	Document name DOCTORAL DISSERTATION
LUND UNIVERSITY Faculty of Medicine Department of Clinical Sciences, Malmö	Date of issue 2022-03-23
	Sponsoring organization
Author(s) Zhiyi Ding	

Title and subtitle

Neutrophil and endothelial cell-mediated inflammation in abdominal sepsis

Abstract

Sepsis is defined as a life-threatening condition caused by a dysregulated host response to infection. Neutrophils are the most abundant innate immune cells of the body and play a key role in septic pathogenesis. During sepsis activated neutrophils release web-like traps decorated with various cellular proteins known as neutrophil extracellular traps (NET). The primary task of NET and NET-associated proteins are to kill pathogens; however, excessive accumulation of NET is known to cause tissue damage. Endothelial cells are important for regulating vascular permeability and barrier functions; however, during sepsis endothelial cells get activated and contribute to tissue damage and organ failure. The four original studies included in this thesis aimed to investigate new mechanisms involved in formation of NET, lung injury and pulmonary endothelial cell activation in abdominal sepsis. In study I, we have found that c-Abl kinase regulate NET formation through ROS signaling pathway. Blocking of c-Abl kinase not only inhibited NET formation but also reduced inflammation and tissue damage in sepsis. In study II, we investigated the role of actin-related protein 2/3 complex (Arp2/3 complex) and found that it regulates neutrophil trap expulsion both in vivo and in vitro. Inhibition of Arp2/3 complex not only reduced the neutrophil infiltration in bronchoalveolar space, but also alleviated lung damage in abdominal sepsis. In study III, we investigated the role of S100A9, a pro-inflammatory alarmin, in regulating inflammation and tissue damage in abdominal sepsis. Inhibition of S100A9 by a specific inhibitor, ABR-238901, decreased sepsis-induced neutrophil activation, cytokine formation as well as damage to the lung tissue. In study IV, we examined global transcriptomic changes in a subgroup of lung endothelial cells during sepsis. We found that sepsis caused transcriptomic changes of genes related to regulation of coagulation, vascular permeability as well as wound healing and lipid metabolic in capillary endothelial cells. In contrast, postcapillary venules were found to be more enriched with genes related to chemotaxis, cell-cell adhesion of integrins, chemokine biosynthesis, regulation of actin polymerization and neutrophil homeostasis after sepsis. Together, these results demonstrated that targeting c-Abl, Arp2/3 complex, S100A9 or endothelial functions could be useful targets to ameliorate neutrophil mediated tissue injury in sepsis.

Key words: Sepsis; lung; neutrophil; endothelial cell; neutrophil extracellular traps; inflammation; capillary; post capillary venules; cytoskeleton; RNAseq

Classification system and/or index termes (if any):

Supplementary bibliographical information:	Language English
ISSN and key title: 1652-8220 Lund University, Faculty of Medicine Doctoral Dissertation Series 2022:60	ISBN 978-91-8021-221-2

Recipient's notes	Number of pages 88	Price
	Security classification	

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Faculty of Medicine
Department of Clinical Sciences, Malmö

Lund University, Faculty of Medicine Doctoral Dissertation Series 2022:60
ISBN: 978-91-8021-221-2
ISSN: 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

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知其雄，守其雌，为天下溪
—《道德经》

Contents

Publications included in the thesis

This thesis is based on the following publications, referred to by their Roman numerals:

I Hawez A, **Ding Z**, Taha D, Madhi R, Rahman M, Thorlacius H. **c-Abl kinase regulates neutrophil extracellular trap formation and lung injury in abdominal sepsis.** *Lab Invest.* 2022 Mar;102(3):263-271.

II **Ding Z**, Du F, Rönnow C.F, Wang Y, Rahman M, Thorlacius H. **Actin-related protein 2/3 complex regulates neutrophil extracellular trap expulsion and lung damage in abdominal sepsis.** *Am J Physiol Lung Cell Mol Physiol.* 2022 Mar 10. In press.

III **Ding Z**, Du F, Averitt V R.G, Jakobsson G, Rönnow C.F, Rahman M, Schiopu A, Thorlacius H. **Targeting S100A9 reduces neutrophil recruitment, inflammation and lung damage in abdominal sepsis.** *Int J Mol Sci.* 2021 Nov 29;22(23):12923.

IV Rahman M*, **Ding** Z*, Rönnow C.F, Thorlacius H. **Transcriptomic analysis reveals differential expression of genes between lung capillary and post capillary venules in abdominal sepsis.** *Int J Mol Sci.* 2021 Sep 22;22(19):10181.

*Equal contributor

Abbreviations

ALI	Acute lung injury
ARDS	acute respiratory distress syndrome
Arp2/3 complex	actin related protein 2/3 complex
BALF	Bronchoalveolar lavage fluid
Bnip3	Bcl2 interacting protein 3
c-Abl	c-Abelson
capEC	capillary endothelial cells
CARS	compensatory anti-inflammatory response syndrome
CLP	Cecal ligation and puncture
CLR	C-type lectin receptor
CXCL-1	C-X-C motif chemokine ligand 1
CXCL-2	C-X-C motif chemokine ligand 2
DAMPs	damage-associated molecular patterns
DAVID	Database for Annotation, Visualization and Integration Discovery
DEGs	differentially expressed genes
EC	endothelial cell
ELISA	enzyme-linked immunosorbent assay
F-actin	filamentous actin
G-actin	globular actin
GO	Gene Ontology
GSEA	Gene Sets Enrichment Analysis
HMGB1	high mobility group box 1 protein
ICAM-1	intercellular adhesion molecule-1
ICU	intensive care unit
IL-6	interleukin 6
JAK/STAT	Janus kinase/signal transducer and activator of transcription
KEGG	Kyoto Encyclopedia of Genes and Genomes
LPS	lipopolysaccharide
MAP	mean arterial pressure
MAPK	mitogen-activated protein kinase
MFI	mean fluorescence intensity

MNL	mononuclear leukocyte
MODS	Multiple organ dysfunction syndrome
MPO	myeloperoxidase
MRP14	migration inhibitory factor-related protein 14
Naif1	nuclear apoptosis-inducing factor 1
NE	neutrophil elastase
NET	neutrophil extracellular trap
NF-κB	nuclear factor-kappa B
NLR	NOD-like receptor
PAD4	protein-arginine deiminase type 4
PAF	Platelet-activating factor
PAMP	pathogen associated molecular pattern
PBS	phosphate-buffered saline
PCA	principal component analysis
PCV	post capillary venules
PD-L1	ligand programmed death ligand-1
PMA	phorbol 12-myristate 13-acetate
PMNL	polymorphonuclear leukocyte
PRRs	pattern recognition receptors
qPCR	Real-time polymerase chain reaction
qSOFA	quick Sequential [Sepsis-related] Organ Failure Assessment
RES	running enrichment score
RLR	Retinoic acid-inducible gene (RIG)-I-like receptor
RNS	reactive nitrogen species
ROS	reactive oxygen species
S100A9	S100 calcium-binding protein A9
SOFA	Sequential [Sepsis-related] Organ Failure Assessment
TFs	Transcription factors
TLR	Toll like receptor
TNF-α	tumor necrosis factor α
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor

Introduction

Sepsis

Sepsis is a life-threatening medical condition caused by body's overwhelming immune response to infections. The beginning of using term 'sepsis'can be traced back to more than 2700 years ago [1]. The definition of sepsis is developing till now, the new definition of sepsis by 45th Critical Care Congress is known as 'Sepsis-3'. According to 'Sepsis -3', sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [2, 3, 4]. A previous study reported that there were 48.9 million cases of sepsis and 11.0 million sepsis-related death in 2017 in the world [5]. Although age-standardized cases of sepsis have went down, overall sepsis-related mortality rate remains stable during the recent decades [5, 6]. Thus, sepsis remained as a big burden for hospital and intensive care unit (ICU) throughout the world [7].

Sepsis is a heterogeneous disorder, the main etiological causes are various microbial infection [8]. It usually develops from severe infections [8]. The most common organ dysfunctions were renal, cardiovascular and respiratory. Depending on the culture conditions, sepsis can be divided into culture-negative sepsis and culture-positive sepsis. The two types of patients have different epidemiology, pathophysiology, and response to treatment, but both of them have similar risk factors for death and are recommended to be treated with broad-spectrum antibiotics as early as possible [9]. A recent study reported more clinical phenotypes of sepsis based on a vast array of symptoms and biological process [10]. The outcomes of sepsis also vary depending on the heterogeneity of patients' immune system, age, and intervention time-point [11]. Despite large numbers of sepsis studies, the pathophysiology of sepsis still remains unclear.

Clinical early detection and recognition of sepsis is crucial for improvement of disease outcomes and survival rate. However, unlike other life-threatening disease such as myocardial infarction, there is no rapid diagnostic methods available for identification of early phase of sepsis. In 2016, an efficient, simple and valid way called quick Sequential [Sepsis-related] Organ Failure Assessment (qSOFA) was proposed as a criterion for quick identification of the suspected sepsis or septic shock, which include three parameters: respiratory rate of 22/min or more, altered mentation, systolic blood pressure of 100 mm Hg or less (Table 1) [12]. Although various of pathophysiological alterations and intervention technics were

evaluated on clinical trials or animal experiments, the better outcomes still rely on immediate resuscitation and broad-spectrum antibiotics treatment [13]. This thesis studied several new mechanisms involved in sepsis and sepsis-induced lung injury in mice, and explore the potential therapeutic strategies to alleviate pathogenic inflammation and pulmonary tissue damage in abdominal sepsis.

Table 1: quick Sequential Organ Failure Assessment (qSOFA) score.

qSOFA Criteria	Points
Respiratory rate $\geq 22/\text{min}$	1
Altered mentation	1
Systolic blood pressure $\leq 100\text{mmHg}$	1

Pathogenesis and pathophysiology of sepsis

Environmental and genetic factors

There are various risk factors associated with sepsis. Social and economic factors are considered as important determinants. For instance, higher rates of sepsis and mortality were found in Africa and Asia [5], indicating significant regional differences based on socio-demographic index. Another study reported high risk of sepsis induced organ dysfunction in black population than white population, indicating racial difference [14]. Although nutritional status, smoking status, and alcohol consumption might account for the variations, involvement of genetic factors deserved more investigation.

One study in 1988 linked infections related death with genetic background [15], gene polymorphisms were reported to be responsible for different outcomes of sepsis [16, 17, 18, 19]. For example, the genetic variation of cytokines and cytokine receptors, especially receptor for tumor necrosis factor α (TNF-α), was linked to the death rate of septic patients [16]. In addition, nucleotide polymorphism in caspase-12 (which is a mediator of apoptosis) was found to be associated with impaired inflammatory and immune response to endotoxins, and contributing to the development of sepsis [20]. Together, the identification of environmental and genetic factors could be an effective way for the precision treatment of sepsis in the future.

Excessive inflammation

The inflammatory response usually supports the clearance of pathogenic microorganisms and tissue repair. However, during sepsis, a large number of activated inflammatory cells

and inflammatory mediators are released into the systemic circulation. The over-activated inflammatory cells next infiltrated into the tissues, release reactive oxygen species (ROS), lysosomal enzymes and inflammatory mediators, which further enhance the inflammatory cascades, leading to serious damage to the tissues and resulting in organ dysfunction. Despite significant studies tried to block inflammatory cascades of sepsis, most anti-inflammatory trials have failed in clinic [21, 22], implying an urgent need for discovering new mechanisms and treatment for sepsis.

There are various types of inflammatory mediators. Cytokines, such as TNF-α, interleukin 6 (IL-6), IL-8; chemokines, such as C-X-C motif chemokine ligand 1 (CXCL-1), C-X-C motif chemokine ligand 2 (CXCL-2) and high mobility group box 1 protein (HMGB1), were found up-regulated in blood and tissues during sepsis [23, 24]. Platelet-activating factor (PAF) is a phospholipid inflammatory mediator with well-known pro-inflammatory effects [25, 26]. Cell adhesion molecules are mainly involved in regulating interactions between endothelial cell (EC) and leukocytes. Sepsis is also associated with activation of complement system and overexpression of anaphylatoxins C3a and C5a [27]. The high levels of ROS and reactive nitrogen species (RNS) formation during sepsis are known to be harmful to the cells and organs [28]. In general, most of the over-produced inflammatory mediators are regulated by different cell signaling pathways, including nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways [29].

Immune response and immunosuppression

Because of uncontrolled exuberant inflammation during sepsis, the importance of understanding the initial innate immune response has grown significantly. The immune response during acute inflammation try to maintain a homeostasis between anti-inflammatory and pro-inflammatory responses. Innate immune cells get activated through pattern recognition receptors (PRRs), including NOD-like receptor (NLR), C-type lectin receptor (CLR), Retinoic acid-inducible gene (RIG)-I-like receptor (RLR), and particularly Toll like receptor (TLR). It has been reported that early TLR2/4 up-regulation plays an important role in the pathophysiology of sepsis [30]. Each PRR recognizes a distinct pathogen associated molecular pattern (PAMP) [31] for sensing and hosting initial defense against pathogens and infections [32]. Other evidence pointed out that some endogenous molecules called damage-associated molecular patterns (DAMPs) or alarmins, can also recognized by PRRs [33], such as S100 proteins [34] and HMGB1 [35]. Recently, levels of alarmins are reported to be highly associated with sepsis and designated as therapeutic targets in some inflammatory diseases [36, 37].

After initial pro-inflammatory response, a compensatory anti-inflammatory response syndrome (CARS) is induced [38], known as an immunosuppression stage of sepsis. The

immunosuppression was characterized by three phases, including the anergy, increasing secretion of anti-inflammatory cytokines, and immune cells death [39]. The shift from production of pro-inflammatory cytokines to anti-inflammatory cytokines impaired the chemotactic and recruitment effect of immune cells, resulting in the poor clearance of pathogens at the local inflammatory site. Moreover, lack of response to antigens and death of immune cells, such as lymphocytes, macrophages and neutrophils, cause an immunosuppressive disorder [40, 41, 42]. Multiple studies have shown strong evidence that immunosuppression disorder in fact contributes to huge number of deaths in sepsis [43, 44]. The understanding of immunosuppression suggests potential targets for improving the survival rate in sepsis.

Sepsis induced lung injury

Multiple organ dysfunction syndrome (MODS) is a major problem in sepsis and is a leading cause of death in sepsis [45]. Acute lung injury (ALI), also known as acute respiratory distress syndrome (ARDS), is the key component of MODS [46]. More than 40% mortality was reported on patients with ALI during septic shock [47], and lung is usually the first organ to be affected during ARDS [46]. The mechanisms of sepsis-induced lung injury is complicated and there is still no effective management strategy for sepsis, so it is important to study the basic mechanism of sepsis and develop new effective therapies against sepsis.

Pathologically, sepsis-induced lung injury might initiate from serval aspects. Pathogens can directly damage the pulmonary epithelial cells, or damage the microvascular endothelial cells [48], break the integrity of alveolar-capillary barrier (also known as blood-gas barrier), and increase the permeability of epithelial and endothelial layers [49]. In addition, large number of activated immune cells, particularly leukocytes, can migrate to the lung tissues and release abundant amount of anti-microbial products, such as cytokines, histones, elastase, and ROS, which might help with pathogens clearance but could also damage the lung tissues [50, 51]. Meanwhile, the barrier dysfunction and production of inflammatory mediators further enhance the generation of thrombin, formation of PAF and vascular endothelial growth factor (VEGF) in the microvascular vessels [52], destabilize the VE-cadherin, which is important for maintaining barrier integrity [53]. All these pathologic changes finally lead to severe abnormalities such as pulmonary edema, hypoxemia, and respiratory acidosis [54].

Treatment of sepsis

Sepsis is medical emergency and recommended to undergo immediate treatment and resuscitation [55]. Earlier diagnosis and identification of sepsis by screening and quick start of supportive therapies have been associated with lower mortality in recent studies [13, 56].

In clinic, Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score is the most important criteria to determine sepsis among ICU[57], but it requires clinical and laboratory tests which are difficult to obtain in time outside of the ICU, while the performance of qSOFA (Table 1) is similar as SOFA but without any need for blood tests [57], indicating that qSOFA is a better criteria to consider the possibility of sepsis in such situations. Thus, it is recommended that a SOFA score of 2 points or more could be the criteria for sepsis and qSOFA could be used in non-ICU settings to assess the suspected sepsis (Figure 1) [57]. The

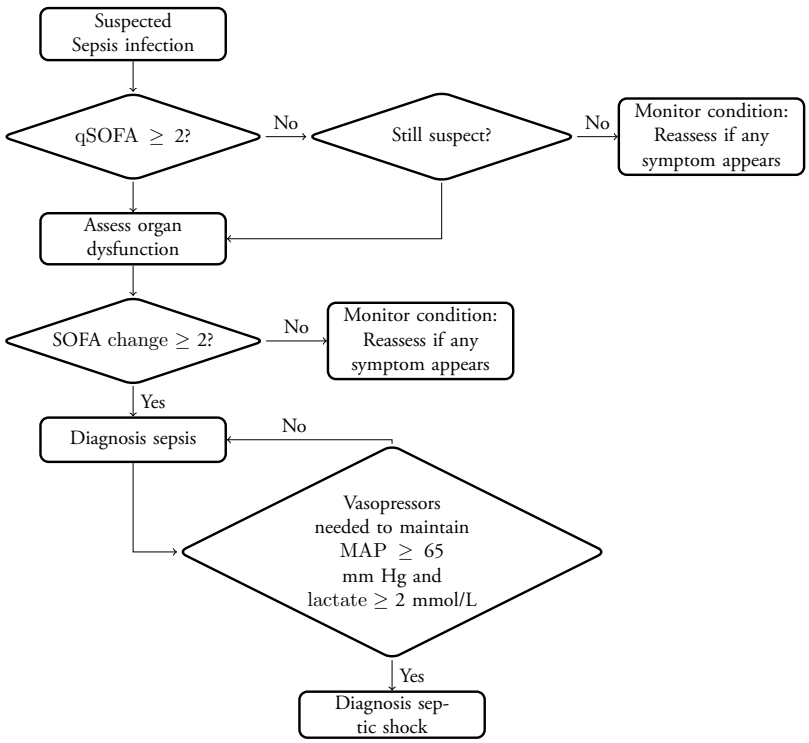

Figure 1: Clinical Diagnostic FlowChart of Sepsis and Septic Shock.

recommended treatment and management of sepsis can be approximately divided into initial resuscitation, administration of antibiotics, infection source control and other supportive therapies [55]. The core recommendation of implementation is the 'Hour-1 bundle', which encourages clinicians to start interventions in the first hour after recognition of sepsis [58], including measurement of lactate, obtaining blood cultures, administration of broad-spectrum antibiotics, rapid fluid resuscitation, and applying vasopressors if mean arterial pressure (MAP) $\geq$ 65 mm Hg [58].

Although the clinical treatment of sepsis is keeping updated, the options are restricted to management of the infection and support to the failing organ systems, most of the experimental therapeutic strategies are remained in laboratory and far away from showing

any clinical efficacy [59]. For example, recombinant human activated protein C had been proposed as an effective choice for severe sepsis in 2001 [60], but later was withdrawn from market because of no significant reduction of mortality [61]. Nowadays, precision medicine strategies begin to draw attention, more and more studies are focusing on specific targets. For instance, gene-based arrays and biomarkers identification promote clinical sub-classification of sepsis [10, 62] and raise the possibility of potential therapeutic target [63]. In addition, reversing sepsis-induced immunosuppression has been examined, for example, application of immune-stimulating cytokines interleukin-7 and interferon-γ have been shown to reduce mortality [64, 65]. Blocking apoptosis of lymphocytes by blocking immune checkpoints such as ligand programmed death ligand-1 (PD-L1) could not only reversed immune dysfunction but also improved the survival rate in animal sepsis models [66, 67, 68]. Moreover, studies which target on epigenetic modifications in sepsis also increase the choice as well. For example, blocking of micro-RNA-155 by antagomir has reported to reduce sepsis-induced inflammation and lung injury through reducing neutrophil recruitment and neutrophil mediated NETosis [69].

Neutrophil response in sepsis

Neutrophil is one type of the innate immune cells, and usually the earliest one to respond to local inflammation [70]. Normally, neutrophil is considered beneficial during inflammation, it identifies pathogens through TLR4, IgGFcR and C3bR/C4bR receptors [71]. Neutrophil cytoplasmic granules contain myeloperoxidase (MPO), phosphatase, calprotectin, lysozyme and defensin, which arm the neutrophil with the function of phagocytosis, also exert anti-microbial effect by oxidative or non-oxidative manner [72, 73]. Lysozyme is known to exert bacteriolytic effect [74], MPO is shown to kill bacteria [75], and calprotectin is required for anti-microbial defense [76]. However, in sepsis, neutrophil may harm the host by inducing excessive release of inflammatory mediators and free radicals [77].

Neutrophil recruitment

Neutrophil recruitment (Figure 2) is considered as a rate-limiting process in sepsis and septic lung injury [78, 79]. It is reported that lung is the predominant site for neutrophil trafficking [80], where lung microvessels, particularly post capillary venules, are the most common sites for the neutrophil transmigration [81]. Rolling is regulated by selectin family, including E-selectin and P-selectin, which are expressed by endothelial cells, and L-selectin is expressed by leukocytes. Selectins are usually overexpressed under inflammatory condition, and mediate neutrophil rolling by interacting with sialyl Lewis X [82, 83]. Firm adhesion then occurred by the interactions between integrin ligands and adhesion

molecules, termed as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [84], finally resulting in transmigration through the venular wall to the surrounded tissues. In addition, neutrophil expresses chemotactic receptors on its surface for recognition of chemokines, such as IL-8 and C5a, which guide neutrophils to the site of infection [84, 85]. The whole process is also regulated by actin polymerization and cytoskeletal rearrangement which facilitate neutrophil's functions [86, 87, 88].

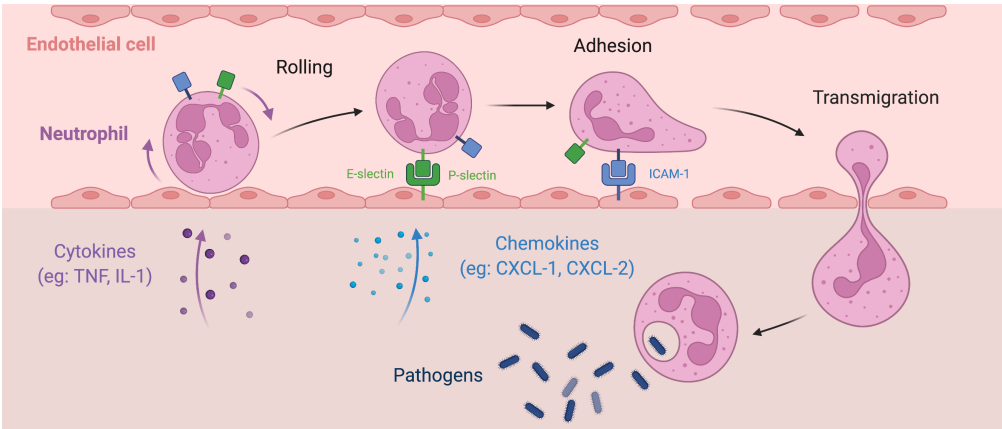

Figure 2: Typical neutrophil recruitment process from the vasculature to surrounded tissues. Cytokines and chemokines generated due to local inflammation can activate endothelial cells to express selectins, such as E-selectin and P-selectin. Selectins interact with their ligands on neutrophils, causing them to roll on the endothelium. Integrins regulate neutrophils adhesion through interaction with adhesion molecules, such as ICAM-1. Finally, neutrophils transmigrate to inflammatory sites, and then pathogens are killed by phagocytosis.

Neutrophil extracellular traps

The conception of neutrophil extracellular trap (NET) was first suggested in 2004 [89], which described a formation of extracellular web-like chromatin structure consists of nuclear and cytoplasmic proteins released by neutrophils. The formation of NETs has been considered as a novel mechanism of programmed cell death [90], termed as NETosis. Programmed neutrophil cell death was confirmed important for regulating the pathogenesis of sepsis. For instance, neutrophil pyroptosis was reported to be the major source of Il-1β production during sepsis[41], and neutrophil apoptosis was shown highly associated with the severity of sepsis [77]. The role of NETosis in mediating the pathogenesis of sepsis is not fully elucidated, but more and more evidence indicates the importance of NETosis in sepsis.

NETosis (Figure 3) can occur in both oxidative-dependent and independent way. In oxidative-dependent pathway, the NADPH oxidase enhances ROS formation, which promotes MPO to stimulate the translocation of neutrophil elastase (NE) [91], NE then binds with filamentous actin (F-actin) and initiates cytoskeleton degradation as well as membrane re-

arrangements, starting the disassembly of the nuclear envelope for translocation to the nucleus [92], further causing the chromatin de-condensation and final expulsion to the extracellular spaces. Another alternative mechanism of chromatin de-condensation is related to the protein-arginine deiminase type 4 (PAD4), which mediates histone citrullination to enable NE access to the chromatin structure [93]. Inhibition of NADPH oxidase was shown to reduce LPS-induced citrullination [94]. PAD4 knockout neutrophils failed to cause histone H3 citrullination and NET formation during ROS stimulation [95], suggest that ROS might function upstream of PAD4 during NET formation. PAD4-induced NETosis was reported as condition dependent, inhibition of PAD4 can block the nicotin-induced NETosis [96] but not cholesterol crystals-induced NETosis [97].

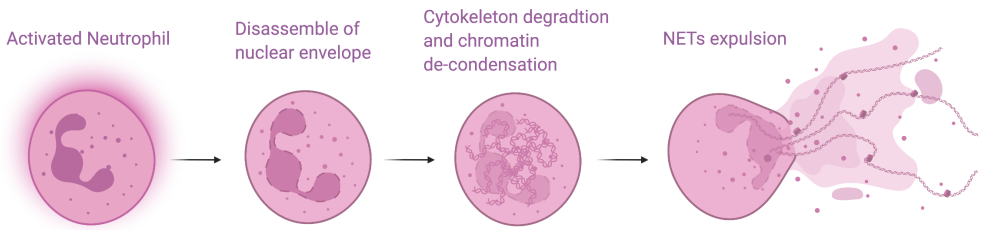

Figure 3: The process of NET formation, NETosis. After neutrophil activation, cells initiate the cytoskeleton degradation as well as membrane rearrangements. Next, the nuclear envelope disassembles, continues with loss of cellular polarization, chromatin de-condensation and plasma membrane rupture. The web-like chromatin structures are finally released into extracellular spaces.

In recent years, NET has been shown to act as a double-edged sword during the inflammatory responses. In some studies, NET was suggested important for phagocytosis of microbial pathogens [89, 98, 99]; on the other hand, NET was found to play a crucial role in disease pathophysiology, such as lung injury [100, 101], and autoimmune diseases, such as systemic lupus erythematosus [102, 103] and rheumatoid arthritis [104, 105]. The ability of NET to damage tissue is dependent on various aspects. NET induced pulmonary epithelial and endothelial cells damage in sepsis can be related to NET-bound proteins, for example, NET-bound histones [106] and defensins [107] was shown to permeabilize eukaryotic cells, NET-NE was reported to disrupt cell junctions [108], and NET-calprotectin was found to promote inflammation in endotoxin-induced shock [109]. Together, these imply that NET and NET-bound proteins may play a detrimental role in sepsis, effective clearance of NET and NET-associated proteins might be beneficial during inflammation progression.

Actin dynamics in neutrophil

Actin is a ubiquitous protein found in most eukaryotic cells and involves in lots of protein−protein interactions [110]. Nucleation and generation of new actin filaments are mostly regulated by actin binding proteins, such as thymosin $\beta 4$ [111], formin family

[112], and actin related protein 2/3 complex (Arp2/3 complex) [113]. Arp2/3 complex is composed of Arp2 and Arp3 and other five subunits. Normally, Arp2/3 complex maintains an inactive state because the Arp2 and Arp3 is too far to generated new filaments, but with the engagement of nucleation-promoting factors (NPFs), Arp2/3 complex acts as actin nucleation factors to initiate generation of new branches of actin filaments along with the existing mother actin filaments [113]. Generally, the regulation of Arp2/3 complex is considered as rate-limiting step during actin polymerization [114, 115].

Actin remodeling based cytoskeleton changes are involved in many biological functions of neutrophil, such as chemotaxis, migration, and secretion [116, 117, 118]. For example, perinuclear Arp2/3 complex was found to facilitate neutrophil migration by regulating nuclear deformation [119], RhoA/Rho kinase pathway-mediated actin polymerization was reported to be necessary for efficient neutrophil chemotaxis to C5a and IL-8 [120, 121]. Depletion of endogenous hematopoietic lineage cell-specific protein 1 (HS1) in the neutrophil was shown to impair the binding between HS1 and Arp2/3 complex, further impaired chemotaxis [122]. In addition, the interaction between neutrophil and endothelial cells during transmigration process is strongly dependent on actin dynamics of both cell types [123]. Furthermore, tyrosine kinase-meditated actin dynamics were shown as important step for neutrophil adhesion to the endothelium [124]. Mutation in ARPC1B, a subunit of Arp2/3 complex, was found to cause defective Arp2/3 complex-dependent actin filaments branching, leading to defective neutrophil transmigration [125].

In recent studies, degradation of actin cytoskeleton was verified during NET formation [92, 126]. In vitro experiments have revealed that cytochalasin D, a powerful inhibitor of F-actin polymerization, is an efficient inhibitor of neutrophil extracellular chromatin release [94, 127]. Actin polymerization is needed in the early phase (15-30 min) of stimulation to induce NET formation [92, 126], which enables the transportation of enzymes and granules through cytoplasm, such as translocation of NE to nucleus to initiate chromatin de-condensation [92]. As PAD4 plays a key role in NET formation, blockade of cytoskeletal changes has been related to the inhibition of PAD4 activity and histone citrullination [94]. The mechanism of how cytoskeletal signaling impacts PAD4 is elusive. One study has reported that the activation of PAD4 is due to the failure of actin-dependent neutrophil phagocytosis. When pathogens are too large to be phagocytosed by neutrophils, PAD4 got activated to initiate NET formation to support clearance of pathogens [98]. Alternatively, it has been shown that actin polymerization is important for activation of NADPH oxidase and ROS generation [128, 129]. The activation of $p40^{phox}$, which is one of the components of active oxidase [130], requires a two-step lipid-independent membrane binding event, followed by activation of the NADPH oxidase. The membrane binding activity has been reported to involve cytoskeletal interactions between F-actin and $p40^{phox}$ [128], indicating that F-actin polymerization plays an important role in initiation of NADPH oxidase induced ROS generation.

c-Abl kinase and neutrophil

c-Abelson (c-Abl) tyrosine kinase is a non-receptor tyrosine kinase, belongs to the Src family [131]. The C-terminal tail of c-Abl kinase contains DNA-binding domain [132] and actin binding domain [133, 134]. c-Abl is available abundantly in both nucleus and cytoplasm of different animal cells with different subcellular localization [131]. Nuclear-localization signal (NLS) is responsible for the localization of c-Abl in the nucleus, and the translocation of c-Abl to the cytoplasm is regulated by nuclear-export signal (NES). The unique distribution of c-Abl in multiple cellular compartments facilitate its ability to involve in various protein-protein interactions [135].

c-Abl participates in biological process through actin cytoskeleton modulation, phosphorylation, and participation in signaling pathways [136, 137, 138]. For example, F-actin localization in the cytoplasm requires the interaction between NES and C-terminal domain of c-Abl [131]; interaction with F-actin facilitate c-Abl to mediate filopodia formation, further influence the cell migration [136]. Moreover, c-Abl tyrosine phosphorylation is needed for regulation of its downstream targets which involve in cell migration [137], inhibition of c-Abl kinase activity markedly reduced the integrin-dependent neutrophil migration and polarization [139]. Similarly, c-Abl kinase phosphorylation activates Vav1, which is a hematopoietic cell protein and required for cell adhesion, polarization and migration [140] by regulating cytoskeletal reorganization [141] and β_2-integrin [138, 142].

In addition, c-Abl kinase plays important roles in RhoA/Rho signaling, NF-κB signaling, and ROS signaling. c-Abl kinase has been reported to regulate activity of small GTPase, which belongs to the Rho family [143, 144], and induce actin cytoskeletal rearrangements. Blocking of c-Abl has shown to decrease NFκB phosphorylation and nuclear translocation, resulting in decreased production of TNF-α, IL-8, and IL-1β in lipopolysaccharide (LPS)-induced ALI model [145]. ROS is known to cause c-Abl activation and nuclear translocation [146], inhibition of c-Abl activity and expression by activation of protein kinase GI has been reported to increase the antioxidant proteins, and help attenuate oxidant-induced tissue damage [146].

S100A9 and neutrophil

S100 calcium-binding protein A9 (S100A9) (also known as migration inhibitory factor-related protein 14 (MRP14)), is involved in many different inflammatory processes and diseases [147, 148, 149]. Its expression varies from nucleus to cytoplasm and plasma membrane for different functional purposes [150]. Together with S100A8, it forms a heterodimer termed as calprotectin. S100A9 was reported to composed 40% of cytoplasmic proteins in neutrophils [151, 152], and is also expressed in monocytes, dendritic cells, and platelets, but at much lower concentration [151, 153, 154].

Since S100A9 release is largely associated with neutrophil, and neutrophil is essential for regulating inflammation and tissue damage in sepsis [78, 155, 156], the functional role of S100A9 in sepsis has driven some attention in recent time. S100A9 is reported to exert important role in inflammation by regulating leukocytes recruitment and cytokines secretion [157, 158]. Besides, S100A9 is also shown to release form elongated neutrophil-derived structures [159], which formed in the vessel lumen and do not contain mitochondria, endoplasmic reticulum, or DNA. The degradation of this structure can cause release of S100A9 and calprotectin in LPS-injected mice and septic patients [159]. In addition, one study suggested that the release of S100A9 is correlated with NET formation, and 30% of the total S100A9 is bound with NET [76], suggesting that S100A9 is an important pro-inflammatory component of NET [160, 161]. Furthermore, accumulating studies have revealed that S100A9 acts as a member of DAMPs and binds with TLR4 and RAGE receptors [109, 162], which are typical PRRss involved in NFκB pathway during inflammation.

In clinics, S100A9 and its heterodimer have been shown to involve in the pathogenesis of different diseases [37, 163, 164], as well as sepsis [165]. Emerging evidence demonstrated that blocking of S100A9 or treatment with anti-S100A9 antibody could be an effective therapeutic approach for the treatment of cardiac inflammation and cancer [37, 163, 164].

Endothelial cell response in sepsis

Perspectives on pathogenesis and pathophysiology of sepsis are traditionally focused on neutrophil-mediated tissue damage; nevertheless, formation of edema in the critical organs of the body due to increased vascular permeability is also identified as a major problem in sepsis [166]. Moreover, endothelial cell dysfunction has been considered as a central event in organ failure, which occurred due to high ROS formation, intercellular junctions damage, leukocyte transmigration, and activation of the coagulation cascade [167]. Notably, pulmonary endothelial barrier dysregulation plays a crucial role in sepsis-induced lung injury [168], as a result to fluid accumulation and vascular leakage [169]. Endothelial dysfunction is one of the most important early indicators of sepsis [170], and a consensus of recovery from septic shock is the reduction of edema and reparation of vascular integrity [166].

Heterogeneity of endothelial cell in microvasculature

Typical microvasculature is composed of terminal arterioles, metarterioles, capillaries, and post capillary venules [171], which support the functions of microcirculation (Figure 4). Microvasculature can be found in major organs, such as brain, liver, kidney and lung. Each

segment of microvessel perform specific functions in specific organ, which can be explained by the structural and functional heterogeneity of endothelial cells in different organs [172]. For example, endothelial cells of the pulmonary arteries were reported to be broader and shorter with a rectangular shape, while endothelial cells of the pulmonary veins were large and round in shape [173]. Moreover, the junctional organization of the endothelial cells along the vascular is organ specific [174, 175]. In the brain, tight junctions are more enriched for strictly controlling permeability and protecting tissues from fluctuations of blood composition to maintain the blood-brain barrier [176], but poorly organized tight junctions are found in the post capillary venules to facilitate the inflammation-induced leukocytes transmigration and fluid exudation [174].

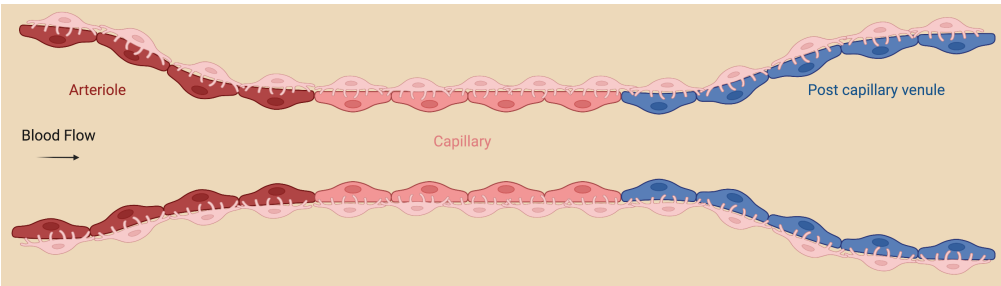

Figure 4: Microvascular bed structure is composed of arteriole, capillary, and post capillary venule.

In addition, it is assumed that the structural variations of pulmonary microvasculature are for different functions. For example, in the lung, post capillary venules are known to be the preferred site for neutrophil trafficking and transmigration during inflammation [81]. Moreover, platelets can also roll on activated post capillary venular endothelium [177]. In contrast to post capillary venules functions, capillaries are the main exchange vessels and responsible for maintaining the blood-gas barrier during pulmonary circulation in the lung [178]. Although one recent study reported that pulmonary capillaries could be involve in neutrophil transmigration [179], capillaries are mainly responsible for slowing blood flow [180] and expressing of angiotensin I [181] to regulate blood pressure in the pulmonary circulation.

Endothelial cell dysfunction in sepsis

Endothelial cells line the interior surface of blood vessels, regulating a series of physiological functions, forming a dynamic barrier between the blood and surrounding tissues, establishing a balance of fluid and substances exchange [182]. Endothelial cells are connected by junction-related proteins, including adherent junctions, tight junctions, and gap junctions [183]. The luminal surface of endothelium is covered by a layer of glycocalyx [184].

The pathophysiological events of endothelial cells dysfunction during sepsis can be de-

scribed on endothelial cell activation, induction of coagulation, leukocyte recruitment and transmigration, disruption of endothelial junctions and endothelial cell death [185]. Endothelial cell activation occurs due to simulation by cytokines, DAMPs, PAF, and ROS, which are produced in response to innate immune defense [186]. After activation, the glycocalyx layer of endothelium get degraded, resulting in the exposure of endothelium for further leukocyte adhesion [187]. Activated endothelial cells express E-selectin, P-selectin, ICAM-1 and VCAM-1 [186], enhancing the interaction between the endothelium and leukocytes. Rolling leucocytes adhere to the endothelium, causing continuous remodeling of intercellular junctions by releasing different type of enzymes and proteins, finally increased permeability and decreased endothelial barrier integrity [188]. In the meantime, endothelial cells release pro-thrombotic glycoproteins, such as tissue factor [189] and von Willebrand factor [190], which initiate the coagulation cascade, activate the production of microvascular thrombosis [191], and promote the recruitment of leukocytes-platelets conjugates in the microvascular environment [190]. Moreover, recruited leukocytes continuously produce inflammatory mediators as well as release NET, also disrupt the cytoskeleton and intercellular junctions of endothelial cells, resulting in the death of endothelial cells and impaired barrier function [106, 192].

Aims

The specific aims of this thesis are:

I. Investigate the role of c-Abl kinase in neutrophil extracellular trap formation and lung injury in abdominal sepsis.

II. Investigate the role of arp2/3 complex in neutrophil extracellular trap formation and septic lung injury.

III. Investigate the role of S100A9 in abdominal sepsis.

IV. Investigate transcriptional changes of lung capillary endothelial cells and post capillary venules in abdominal sepsis.

Material & Method

Animals

All experimental methods were carried out in accordance with the guidelines of Regional Ethics Committee for Animal Experimentation (Permit number: 5.8.18-08769/2019) at Lund University. Male C57BL/6 mice aged 8-9 weeks (weight 20-25 g) were kept in a 12-hour standardized light–dark cycle at 22°C, fed a laboratory diet, and given free access to water. An intraperitoneal (i.p.) injection of 75 mg/kg ketamine hydrochloride (Hoffman-La Roche, Basel, Switzerland) and 25 mg/kg xylazine was used to anesthetize the animals (Janssen Pharmaceutica, Beerse, Belgium). Subcutaneous injections of buprenorphine hydrochloride (0.5 mg/kg; Schering-Plough, Berkeley Heights, NJ) was done to give relieve from pain. In all in vivo experiments, mice were randomly assigned to various groups. For all animal research, the ARRIVE criteria were followed [193].

Animal model of sepsis

Cecal ligation and puncture (CLP) was used to induce abdominal sepsis in mice. In order to expose the cecum, the animals were sedated and a midline incision in the abdomen wall was made. The cecum was filled with feces from the ascending colon, 75% of the cecum was ligated with 5-0 silk suture, soaked using phosphate-buffered saline (PBS) (Qiagen, Hilden, Germany), and punctured twice with a 21-gauge needle. A small amount of feces was gently pulled out of the cecum from the perforated locations. Following that, the cecum was returned to the peritoneal cavity and the abdominal incision was sutured. The cecum was neither ligated nor perforated in sham mice, but they received the same laparotomy and resuscitation protocols.

Bacterial culture

Blood and lung tissues were harvested 24 h after sham or CLP procedure. Lung tissues were weighted and homogenized by a tissue lyser (TissueLyser II, Qiagen, Hilden, Germany) aseptically. After 10 times dilution, blood and homogenized lung tissues were plated on Blood Agar (TSA with Sheep Blood) Medium plates (PB5012A, ThermoFisher Scientific, Waltham, Massachusetts, USA) and incubated for 24 hours at 37°C. Colony-forming unit

(CFU) /ml blood and CFU/g lung tissue were used to count and evaluate the number of bacterial colonies in the blood and lung, respectively.

Bronchoalveolar lavage fluid

The trachea was exposed after putting the animals in sleep by anesthesia, and a PE50 catheter was inserted and sutured into the trachea. Bronchoalveolar lavage fluid (BALF) was obtained by washing it five times with 0.8 ml cold PBS containing 0.5 mM EDTA. BALF was centrifuged at 1400 rpm for 5 minutes, the supernatant was collected for DNA-Histone complex assay and the pellet was resuspended in 200 µL PBS for leukocytes counting. Leukocytes were counted in a Burker chamber and classified as mononuclear leukocyte (MNL) or polymorphonuclear leukocyte (PMNL).

Histology

Lung samples were fixed in 4.0% formaldehyde for 24-48 hours at 4°C before being dehydrated with ethanol. Tissues were fixed in paraffin, then sliced into 5mm-thick sections and stained with hematoxylin and eosin. The histological evaluation was done in a double-blinded way by using a pre-determined scoring system. Briefly, four parameters were evaluated, including extent of alveolar gaps, the thickness of alveolar septa, alveolar hemorrhage, and neutrophil infiltration. Each parameter was given a score ranging from 0 (absent) to 4 (extensive).

MPO Activity

Lung tissues were weighed and homogenized in 1 ml of PBS by TissueLyser II and centrifuge at 14000 rpm for 10min. Pellets were resuspended in 0.2 M phosphate buffer (PB) pH7.4, then centrifuged, the pellets were again suspended in 1 ml of 0.5% hexadecyltrimethylammonium bromide buffer (HTAB). The samples were frozen overnight, thawed, sonicated for 90 seconds and put for water bath 2 h at 60°C. MPO activity of the samples was evaluated spectrophotometrically by measuring the change in absorbance in the redox reaction of H_2O_2 (450 nm, with a reference filter of 540 nm, 25 C). MPO units per gram of tissue are used to interpret the results.

Lung edema

The left lung was collected, with all extrapulmonary tissues being removed, and then gently dried using blotting paper before the wet weight was measured. Each lung sample was

placed in a dish and dried at 60°C for 72 hours before being weighed as dry weight. Lung edema was measured as a ratio of wet to dry weight (wet/dry).

Transmission and scanning electron microscope

Deparaffinized lung tissues were fixed in 2.5% glutaraldehyde in 0.15 M/L sodium cacodylate, pH 7.4, for 30 minutes at room temperature (cacodylate buffer). After fixation, samples were rinsed with cacodylate buffer and dehydrated using an ethanol concentration series ranging from 50.0% (vol/vol) to absolute ethanol (10 min/step). After that, the samples were dried at critical point in carbon dioxide with 100.0% ethanol as an intermediate solvent. Mounting was done with aluminum holders, and the samples were then sputtered with 20 nm palladium/gold. JEOL 1400 PLUS transmission electron microscope and HITACHI SU3500 scanning electron microscope were used for examining the samples at Microscopy Facility at Department of Biology, Lund University. In an ultramicrotome, the coverslips were embedded in Epon 812 and cut into 50 nm-thick ultra-thin sections using a diamond knife. Sections were incubated with primary antibodies against elastase (ab68672, 10 g/ml, Abcam, Cambridge, UK) and citrullinated histone 3 (ab5103, 10 g/ml, Abcam, Cambridge, UK) overnight at 4°C. The grids were then treated with gold-conjugated secondary antibodies specific to the species. After that, the sections were post-fixed in 2.0% glutaraldehyde and dyed with 2.0% uranyl acetate and lead citrate. At high magnification, characteristic web-like fibrillar NET structures were identified, which were later validated using TEM/gold-labelled immunostaining for NET components. Adobe Photoshop CS5 was used to evaluate the NET regions. Briefly, the Ruler Tool was used to calculate the number of pixels per square micrometer, and the Magic Wand Selection Tool was used to convert NET areas to pixel numbers. Finally, the fractions of NET areas relative to the total area of a given electron micrograph was calculated.

Enzyme-linked immunosorbent assay

TissueLyser II was used for lung tissues homogenization. Blood was collected from the inferior vena cava with acid citrate dextrose, centrifuged 2000 g for 10 min at room temperature, and the plasma was collected. The levels of neutrophil chemoattractant, CXCL-1, CXCL-2 and systemic inflammation and sepsis severity indicator, IL-6 in lung and plasma were determined by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Europe, Abingdon, Oxon, UK) according to the manufacturers'instructions. BALF and plasma levels of DNA–histone complexes were measured by a Cell Death Detection ELISA kit (11544675001, Roche Diagnostics, Mannheim, Germany).

Systemic leukocytes differential count

Blood was taken from the tail vein of mice and mixed immediately with Turks solution (1:20 dilution). Leukocytes were counted in a Burker chamber and classified as MNL or PMNL.

NET formation in vitro

Bone marrow neutrophils were isolated from the femur and tibia of C57BL/6 mice. The femurs and tibias of mice were removed, and the bone marrow was flushed three times with ice cold DMEM media to collect the bone marrow cells. After that, the bone marrow cells were added to 4 ml Ficoll-Paque and centrifuged at 400 g for 30 minutes without break. The pellets were collected after centrifugation and resuspended in 2 ml ACK for 5 minutes to lyse red blood cells, then centrifuged for 5 minutes at 1400 rpm. Finally, the bone marrow neutrophils were collected and resuspended in 1 ml DMEM medium and filtered by a 40 μm filter for further experiments. Freshly isolated bone marrow neutrophils (1 million per well) were stimulated with phorbol 12-myristate 13-acetate (PMA) (P1585, Sigma-Aldrich, Stockholm, Sweden) at a concentration of 100 nM for 3 hours at 37°C to generate NET.

Flow cytometry and cell sorting

For flow cytometry, blood was harvested from the inferior vena cava of septic and control animals, cells were blocked with anti-CD16/CD32 (1:200) (553124, BD Bioscience, San Diego, CA, USA) to block FcγRIII/FcγRII to reduce nonspecific binding, then stained with FITC-conjugated anti-CD11b (553310, BD Biosciences, San Diego, CA, USA) and APC-conjugated anti-Ly6G (127614, Biolegend, London, UK) for Mac-1 expression, or stained with PE-conjugated anti-Ly6G (1:200), FITC-conjugated anti-myeloperoxidase (1:200) (ab90812, Abcam, Amsterdam, The Netherlands) and anti-Histone H3 (citrulline R2 + R8 + R17) (ab5103, Abcam, Amsterdam, The Netherlands) as primary antibodies overnight (4 °C) and APC-conjugated anti-rabbit (IgG, goat, 1:400) (A-10931, ThermoFisher Scientific, Waltham, Massachusetts, USA) as secondary antibody for detection of NET components on the surface of circulatory neutrophils. In in vitro experiments, freshly isolated bone marrow neutrophils were blocked in the same way and stained with PE-conjugated anti-Ly6G antibodies, dihyrorhodamine 123 (10 μM) (DHR-123, 85100, Cayman Chemical, USA) for ROS detection. For F-actin detection, cells were labeling with Alexa Fluor 647 phalloidin (1:400) (A-22287, ThermoFisher Scientific, Waltham, Massachusetts, USA) and PE-conjugated anti-Ly6G (1:200) (128017, Biolegend, London, UK). For endothelial subsets sorting, lung tissues from mice were digested to

single cell suspensions. Hematolymphoid cells and epithelial cells were depleted with anti-CD45 and anti-CD326 micro-beads (Miltenyi Biotech, Bergisch Gladbach, Germany) from the cell suspensions. Anti-CD31 was used as the primary defining EC marker and anti-gp38 to distinguish between lymphatic ($gp38^{+}CD31^{+}$) and blood ($gp38^{-}CD31^{+}$) ECs. Hematolymphoid, epithelial, stromal, and dead cells were excluded from the analysis using a combination of lineage markers (anti-CD45, -CD11a, -TER119, -EpCAM) and 7-AAD. $CD31^{+}Icam1^{+}Vcam1^{-}$ EC was identified as capillary endothelial cell subset, while $CD31^{+}Icam1^{+}Vcam1^{+}$ EC was identified as post capillary venule endothelial cell subset.

Confocal imaging

Freshly isolated bone marrow neutrophils (1 million per well) were seeded on glass cover slips in 24-well plates, and NET formation was induced as mentioned above. For NET visualization, cells were fixed with 4% formaldehyde at various time points. The cells were then permeabilized for 5 minutes at 4 °C with a 0.1% Triton X-100 solution, washed, and blocked with 5% BSA. After blocking with anti-CD16/CD32 (1:200) for 5 minutes, cells were stained with FITC-conjugated anti-myeloperoxidase antibody (1:200) and anti-Histone H3 antibody (1:200) as primary antibodies overnight (4°C) and visualized with APC-conjugated anti-rabbit (1:400) as secondary antibody. For SiR-Actin staining, cells were not permeabilized and were stained immediately with 2.5 µM SiR-Actin (SC001, Spirochrome AG/Tebu-bio) after washing with PBS. After that, Hoechst 33342 was used to stain nuclear DNA (1:2500). Prolong Diamond Antifade Mountant (P-36965, ThermoFisher Scientific, Waltham, Massachusetts, USA) was used to mount the cover slips on the microscope slide. Samples were photographed with an LSM 800 confocal microscope after they were dried completely (Carl Zeiss, Jena, Germany). The pinhole was set ∼1 airy unit and the scanning frame was 1024×1024 pixels. A Z-stack setting was used to collect the 3D data. All Z-stack slices (∼10 µm) were processed with ZEN light 3.1 (blue edition) software (Carl Zeiss, Jena, Germany) to create a 2D orthogonal projection.

Western Blot

Isolated neutrophils were lysed in RIPA buffer which contained proteinase inhibitor (Complete mini proteinase inhibitor cocktail, Roche). The insoluble cell debris or lipid fraction was removed by centrifugation. Protein concentration was measured by BCA protein assay kit (Thermo Fisher Scientific). The samples were then boiled in SDS sample buffer at 95°C for 5min, and loaded into 8%−16% stain-free gel (Bio-Rad), transferred to PVDF membrane (Millipore, Bio-Rad), and analyzed by immunoblotting. Antibody against c-Abl kinase (2862, 1:1000, Cell signaling technology, Danvers, USA) and anti-phosphotyrosine

antibody 4G10®Platinum (16-452, 1:1000, Merckmillipore, Darmstadt, Germany) were used as primary antibodies. For detection of phosphor-c-Abl kinase, membranes were first treated with HRP-conjugated anti-biotin secondary antibody (7075P5, 1:1000, Cell Signaling, Leiden, Netherlands). The same membranes were stripped first and then treated with an anti-rabbit HRP-conjugated secondary antibody to detect total c-Abl kinase (7074, Cell Signaling, Leiden, Netherlands). Signal was detected using the ECL system (Bio-Rad) according to the manufacturer's instructions.

RNA sequencing and data analysis

Total RNA was extracted from the sorted cells with RNeasy Plus Micro kit (Qiagen) and the quality of RNA was evaluated by Bioanalyzer RNA 6000 pico assay (Agilent, California, USA). RNA sequencing was done at the Center for Translational Genomics (CTG) at Lund University and Clinical Genomics Lund, SciLifeLab. Briefly, the sequence library was created using the SMARTer®Stranded Total RNA-Seq Kit v2-Pico Input Mammalian (634411, Takara Bio USA, Inc.). The double-stranded cDNA was sequenced on NextSeq 500 (SY-415-1001, Illumina) using the (read one-index reads-read two, bp): 75-8-8-75 configuration. Bcl2fastq2-hisat2-StringTie-DESeq2 pipeline was used to analysis the data, the reference genome sequence was from the Ensemble database, the Mouse 38, and the annotation (GTF) was from the release 93. Variables with more than three samples having zero counts were filtered out before analysis. The Gene Ontology (GO) biological process terms were based on biological process of Molecular Signatures Database (v7.2 MSigDB), the Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed using the top 500 DEGs from capillary endothelial cells and post capillary venule endothelial cells in Database for Annotation, Visualization and Integration Discovery (DAVID) (version 6.7; http://david.abcc.ncifcrf.gov), the Gene Sets Enrichment Analysis (GSEA) was done by Qlucore Omics Explorer software version 3.6.

RT-qPCR

Total RNA was isolated from freshly obtained lung tissue using TRIzol (Invitrogen, Thermo Fisher Scientific, Inc.) and purified using the Direct-zol RNA extraction kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's guidelines for RNA sequencing data validation. The RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific™, Milford, USA) was used to make cDNA from 0.5 µg total RNA. Real-time polymerase chain reaction (qPCR) was performed using TB Green Advantage qPCR Premix (Clontech, Mountain View, CA, USA) in the MX 3000P detection system (Stratagene, AH diagnostics, Stockholm, Sweden). The $2^{-\Delta\Delta CT}$ approach was used to measure the expression of target genes in comparison to GAPDH.

Statistics

GraphPad Prism 8, Qlucore Omics Explorer (version 3.6) and R (version 4.0.2) were used for data analysis and visualization. Statistical comparisons were performed using non-parametrical tests (Mann–Whitney or Kruskal–Wallis on ranks followed by Dunn's multiple comparisons). $p < 0.05$ was considered significant and n represents the number of animals or experiments.

Results & Discussion

Study I

c-Abl kinase regulates neutrophil extracellular trap formation and lung injury in abdominal sepsis

c-Abl kinase has been implicated in number of cellular processes, including actin cytoskeleton modulation, the DNA-damage response, and the cell cycle regulation. The functions of c-Abl kinase to regulate essential components of inflammation, such as neutrophil adhesion and endothelial cell integrity have also been confirmed [138, 142]. Convincing data has shown that c-Abl kinase plays an important role in human leukemias [194] and solid tumors[195]. Moreover, inflammatory diseases have also been linked to c-Abl kinase, such as nephrotoxicity and endotoxin-induced vascular leakage [196], allergic lung inflammation[197]. Recent studies pointed out the inhibition of c-Abl kinase could be a potential therapeutic target for Parkinson's Disease [198]. Taken together, increasing evidence indicate that c-Abl kinase might play a key role in regulating neutrophils functions and inflammation in diseases models, such as sepsis.

c-Abl kinase activity in neutrophils

Knowing that c-Abl kinase is an important regulator of inflammation, we first examined the activity of c-Abl kinase in neutrophils in vivo. The Western Blot data analysis suggested the phosphorylation of c-Abl kinase has been evoked in circulating neutrophils after induction of sepsis (Figure 5). In addition, injection of a potent c-Abl kinase inhibitor (Figure 5), GZD824, greatly decreased the c-Abl kinase phosphorylation in CLP group, while treatment of GZD824 on the sham group didn't show any effect (Figure 5), suggesting that GZD824 is an efficient inhibitor of c-Abl kinase.

It has been reported that c-Abl kinase activity is mediated by phosphorylation [199]. According to the crystal structure and functional data of c-Abl, several phosphorylation sites in the breakpoint-cluster region were identified to be required for c-Abl activation [199]. In our study, we confirmed c-Abl kinase activation through phosphorylation in septic neut-

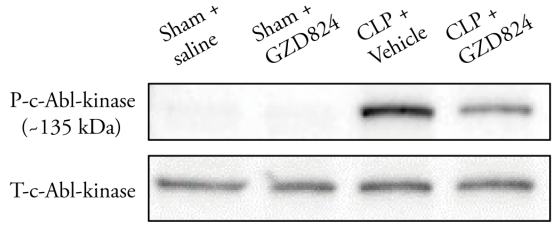

Figure 5: Phosphorylation of c-Abl kinase in isolated circulating neutrophils. Mice were treated with GZD824 or vehicle prior to sham or CLP operation, there was no CLP operation in sham mice and served as a negative control.

rophils, this finding is consistent with a previous study where c-Abl kinase activation was shown to regulate LPS-induced inflammation and lung injury [200].

c-Abl kinase regulates lung injury and systemic inflammation in abdominal sepsis

The effect of c-Abl kinase in different diseases have been well studied as mentioned above, but not well investigated in animal sepsis model. Next, we examined the effect of GZD824 in sepsis induced lung injury. Compared to the sham mice (Figure 6.A and C), CLP operation generated severe lung injury, induced tissue microarchitecture destruction, interstitial issue edema, and neutrophil infiltration (Figure 6B). Treatment of GZD824 significantly reduced the pulmonary damage (Figure 6D) according to the double-blinded histological score as well as the lung edema formation in CLP operated mice. At the same time, the number of BALF neutrophils has been counted and the pulmonary levels of cytokines have been measured. Administration of GZD824 markedly decreased BALF neutrophils and the secretion of CXC chemokines, indicating the role of a-Abl kinase signaling in neutrophil recruitment and tissue damage. In addition, plasma levels of CXC chemokines were decreased significantly in the treatment group.

Recent studies mentioned the role of c-Abl in regulating integrity of the vascular barrier [196, 201], which contributes to the pathogenesis of lung damage as well. Another study has reported that mice treated with c-Abl kinase inhibitor recovered quicker from pathogen-induced lung damage [202]. These are in line with our findings that c-Abl kinase can control the edema formation in the lung and inflammation induced lung damage. Interestingly, IL-8, IL-6 was reported to be elevated by a Bcr-Abl dependent manner during chronic myeloid leukemia [203, 204]. In our study, the CXCL-1, CXCL-2, and IL-6 expression were found decreased in lung and plasma after inhibition of c-Abl kinase, suggesting that c-Abl kinase regulates CXCL-1, CXCL-2, and IL-6 formation in abdominal sepsis.

Moreover, the c-Abl kinase is known to regulate the downstream activity of small GTPase,

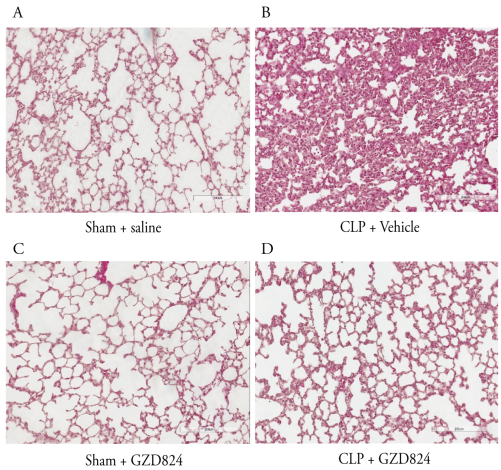

Figure 6: Representative histology sections of the lungs. Mice were treated with GZD824 or vehicle prior to sham or CLP operation, there was no CLP operation in sham mice and served as a negative control. Scale bar = 200 µm

such as Rac1 and RhoA [143, 144] which belongs to the Rho family and shown to regulate the lung damage in sepsis [205, 206]. Although our findings imply that c-Abl kinase activation in neutrophils is important for septic lung damage, we could not exclude the possibility that the c-Abl kinase activity in other cells can play a role, such as endothelial cells [207]. Furthermore, prior research found that inhibiting c-Abl kinase reduces endotoxin-induced pulmonary inflammation but increases ventilator-induced lung injury, indicating that the pro-inflammatory role of c-Abl kinase may be context-dependent [208] and need careful evaluation before translating to clinic.

c-Abl kinase mediates NET formation

As c-Abl kinase-mediated neutrophil recruitment was considered as an important component for tissue damage, we next evaluated the role of c-Abl in regulating neutrophil derived NET formation. NET formation is considered as one of the pro-inflammatory responses during neutrophil mediated immune responses [209, 210]. We observed that neutrophil derived web-like structures and neutrophil-derived nuclear citrullinated histone 3 as well as elastases were increased in the lung of septic animals and administration of GZD824 reduced the web-like NET structure in the septic lung (Figure 7). Similarly, the DNA-Histone complex formation in the plasma was decreased after treatment of GZD824, suggesting that in abdominal sepsis, c-Abl plays a significant role in NET formation.

Herein, the next question was whether c-Abl kinase regulates NET formation in neutrophils directly. We used TNF-α, a well-known activator of c-Abl kinase pathway, to stim-

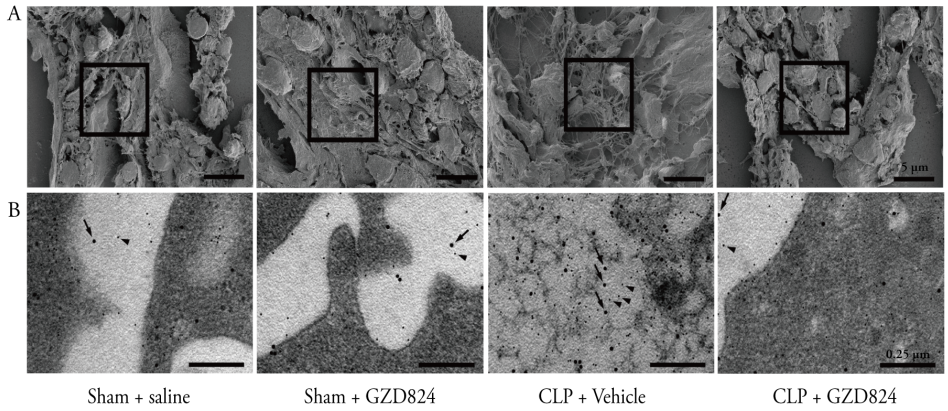

Figure 7: Mice were treated with GZD824 or vehicle prior to sham or CLP operation, there was no CLP operation in sham mice and served as a negative control. A. Representative Scanning electron microscopy showing extracellular web-like structures in the lungs, Scale bar = 5 µm. B. Transmission electron microscopy of the indicated area of interest from Figure 7A, incubated with gold-labeled antibody against citrullinated histone 3 (large gold particles) and anti-elastase (small gold particles) antibodies. Scale bar = 0.25 µm.

ulate isolated bone marrow neutrophils. We discovered that co-incubation with GZD824 reduced TNF-induced co-expression of MPO and citrullinated histone 3 on neutrophil derived DNA and DNA-histone complexes in isolated neutrophils (Figure 8), implying that c-Abl kinase regulates NET formation directly in neutrophils. Furthermore, ROS has been reported to play an important role in regulating c-Abl kinase phosphorylation [211]. Another study found ROS formation is critical for the production of NET in neutrophils stimulated by TNF-α [212], we then revealed that inhibition of c-Abl kinase significantly decreased TNF-α-induced ROS production in neutrophils. Thus, the TNF-α induced production of NET might be dependent on c-Abl kinase mediated ROS formation.

To summarize, our findings reveal that c-Abl kinase is involved in the formation of NET in neutrophils as well as sepsis-induced lung damage and systemic inflammation. Thus, this study not only identifies a new signaling pathway that regulates NET formation in sepsis, but it also suggests that inhibition of c-Abl kinase could be a useful pharmaceutical target for treating infectious disorders in which excessive NET production harms human organs and tissues. Nevertheless, one concern about the findings is that the bacterial clearance wasn't clearly identified in this study. NET not only cause tissue damage and organ failure [209, 213], but also can capture and destroy bacteria [98, 214]. Thus, it would be interesting to know whether the bacterial clearance functions are influenced by c-Abl kinase inhibition in the further research. Another limitation is the time of intervention. Only pre-treatment was done to investigate the functional role of c-Abl in NET formation and abdominal sepsis, but the post-treatment would be better to reflect the sepsis situation.

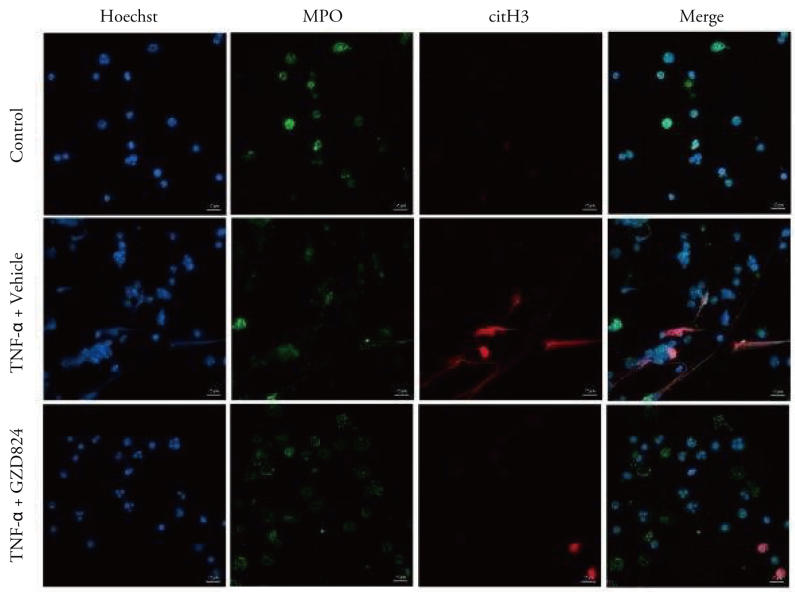

Figure 8: NET formation in vitro. Isolated bone marrow neutrophils were treated with GZD824 or vehicle prior to TNF-α stimulation. NET was visualized by confocal microscopy using antibodies against myeloperoxidase (MPO), citrullinated histone 3 (citH3), and Hoechst 33342 (Hoechst).

Thus, our findings provide some insights for future research to study the time-point for treatment and the combination therapy with antibiotics or other drugs.

Key findings:

- c-Abl kinase phosphorylation occurrs in circulatory neutrophils during sepsis.
- c-Abl kinase regulates lung injury and systemic inflammation in sepsis.
- c-Abl kinase mediates NET formation in sepsis.
- c-Abl kinase regulates NET formation through ROS signaling pathway.

Study II

Actin-related protein 2/3 complex regulates neutrophil extracellular trap expulsion and lung damage in abdominal sepsis

Neutrophil activities such as phagocytosis, migration, and vesicular trafficking and secretion are highly dependent on cytoskeletal dynamics [116, 117, 215]. According to some NET kinetic studies, an increased F-actin polymerization followed by global actin cytoskeleton disruption is important for NET expulsion [126, 216]. The assembly of monomers for polymerization and branched filament growth are required for the generation of new actin filaments, known as nucleation, which is the rate-determining step of actin filaments assembly controlled by actin nucleation factors [115]. Arp2/3 complex is the first revealed actin nucleation factor which has been reported to play a major role in regulating actin polymerization [113]. However, the role of the Arp2/3 complex in NET expulsion during sepsis is not studied yet.

Arp2/3 complex regulates F-actin polymerization in neutrophils

In order to show the dynamic changes of F-actin polymerization in neutrophils, PMA was used to stimulate the neutrophils. Both mean fluorescence intensity (MFI) determined by flow cytometry and cell imaging by confocal microscopy (Figure 9) suggest that the F-actin

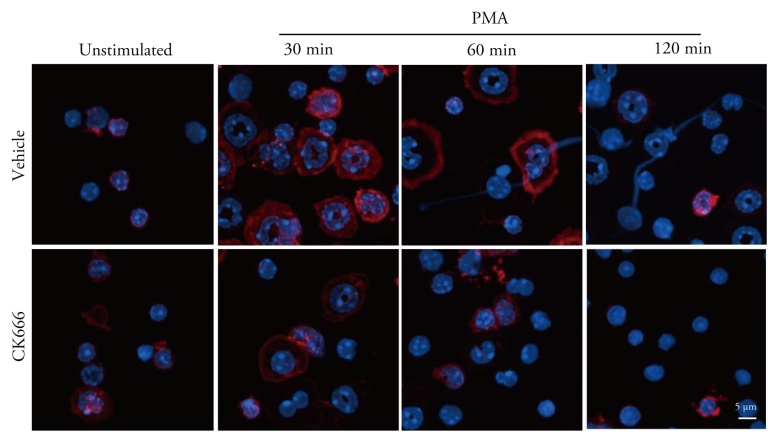

Figure 9: Isolated bone marrow neutrophils were treated with CK666 or vehicles prior to PMA stimulation. F-actin formation was induced from 0 to 120 min and visualized by confocal microscopy using an antibody against F-actin (SiR Actin, red), and Hoechst 33342 for nuclear staining (blue), scale bar = 5 µm.

polymerization is a time-dependent process which was extremely evoked at 30 min after stimulation, and spontaneously return to the base line 120 min after simulation, which is consistent with a previous finding [126]. The F-actin activities were observed being partly blocked by using of an effective inhibitor of Arp2/3 complex called CK666 (Figure 9). In addition, a previous study reported that neutrophil elastase regulates F-actin polymerization during the process of NETosis in response to *C. albicans* and the peak is around 7 to 15 min [92]. This finding demonstrates a similar tendency of F-actin polymerization as our findings, and also implies a potential intervention time-point of F-actin polymerization, which might be crucial for targeting NET formation.

Actin polymerization can start from globular actin (G-actin), which includes a slow phase of actin nucleation action [217]. The nucleating factor, Arp2/3 complex remains attached to the sidewalls of pre-existing actin filaments and a new filament nucleated to help with maintaining the stability of the kinetic process of actin polymerization [218]. Arp2/3 complex consists of actin related Arp2 and Arp3 as well as five other subunits [219]. The compound CK666 works as an allosteric effector to block the movement of Arp2 into the short pitch conformation, which help to stabilize the inactive state of the Arp2/3 complex, and further inhibit the actin polymerization [220].

Arp2/3 complex controls NET formation in vitro

Knowing that NET formation is linked with the actin cytoskeleton dependent dynamic morphological alterations [126, 221], we next asked the role of Arp2/3 complex in neutrophil derived NET formation in vitro. The isolated bone marrow neutrophils were pretreated with CK666, then challenged with PMA to check the NET formation by confocal imaging and DNA-Histone complex measurement in the supernatants. It was observed that PMA induced excessive formation and expulsion of web-like structures, and the expulsion of web-like structures were markedly blocked by inhibition of Arp2/3 complex (Figure 10). In addition, it was observed that the size of cells increased, rounded and unpolarized (Figure 10), indicating the functional role of F-actin filaments in regulating morphological changes. Likewise, the DNA-histone complex formation in the cell culture medium was increased by PMA stimulation and decreased by treatment with CK666, suggesting that Arp2/3 complex plays an important role in NET expulsion. These findings are consistent with previous studies which showed the effect of cytochalasin D, another inhibitor of F-actin polymerization, in reduction of chromatin expulsion from activated neutrophils [94, 127]. It should be noted that the mechanism of CK666 is different from cytochalasin D. Cytochalasins are very potent inhibitors of F-actin polymerization, they can disrupt the cytoskeleton by breaking of actin filaments, known as 'Severing'[222]; In contrast, CK666 inhibits actin filament-filament interactions and branching instead of breaking or severing the filaments [223]. Too much disruption of cytoskeleton may cause

possible inflammation or influence the tight junctions of the normal cells [224, 225].

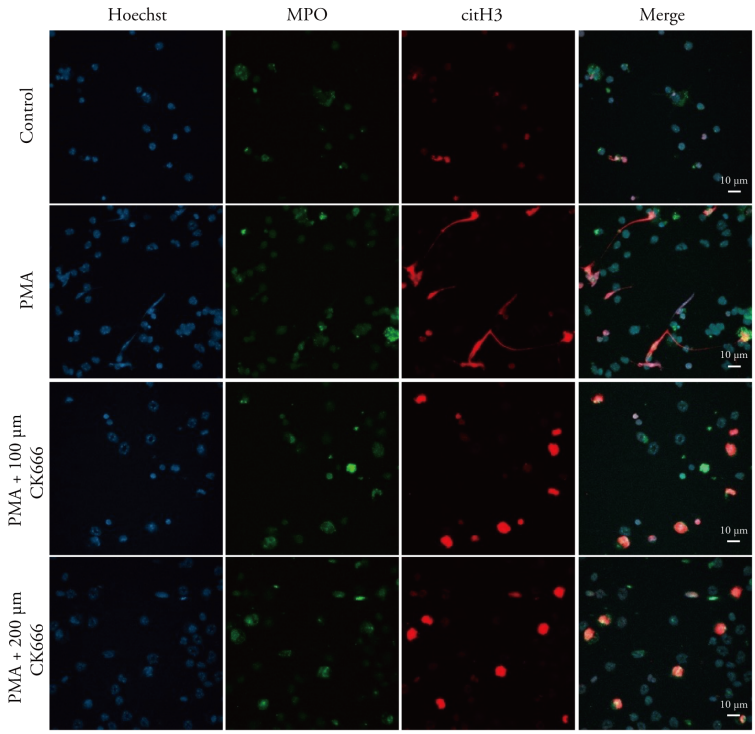

Figure 10: NET formation in vitro. Isolated bone marrow neutrophils were treated with CK666 or vehicles prior to PMA stimulation. NET was visualized by confocal microscopy using antibodies against myeloperoxidase (MPO), citrullinated histone 3 (citH3), and Hoechst 33342 (Hoechst), scale bar = 10 µm

Another possible explanation of the effect could be the relation of actin polymerization and ROS formation. Actin polymerization was found to be important in the early phase of neutrophil activation during NETosis [126]. It was reported that actin polymerization promotes the ROS formation in neutrophils [128, 129]. Furthermore, ROS formation was shown to mediate actin and tubulin polymerization and then regulate formation of NET [226]. Thus, actin polymerization and ROS formation might play like a feedback loop in NET formation. Interestingly, we didn't find any effect of CK666 on PMA-induced ROS formation, indicating that the Arp2/3 complex-mediated NET formation is not related to the inhibition of ROS formation.

Arp2/3 complex controls NET formation in vivo and lung tissue damage

Actin polymerization was related to a lot of neutrophil-associated dysfunctions and diseases [227, 228]. Increasing number of studies indicate that neutrophil derived NET generation

is crucial during septic pathophysiology [209, 214, 229]. Herein, we found CK666 treatment reduced neutrophil-derived web-like NET structures and associated proteins, such as citrullinated histone 3 or elastases in the lungs (Figure 11A, B, C). In addition, CK666 reduced the DNA-Histone complex formation in the BALF, suggesting Arp2/3 complex regulates the pulmonary NET formation. Likewise, we observed treatment with CK666 decreased the circulating $\mathrm{citH_3^+MPO^+}$ neutrophils in septic mice, indicating Arp2/3 complex might mediate the expulsion of NET in circulating neutrophils too.

We further examined histological changes of lung tissues by analyzing hematoxylin and eosin stained histology sections (Figure 11D), and found treatment with CK666 greatly decreased the lung injury scores and lung edema formation, indicating that Arp2/3 complex is also involved in mediating tissue damage in the lungs. These findings are in accordance with recent studies which showed Arp2/3 complex regulates vascular integrity by controlling vascular permeability of epithelial and endothelial cells [230, 231].

Bacterial clearance during inflammation is carried out by neutrophil through phagocytosis [232], ROS production [233], and the release of granular enzymes [234]. Neutrophils also target extracellular pathogens by NET [89]. NET serves as a dual sword in inflammation, indicating the fact that blocking of NET formation might generate difference consequences

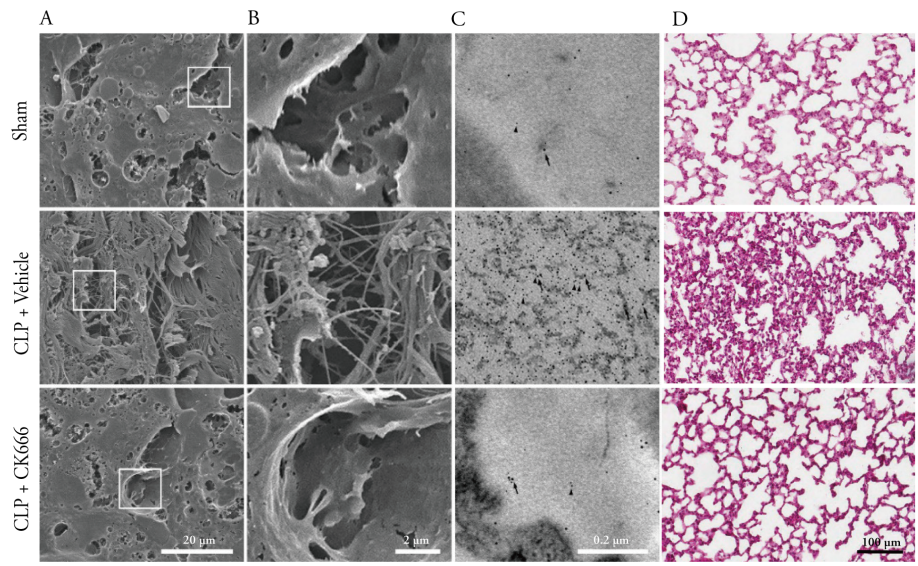

Figure 11: Mice were treated with CK666 or vehicle prior to CLP operation, there was no CLP operation in sham mice and served as a negative control. A. Scanning electron microscopy showing extracellular web-like structures in the lungs, scale bar = 20 µm. B. Transmission electron microscopy of the indicated area of interest from Figure 11A, scale bar = 2 µm. C. Transmission electron microscopy of the indicated area of interest incubated with gold-labeled antibody against citrullinated histone 3 (large gold particles) and anti-elastase (small gold particles) antibodies (C, scale bar = 0.2 µm). D. Representative hematoxylin-eosin stained sections of the lungs, scale bar = 100 µm.

in different situations. In this context, it is not surprising that there was no significant effect of CK666 on bacterial clearance in both blood and lung tissues, although there were some decreases by the treatment. The impaired bacteria clearance could be explained by the fact that CK666 was found to block NET release, hence impair the ability of bacteria trapping function of neutrophils. Nevertheless, CK666 is known to work in a reversible manner [235], the little decrease might be due to the reversion of the drug effects after some time point and thus neutrophil might recover its ability to generate NETs again and kill bacteria.

Arp2/3 complex mediates neutrophil recruitment in lung injury and systemic inflammation in sepsis

infiltration. Given that neutrophil infiltration is an important step in sepsis-induced lung injury progression [78, 79], we next examined the MPO level in the lungs. CLP evoked MPO activity (Figure 12A), and CK666 administration decreased the MPO activity (Figure 12A), suggesting Arp2/3 complex is an efficient regulator of neutrophil infiltration in sepsis. In addition, CXC family is considered as chemoattractant for neutrophils, and play a significant role in mediating neutrophil recruitment and NET formation in sepsis [24]. IL-6 is considered as an important indicator of severity in sepsis [236].In the present study, administration of CK666 significantly reduced the pulmonary production of CXCL-1 and IL-6 in the lung (Figure 12B, C), indicating that Arp2/3 complex might mediate cytokines

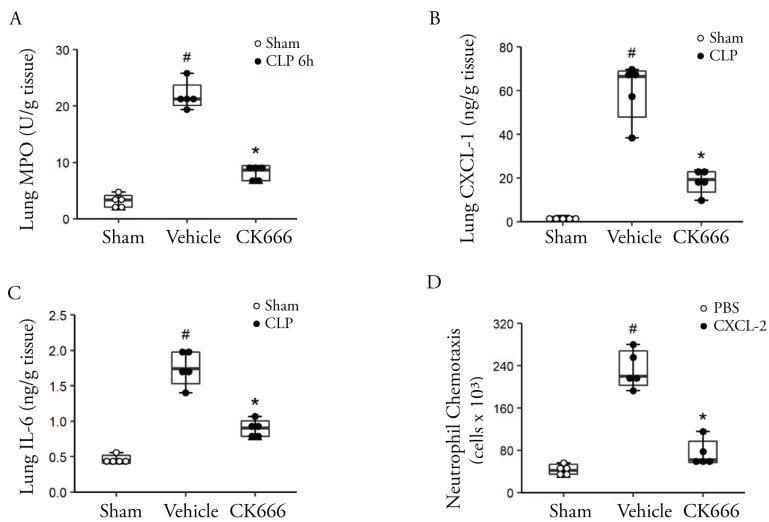

Figure 12: Mice were treated with CK666 or vehicle prior to CLP operation, there was no CLP operation in sham mice and served as a negative control. A. Lung levels of MPO activity 6h after CLP detected by colorimetric method. B. Lung levels of CXCL-1 and C. lung levels of IL-6 24h after CLP detected by ELISA. D. Migration assay on isolated bone marrow neutrophils. $^{\#}p < 0.05$ vs. Sham, $^{*}p < 0.05$ vs. Vehicle, n = 5.

formation in sepsis. Similarly, we found that inhibition of Arp2/3 complex decreased CXCL-2-induced neutrophil chemotaxis (Figure 12D). Taken together, these findings help to explain the inhibitory effect of CK666 on pulmonary neutrophils.

We also studied the effect of CK666 on systemic inflammation during abdominal sepsis. The circulating cytokines (CXCL-1, IL-6) generation was decreased along with cytokines in the CLP lung, indicating the direct role of Arp2/3 complex in regulating neutrophil migration to the local sites through formation and function of cytokines.

It should be noted that the mechanisms of neutrophil recruitment and infiltration in septic lung damage is a complex process and could be influenced by multi-factors. Apart from the reduction of cytokines and NET formation during sepsis, the impaired mechanical forces could also decrease neutrophils trapping in pulmonary microvessels and transmigration to lung tissues. For example, during inflammation, cytoskeletal alteration is known to enhance leukocyte mechanical trapping in the pulmonary microvasculature [237, 238]. It is because the capillaries in the lung have a smaller spherical diameter (6 μm) than neutrophils (7 μm), forcing neutrophils to deform and get mechanically trapped when passing through the narrow lung capillaries [239]. The role of Arp2/3 complex in regulating leukocytes to rapidly and efficiently migrate through narrow gaps has been well studied [119]. A number of investigations have confirmed the hypothesis of F-actin-mediated stiffening and mechanical trapping of neutrophils in the lungs [116, 117, 215, 240, 241]. For instance, one study found that co-incubation of cytochalasin B with activated neutrophils not only reduced F-actin polymerization but also reduced pulmonary accumulation in septic mice [240]. In line with this finding, Arp2/3 complex was found to regulate actin polymerization and cytoskeleton alterations, and thus lead to reduction of inflammation and tissue injury in our study. Although neutrophil recruitment has been shown to be dependent on adhesion molecules, such as P-selectin and Mac-1 expression [78, 242, 243], in our study we found that CK666 has no influence on up-regulation of Mac-1 on neutrophil after stimulation with PMA, indicating that the inhibitory effect of CK666 on neutrophil accumulation is independent of adhesion molecule expression.

In addition, the time of treatment could be a concern of this study. We found pre-treatment works very well but late treatment didn't have any effect on neutrophil recruitment, or plasma formation of cytokines in CLP animals. Further study should investigate the late treatment combined with antibiotics to reflect more precise situation of sepsis therapy and more assessment on other clinical parameters, such as survival rate.

In present study, we demonstrated that Arp2/3 complex not only regulates F-actin polymerization but also induces intracellular retention of NET, suggesting that the actin cytoskeleton is involved in NET expulsion. Furthermore, we discovered that Arp2/3 complex

not only mediates NET formation in vivo, but also controls neutrophil recruitment and tissue destruction in abdominal sepsis. Targeting expulsion of NET might be a potential therapeutic strategy for ameliorating tissue damage and pathological inflammation in abdominal sepsis.

Key findings:

- Arp2/3 complex regulates F-actin polymerization in neutrophils.
- Arp2/3 complex regulates NET expulsion in vitro.
- Arp2/3 complex regulates NET formation in vivo and septic lung injury.
- Arp2/3 complex mediates neutrophil recruitment in septic lung injury and systemic inflammation in abdominal sepsis.

Study III

Targeting S100A9 reduces neutrophil recruitment, inflammation and lung damage in abdominal sepsis

S100A9 is a calcium-binding protein belongs to the S100 family, which is mainly derived from immunocytes, such as neutrophils and macrophages [157]. S100A9 is considered as an alarmin that helps to alert the host of impending danger [244]. Interestingly, several studies have shown that S100A9 is abundantly expressed in neutrophils [165, 245], the expression of S100A9 has also been shown up-regulated in various infections and inflammatory diseases [37, 160, 246]. In addition, several studies demonstrated that the interaction of S100A9 with different receptors can mediate recruitment of leukocytes, expression of cytokines as well as cancer cells migration [158, 247, 248]. Taken together, S100A9 appears to be an important pro-inflammatory component of neutrophil and mediator in different diseases [160, 161]. However, the role of S100A9 in abdominal sepsis is unclear.

Septic levels of S100A9

S100A9 plays a role in a variety of inflammatory diseases, including arthritis [249], myocardial infarction [37, 250], endotoxin-induced lung damage [251], and acute pancreatitis [252]. In present study, we examined the septic levels of S100A9, and found CLP markedly evoked the S100A9 levels in plasma and lung (Figure 13), which is in line with the clinical observation that S100A9 is increased in sepsis [253] and COVID-19 patients [254]. Blocking of S100A9 with a specific S100A9 inhibitor, ABR-238901 (ABR), greatly reduced CLP-induced elevations of S100A9 in both plasma and lungs (Figure 13), suggesting that S100A9 might play an important role in sepsis.

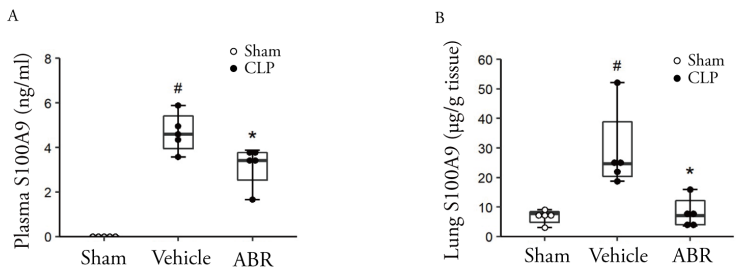

Figure 13: Mice were treated with ABR or vehicle prior CLP operation, there was no CLP operation in sham mice and served as a negative control. A. Plasma levels of S100A9 and B. lung levels of S100A9 24h after CLP detected by ELISA. $^{\#}p < 0.05$ vs. Sham, $^{*}p < 0.05$ vs. Vehicle, n = 5.

S100A9 mediates septic lung injury

S100A9 is also linked to tissue damage in various inflammatory disorder, such as liver necroinflammation [255], myocarditis [256]. Herein we hypothesized that S100A9 is related to the septic lung damage as well. In our study, CLP operation induced systemic neutrophil activation, pulmonary chemokines and edema formation in the lung (Figure 14B), and blocking of S100A9 helps to attenuate these symptoms (Figure 14C), indicating that S100A9 is crucial for regulation of sepsis-induced inflammation and lung damage, which is consistent with previous studies showing that inhibition of S100A9 reduces LPS-induced lung injury [251, 257]. Our current findings are also in line with a previous study which showed that mice lacking S100A9 had better survival and less liver damage during an Escherichia coli induced model [258]. Although post-treatment of ABR-238901 did not show any effect on septic lung damage and inflammation, further studies could be done combined with antibiotics to investigate the role of S100A9 in a situation which is closer to clinical settings.

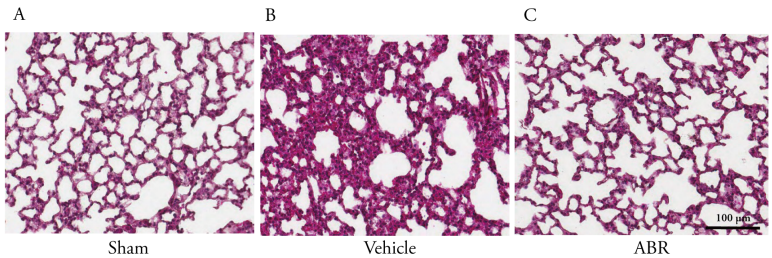

Figure 14: Mice were treated with ABR or vehicle prior to CLP operation, there was no CLP operation in sham mice and served as a negative control. Representative of hematoxylin-eosin stained sections of the lungs, scale bar = 100 μm.

In contrast, several studies reported protective effects of S100A9. For example, one study revealed that administration of S100A9 could reduce lung injury in response to LPS [259], the possible reason is unclear but could be related to different sepsis models. LPS was found in the outer membrane of Gram-negative bacteria, known to induces a strong inflammatory response [260]. Injection of LPS has been used a lot on animal research, but it has been reported that LPS-induced sepsis model doesn't show similar clinical manifestation as in CLP model [261, 262]. In contrast, CLP model destroys the normal barriers of the gastrointestinal tract by surgical operation which is almost similar to clinical situations [261]. CLP model also enables the translocation of mixed enteric bacteria into the blood, closely mimic the pathophysiology in septic human patients. That is why the CLP model is considered as the golden standard in experimental sepsis research [263]. Further investigation is needed to clarify the specific role of S100A9 in inflammation under different contexts. Another two studies indicating that lacking of S100A9 promotes inflammation in invasive pneumococcal pneumonia [264] and staphylococcal pneumonia [265]. These findings again imply that the functional role of S100A9 might be different based on the

contexts.

S100A9 regulates neutrophil recruitment and systemic inflammation

Since neutrophil infiltration has been identified as a crucial component in the pathogenesis of septic lung damage in various studies [156, 266], we next asked the effect of ABR-238901 on neutrophil recruitment during abdominal sepsis. We observed that treatment of ABR-238901 markedly decreased CLP-induced MPO activity in the lungs (Figure 15A), suggesting the S100A9 plays an important role in regulating neutrophil infiltration in sepsis-induced lung damage. A lot of studies have demonstrated that neutrophil recruitment during sepsis is mediated by adhesion molecules, such as P-selectin and Mac-1 [78, 242, 243], which support the cell interactions between endothelial cells and leukocytes [267]. Herein

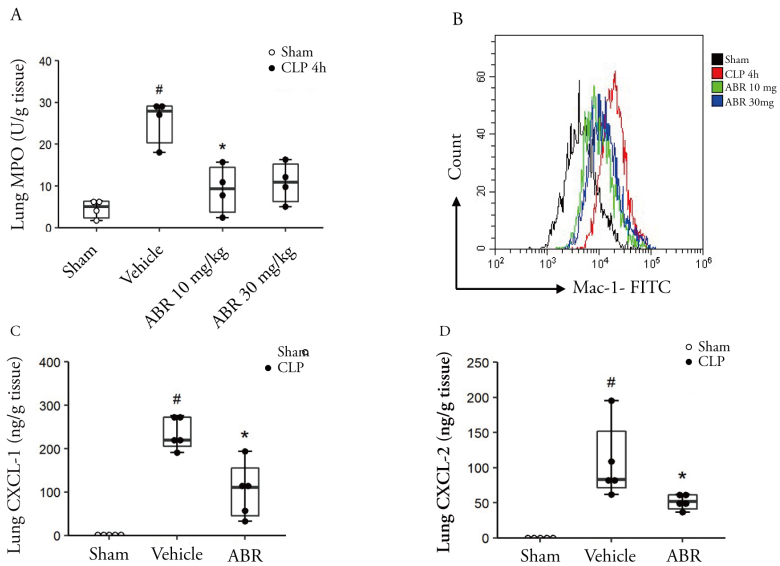

Figure 15: Mice were treated with ABR or vehicle prior to CLP operation, there was no CLP operation in sham mice and served as a negative control. A. Mac-1 expression (MFI) on blood neutrophils detected by flow cytometry. B. Lung levels of MPO 4h after CLP detected by colorimetric method. C. Lung levels of CXCL-1 and D. lung levels of CXCL-2 24h after CLP detected by ELISA.

we examined the Mac-1 expression and found that treatment of ABR-238901 resulted in significant reduction of CLP-induced Mac-1 expression on circulating neutrophils (Figure 15B), suggesting the S100A9 is also involved in neutrophil activation by regulating Mac-1 expression. CXCL-1 and CXCL-2 belongs to the CXC family, which are tightly associated with regulation of neutrophil recruitment and infiltration [268, 269], S100A9 has been reported to promote CXC chemokines secretion from macrophages [270]. In this context, we found the secretion of CXCL-1 and CXCL-2 in lung tissues were decreased

after blocking of S100A9 by ABR-238901 (Figure 15C, D), indicating that S100A9 also controls the production of CXC chemokines in the inflamed lungs.

Increase of plasma levels of cytokines and leukcytopenia were also reported as an indicator of systemic inflammation in sepsis [156]. We observed that blocking of S100A9 reduced CLP-induced plasma levels of CXCL-1, CXCL-2, IL-6 and leukcytopenia as well, indicating the S100A9 also mediates systemic inflammation during sepsis.

In present study, we found that S100A9 regulates neutrophil recruitment by regulating Mac-1 expression and CXC chemokines production in abdominal sepsis. In septic lungs, blocking of S100A9 reduced neutrophil activation and recruitment, edema formation and septic lung injury. Taken together, these findings help us to explain the pro-inflammatory role of S100A9 in septic induced lung injury, and suggesting that blocking of S100A9 could be a potential therapeutic strategy in abdominal sepsis. Nonetheless, there are some limitations that should be noted here. For example, in most studies, S100A9 presented together with S100A8 as S100A8/A9 heterodimer, which is measured in different studies [250, 271, 272]. We couldn't exclude the possibility that S100A8/A9 heterodimer could function differently than S100A9 alone and inhibition of S100A9 by ABR-238901 could also inhibit S100A8/A9 heterodimer function. In addition, one study shown that S100A9 release is correlated with NET formation and about 30% of the total S100A9 is bound with NET [76], thus, it will be interesting to further investigate the pro-inflammatory role and mechanisms of NET attached S100A9 in abdominal sepsis.

Key findings:

- The levels of S100A9 in plasma and lung are significantly increased during sepsis.
- S100A9 mediates lung tissue damage in abdominal sepsis.
- S100A9 regulates neutrophil recruitment and systemic inflammation in abdominal sepsis.

Study IV

Transcriptomic analysis reveals differential expression of genes between lung capillary and post capillary venules in abdominal sepsis

EC plays an important role in systemic and local immune responses during inflammation. Regulation of barrier integrity, coagulation, and homeostasis have been considered as the main functions of EC in resting conditions, however, in response to sepsis all of these functions have shown to get dysregulated [186, 273, 274]. The microvasculature beds in different organs are composed of structurally and functionally different endothelial subsets, such as, arterioles, capillaries (cap) and post capillary venules [275]. In addition, same microvessel was found different in different organs, for instance, capillaries were found fenestrated in liver and kidney but not in lung and brain [276]. Considering their diversity in different organs, we intended to investigate the transcriptional differences between capillaries and post capillary venules in the lung of mice and total transcriptional changes after induction of sepsis.

Cluster analysis of lung EC subsets

Our flow cytometric analysis revealed that lung ECs uniformly expressed Icam1 and Ly6C; anti-Vcam1 and CD63 antibodies together with anti-Icam1 or Ly6C antibodies could separate lung capillary endothelial cells (capEC) from post capillary venules (PCV). Therefore, to analyze the transcriptomic changes of lung EC subsets, we first separated capEC and PCV by using of monoclonal antibodies. The $\mathrm{CD31^{+}Icam1^{+}Vcam1^{-}}$ EC was identified as capEC subset, while the $\mathrm{CD31^{+}Icam1^{+}Vcam1^{+}}$ EC was identified as the PCV subset. Following differential gene expression analysis between sham capEC and PCV, it was revealed that the two EC subsets expressed different endothelial marker genes and adhesion molecules, confirming that lung capEC and PCV are transcriptionally different. For example, sham capEC were found to express CD36, Nes, Hey1 and Notch4, while sham PCV were found to express CD63, Bst1, Tlr4 and Nr2f2. Moreover, principal component analysis (PCA) showed that capEC and PCV biological replicates were cluster together respectively (Figure 16A, B), while capEC and PCV groups were discrete (Figure 16C), suggesting the gene expression of the two subsets have minor internal variance but significant difference between groups. These findings are in line with the previous observations [277].

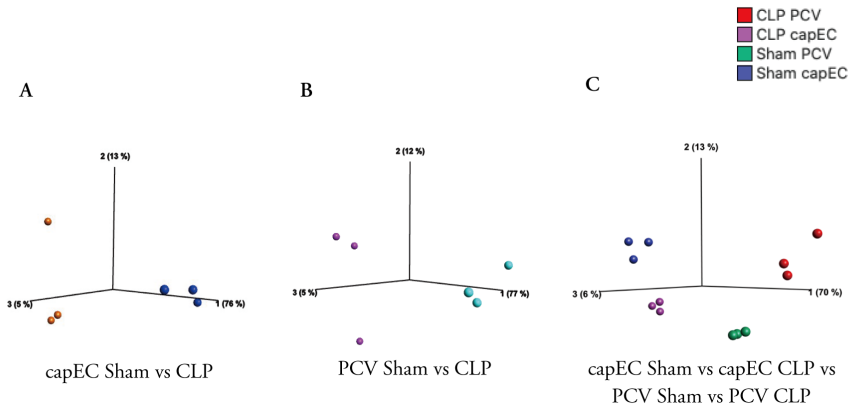

Figure 16: Sepsis was induced by CLP, there was no CLP operation in sham mice and served as a negative control. PCA showing samples and variables (top 500) of A. capEC and B. PCV. C. PCA showing samples and variables (top 500) of all 4 groups.

Identifying DEGs during sepsis between capEC and PCV

Top 100 (Figure 17) and top 500 DEGs were identified by comparing gene expression of sham and CLP groups in the two subsets respectively. We next compared the top 100 differentially expressed genes (DEGs) between capEC and PCV, and found only 37 common genes. Moreover, we also compared the top 500 DEGs, and found only 185 common genes. Together, these findings also support our results that lung capEC and PCV are transcriptionally different. In addition, these DEGs could serve as target genes to investigate their roles in sepsis-induced lung damage.

Among these DEGs, some of them were already verified by previous studies. For example, Bcl2 interacting protein 3 (Bnip3) has been found to play a role in regulating human microvascular endothelial cells [278], over-expression of nuclear apoptosis-inducing factor 1 (Naif1) has been demonstrated to induce cancer cell apoptosis [279]. Nonetheless, most of the DEGs were not studied in sepsis and needed further investigation. For example, we found that a gene termed as Cldn1 or Claudin-1 has not been studied in abdominal sepsis yet, which was reported to be associated with leukocyte vacuoles, cell junction and adhesion [280, 281]. DAMPs were reported to involve in pathological inflammation [282], likewise, here we found some DEGs were associated with or response to DAMPs family, such as S100 gene family. S100 proteins act as DAMPs through interacting with TLR2, TLR4, and RAGE, which could be interestingly targets to investigate how S100 proteins regulate endothelial cell functions during inflammation.

To verify whether the sample preparation techniques, such as, enzymatic digestion, cell sorting, had any influence in the RNA-sequencing data, freshly collected lung samples

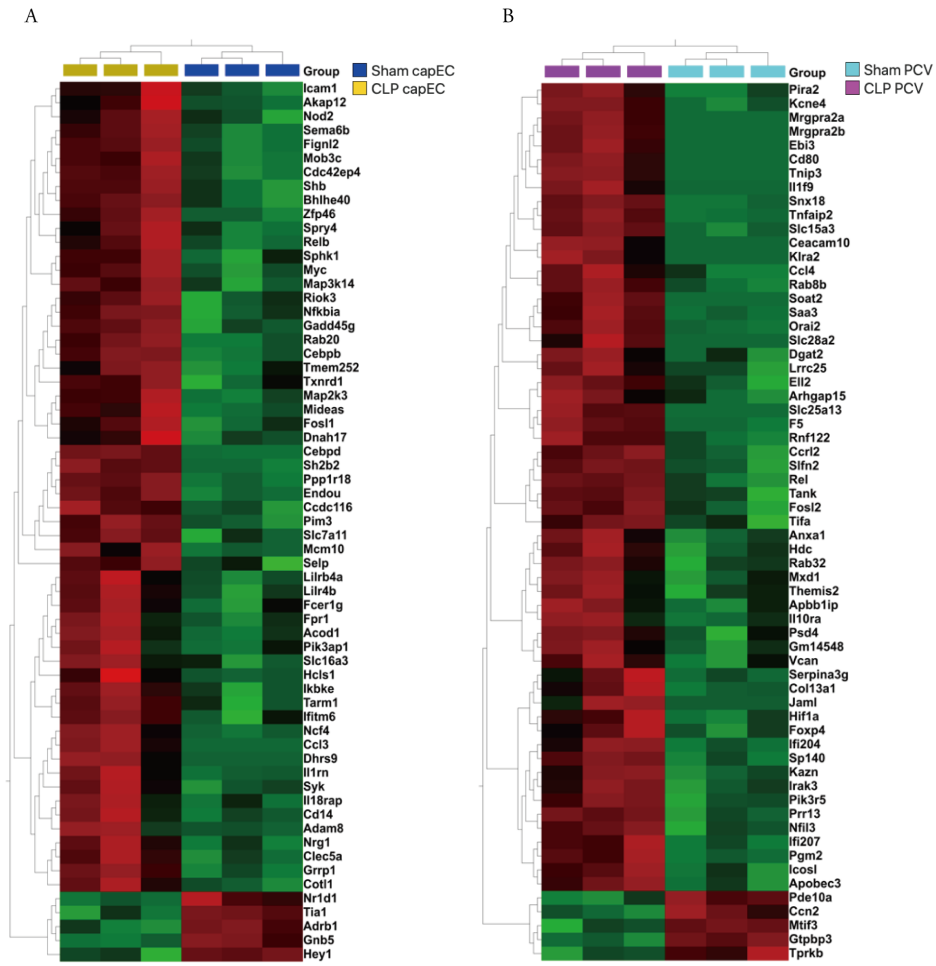

Figure 17: Sepsis was induced by CLP, there was no CLP operation in sham mice and served as a negative control. Top 100 DEGs in A. capEC and B. PCV.

were directly used for RNA extraction, and 6 genes were randomly chosen for validation by RT-qPCR. The results showed consistency with our DEGs analysis.

GO enrichment analysis for DEGs

To further study and understand the gene expression response to sepsis, we performed GSEA analysis based on biological process of gene ontologies. 26 and 28 GO terms were uniquely identified in capEC and PCV, respectively. Three most significant GO terms from each subset were present to show the running enrichment score (RES). Defense response to bacterium, positive regulation of chemokines production, and cell-cell adhesion via plasma

membrane adhesion molecules (Figure 18B) were top relevant gene sets in PCV, which were consist with previous studies [283, 284, 285]. In contrast, VEGF receptor signaling pathway, regulation of coagulation (Figure 18C), and LPS mediated signal pathway were top relevant gene sets in capEC, which strongly support the previous findings that VEGF is a key regulator of blood vessel growth and plays a crucial role in microvasculature maintenance and endothelial survival [286]. Coagulation and LPS mediated signaling pathway were also pivotal in response to host defense and development of organ dysfunction [287, 288].

Interestingly, the actin filament process related genes sets were found largely enriched in

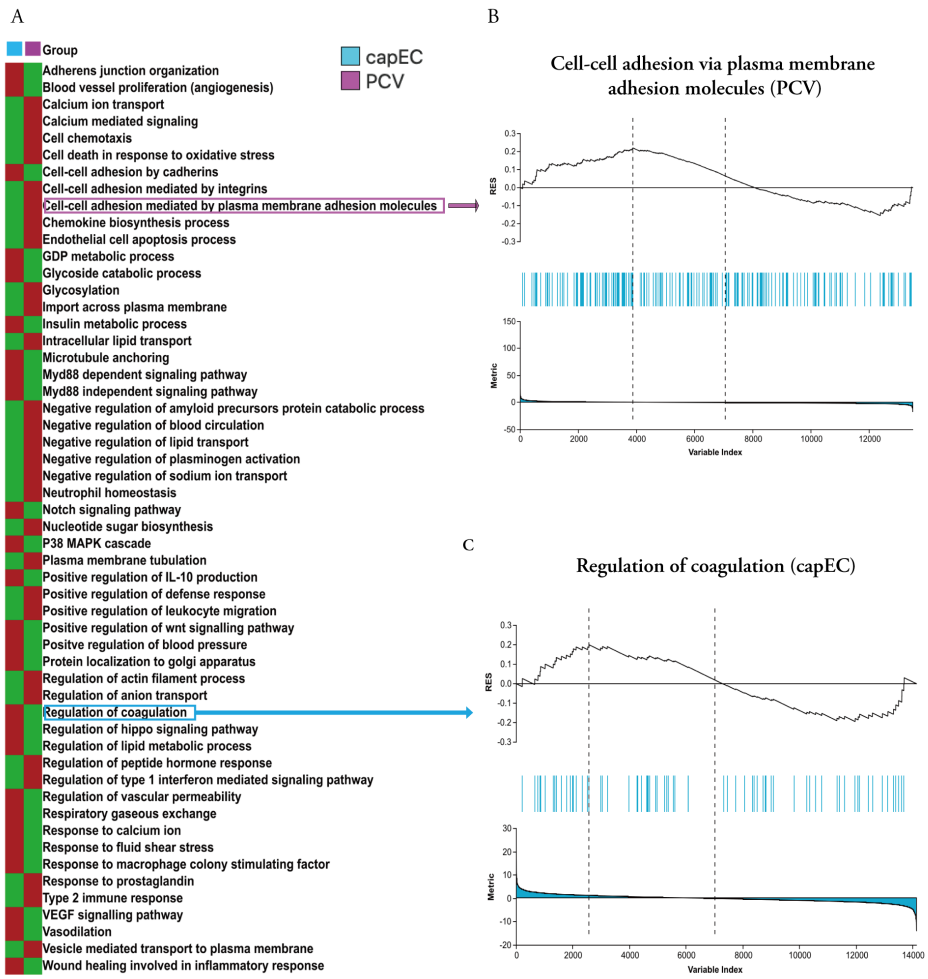

Figure 18: Gene sets enrichment analysis. A. Heatmap showing differentially enriched GO pathways based on normalized enrichment score in lung capEC and PCV after induction of sepsis. B. Representative RES of top relevant enriched gene sets (human orthologs) in lung PCV. C. Representative RES of top relevant enriched gene sets (human orthologs) in lung capEC.

PCV, which contributes to the interaction between endothelial cells and leukocytes, to enable the leukocytes to reach the perivascular spaces in organs vessels through endothelium gaps [123, 289]. The participation of cell-cell adhesion regulated by integrins and adhesion molecules [290] are important for leukocyte migration through endothelial cells, all these molecules are found highly enriched in PCV subsets. Together, our findings strongly support that the transmigration of neutrophils mostly happens in PCV [81].

KEGG pathway enrichment analysis of capEC and PCV

Next, we performed KEGG pathway enrichment analysis of top 500 DEGs. The KEGG pathway analysis revealed that DEGs in capEC were significantly related to the pathways including TNF signaling pathway, NOD-like receptor signaling pathway, toll-like receptor signaling pathway, apoptosis pathway, NF-kappa B signaling pathway, cytokine-cytokine receptor interaction, MAPK signaling pathway, p53 signaling pathway, TGF-beta signaling pathway and chemokine signaling pathway. Moreover, DEGs in PCV were highly related to the pathways including TNF signaling pathway, NF-kappa B signaling pathway, toll-like receptor signaling pathway, NOD-like receptor signaling pathway, cytokine-cytokine receptor interaction, apoptosis, cell adhesion molecules, JAK-STAT signaling pathway, HIF-1 signaling pathway, TGF-beta signaling pathway.

Transcription factors (TFs) control chromatin and transcription by recognizing certain DNA sequences, forming a complex mechanism that regulates gene expression. Given that TFs are up-regulated in response to sepsis, prediction of TFs targets using top DEGs were also performed. For example, in capEC, TFs related to regulation of focal assembly: S100a10, Sdc4; regulation of apoptosis: Nfkbia, Birc3, Ier3, Map3k8, Ube2d3; and response to cytokine: Rel, Relb, etc, have been identified. In PCV, TFs related to regulation of ubiquitination: Mid1; and regulation of nodal signaling pathway: Tgif1, Tgif2, etc, have been identified for further investigation. Despite more and more interests have grown in understanding the role of TFs in gene expression process, lack of information on genomic binding sites for TFs and corresponding transcription regulation after TF binding are largely unknown. Thus, further studies are needed to answer these questions.

To summarize, our results expanded the molecular understanding of sepsis-induced lung injury. We found that sepsis caused massive transcriptional changes in lung EC subsets, and many of these transcriptomes are known to play an important role in maintaining barrier dysfunction, regulation of coagulation, cell-cell interactions, etc. Combined with GO and KEGG analysis of top DEGs, we predict some useful targets, such as Mid1, which could be potential targets in septic research. Although promising, further studies focusing

on gene silencing or genome editing is needed to fully understand the specific roles of these identified genes in abdominal sepsis.

Key findings:

- Lung capEC and PCV are transcriptionally different.
- CapEC are enriched with genes related to regulation of coagulation, vascular permeability, lipid metabolic process after sepsis, accordingly enriched with MAPK signaling pathway, p53 signaling pathway, and chemokine signaling pathway.
- PCV are enriched with genes related to cell chemotaxis, cell-cell adhesion by integrins, regulation of actin filament process after sepsis, accordingly enriched with cell adhesion molecules, JAK-STAT signaling pathway, and HIF-1 signaling pathway.
- Several transcriptional factor targets regulating DEGs were predicted and those could be targeted to control sepsis induced lung damage.

General discussion

Sepsis is considered as one of the most challenging public health problems and has become a global burden due to high direct and indirect cost. Here in this thesis, we studied four different mechanisms behind the early development of septic lung injury. The focuses were to identify new mechanisms that regulate neutrophil-mediated lung damage and pulmonary endothelial cell activation in abdominal sepsis.

In study I, an intracellular signaling molecule, c-Abl kinase, has been shown as a potent regulator of NET formation. It is clear that c-Abl plays a role in sepsis pathogenesis, however, it is not clear how c-Abl regulates septic pathology and what is its connection with neutrophil recruitment and tissue damage. We have found that blocking of c-Abl kinase not only inhibited NET formation but also reduced inflammation and tissue damage in sepsis. Interestingly, c-Abl was reported to regulate vascular leakage during sepsis through phosphorylation of cytoskeletal effectors that mediate vascular permeability [196], implying the involvement of multiple c-Abl dependent mechanisms. A point of concern is that c-Abl is reported play distinct roles depending on its location in nuclear or cytoplasm [131], which raises question in the prospect of c-Abl kinase as a potential therapeutic target.

Knowing that actin polymerization plays a role in NET expulsion and Arp2/3 regulates action polymerization [113, 126], the role of Arp2/3 complex was investigated in study II. Arp2/3 complex was found to regulate NET expulsion and lung damage in abdominal sepsis. We concluded that actin dynamics of neutrophils play an important role in immune and inflammation regulation, affect the outcomes of sepsis and may be a potential therapeutic target. Actin dynamics are mostly studied in endothelial barrier function and integrity in recent years. We did not investigate the role of Arp2/3 in EC function in our study and don't exclude the possibility that Arp2/3 complex inhibitor might also affect endothelial function in sepsis. We speculate that Arp2/3 complex inhibitor might also help to maintain the endothelial barrier stability and reduce the vascular permeability of ECs, thus help to alleviate tissue damage and systemic inflammation. The precise mechanisms of regulating different cells remain elusive and it's difficult to evaluate all the targets in a single research project. Although serval pharmacological compounds targeting actin binding proteins have been studied in preclinical research [291, 292, 293], none of such therapeutic agent has been used in the clinic yet.

In study III, role of S100A9, a pro-inflammatory alarmin, in regulating inflammation and tissue damage was investigated in abdominal sepsis. Inhibition of S100A9 by a specific inhibitor, ABR-238901, decreased sepsis-induced neutrophil activation, cytokine formation

as well as damage to the lung tissue. In most studies, S100A9 is evaluated as heterodimer S100A8/A9 [245], Moreover, S100A9 was shown to play important role in stabilization of S100A8 protein [294, 295], and absence of S100A8 was found to degrade S100A9 [296], indicating importance of S100A9 and S100A8 for each other's stability. Thus, targeting S100A9 might affect the functions of S100A8 and subject of future study.

Since EC plays major role in sepsis, we investigated the transcriptomic changes in two major subgroups of endothelial cells during sepsis in study IV. Considering the genetic heterogeneity of sepsis patients, understanding the difference gene expression levels among the septic patients are indispensable for recognition of different phenotypes of sepsis and precision medicine treatment. It should be noted that the analysis of differential expression genes is limited by the algorithm and mathematics development, and rely on the threshold setting, such as p value. Thus, one should not ignore those genes which did not show any significant transcriptional changes because of the set threshold and limited number of experiments. One should keep in mind that protein activity rather than the mRNA abundance is important for biological functions of a gene, thus, further study should be taken before translating the results to the preclinical or clinical settings. Another limitation of transcriptional analysis is that sample preparations might influence the transcriptomic changes. Although we have tested few selected genes in freshly collected whole tissue, it does not exclude the possibility that some of the gene changes might be due to harsh sample processing. Nevertheless, our study provided details transcriptomic changes in lung endothelial subsets in sepsis and might provide new therapeutic strategies for sepsis patients.

Future perspective

Variations in incidence, prognosis and lack of uniformity in clinical criteria have imposed great challenge in developing effective therapies for sepsis patients. Diagnostic techniques should be simplified to accelerate the early detection of suspected patients. Timely ICU care and comprehensive inspection are required for patients with lung dysfunction. Standardization of immunological test to predict the clinical deterioration during sepsis might be a potential method to improve the differential diagnosis. Although mechanical ventilation is vital for treatment of septic-induced respiratory disturbance, ventilator-associated pneumonia remains as a major concern in ICU. The better treatment often needs to be supplemented with other supportive therapies.

In recent time, a great deal of attention has been given to the innate immune mechanisms of sepsis. Neutrophil acts as a first line of defense for killing the invading pathogens. Neutrophil related inflammatory mediators, such as procalcitonin and IL-6, have been considered as useful biomarkers for assessing the severity of sepsis. In sepsis patients, procalcitonin level measurement has been recommended to determine the duration of anti-microbial treatment [55, 297]. S100A9 and its heterodimer have the similar potential to be used as diagnosis biomarkers as well as therapeutic targets, but more studies are required to strengthen the hypothesis. To defend the host from pathogens, neutrophils release NETs. However, NETs can cause damage to the vasculature and pulmonary tissues. Interestingly, recombinant human DNase (rhDNase) has been used for the treatment of cystic fibrosis since 1990, which was found to successfully lower the risk of clinical exacerbation [298]. One study reported that rhDNase (Pulmozyme®) help to reduce the number of most microorganisms [299]; On the other hand, another study argued that the effect of Pulmozyme® is accomplished by decreasing the excessive DNA released by disintegrated leukocytes [299]. Thus, treatment targeting NETs seems to be a useful strategy. Nevertheless, *S. pneumoniae* was found to increase in the rhDNase treated group [299]. Similarly, another study reported that Pulmozyme® treatment enhanced bacterial dissemination in the lung and peritoneal cavity 6h after CLP, and did not alter overall survival [300]. Although DNase treatment effectively digested NETs in the circulation, the treatment also totally abandoned the antibacterial function of NETs, especially in the early phase of infection. Thus, more investigations are needed to establish effective NET-specific treatment strategy based on different conditions, such as intervention time-points, type of infections etc.

Actin dynamics are complex and widely needed for a variety of cellular functions. Actin

remodeling have been involved in both neutrophil and endothelial cell functions, especially in regulating neutrophils transmigration through the endothelium. It has been suggested that the transmigration of leukocytes can also be regulated by molecules that doesn't participate in the process of rolling and adhesion [301, 302], indicating that actin might be also involved in neutrophil transmigration. Actin dynamics might also be involved in increased endothelium permeability in sepsis, thus, functions of actin in these two cell types are needed to be studied independently. Several pharmaceutical actin manipulating molecules have emerged to target endothelium barrier functions; effect of these molecules need to be verified in neutrophils as well. Moreover, the effect of actin in blocking NET formation also raises some concerns. Since NET plays dual functions during the immune response, balancing its functions might be critical for desired outcomes. Thus, understanding the effect of new therapies in both neutrophils and endothelial functions during sepsis is important for better and safe outcomes.

Despite significant technological improvements, sepsis still remains as a global health problem. Although current therapeutic strategies in clinic focusing on restoring and/or maintaining adequate organ functions, a better understanding of the mechanisms regulating sepsis and septic lung injury would be very helpful to optimize current therapies for sepsis. In addition, both neutrophils and endothelial cells work synergistically in the body and involve in pathophysiology of many diseases. Although their functions under different conditions could vary a lot, we anticipate that our findings could be extended to other disease processes and might be useful to control excessive inflammation in future.

Populärvetenskaplig sammanfattning

Ordet ”Sepsis”kommer från det grekiska ordet ”Sepo”som betyder ”jag ruttnar”. Beskrivningar av sepsis kan spåras tillbaka till tiden för den store läkaren Hippokrates (470 f.Kr.). Enligt honom är sepsis en farlig ruttnande process i vår kropp. Med medicinska framsteg definieras sepsis nu som ett syndrom av livshotande organdysfunktion orsakad av ett dysregulerat värdsvar på infektion. De organ som drabbas mest av sepsis är lungor, njurar och det kardiovaskulära systemet. Med en dödlighet på 40 till 60% på intensivvårdsavdelningar har sepsis och septisk chock blivit det största problemet och bördan för offentliga hälsovårdsutgifter.

Trots förbättringar i den kliniska behandlingen av sepsis har en ökning av sepsis observerats under de senaste decennierna. Nuvarande terapeutiska metoder är begränsade till stödjande vård, det finns ingen specifik och effektiv behandling än så länge. En av de viktiga orsakerna kan vara att sepsis förvandlas till flera processer på mycket kort tid. Ett annat problem är att särskilja och förutsäga de efterföljande infektionsutfallen i den tidiga fasen av sepsis. Ökningen av den gamla befolkningen, åtföljda kroniska sjukdomar, nedsatt immunförsvar och utbredd resistens mot antibiotika gör alla sepsis situationen komplex och svår att behandla med befintliga terapier.

Okontrollerade överdrivna inflammatoriska svar är den främsta orsaken till sepsisinducerad organskada. Värdceller, såsom neutrofiler, endotelceller, makrofager, är huvuddeltagarna i det tidiga inflammatoriska svaret. Neutrofiler är en av de viktigaste immuncellerna som är utrustade med flera funktioner, såsom antimikrobiell, fagocytos och kemotaxi. Under inflammation frisätter neutrofiler nätliknande struktur för att döda invaderande patogener, så kallade neutrofila extracellulära fällor (NET). NET är sammansatt av nätliknande kromatinstruktur dekorerad med olika nukleära och cytoplasmatiska proteiner. Endotelceller utgör det inre slemhinnan i våra blodkärl, som reglerar ämnesutbytet mellan blod och vävnader, cell-cell-interaktioner och koagulation. Grundläggande cellulär och molekylär biologi har varit ett nyckelområde för sepsisstudier med målet att förstå de specifika mekanismerna bakom värdens svar på infektion.

I den första studien fokuserade vi på c-Abl kinas och regleringen av NET bildning och lungskada under sepsis. Vi undersökte aktiviteten av c-Abl i den septiska lungan och fann att den är signifikant ökad under sepsis. Sedan kontrollerades c-Abls roll för NET bildning, och fann att hämning av c-Abl inte bara hämmade NET-bildningen in vitro utan också minskade lungskadan. Dessutom visade det sig att syreradikaler var viktiga för NET bildningen. Aktinrelaterat protein 2/3-komplex (Arp2/3-komplex) är den huvudsakliga

kärnbildningsfaktorn som ansvarar för aktinpolymerisation. Vi fann att hämning av Arp 2/3-komplex signifikant minskade F-aktinpolymerisationen, vilket effektivt stoppade NET utsöndringen in vitro och minskade NET-bildningen i lungvävnader på motsvarande sätt. Intressant nog observerade vi också minskning av neutrofilinfiltrationen i det bronkoalveolära utrymmet, vilket kan bero på det faktum att neutrofiler använder aktindynamik under transmigrering. Dessa fynd antydde att NET utsöndring och neutrofilmigrering regleras av Arp 2/3-komplexe under sepsis.

Genom att känna till NET pro-inflammatoriska roll fokuserade vi i den tredje studien ett NET-relaterad cytoplasmatiskt protein, kallat S100A9. S100A9 tillhör alarmin-familjen och är känt för att reglera pro-inflammatoriska funktioner i många sjukdomar, såsom vid hjärtinfarkt och cancer. Vi fann att nivåerna av S100A9 ökade signifikant både i plasma och lungvävnad hos septiska djur. Blockering av S100A9 med en specifik hämmare, ABR-238901, minskade sepsisinducerad neutrofilaktivering, cytokinbildning samt skada på lungvävnaden. De underliggande mekanismerna, hur S100A9 reglerar pro-inflammatorisk processer återstår att utforskas i framtida studier.

Lungskada under sepsis förmedlas av flera processer. Förutom neutrofilinducerad vävnadsskada spelar endotelceller i mikrokärlet också en avgörande roll i sepsis-processen. Det finns grundläggande strukturella och funktionella variationer mellan mikrovaskulära segment i samma organ eller mellan organ. I den fjärde studien undersökte vi de globala transkriptomiska förändringarna i lungkapillärendotelceller och lungpostkapillära venoler efter induktion av sepsis genom nästa generations sekvensering. Vi fann att dessa två endotelsubtyper är transkriptionellt olika och svarar olika vid sepsis. Till exempel visade sig kapillära endotelceller vara berikade med gener som reglerar koagulation, vaskulär permeabilitet och sårläkning; medan postkapillära venoler berikades med gener som reglerar kemotaxi, cell-celladhesion och aktinfilamentpolymerisation. Vi identifierade flera nya gener och kandidattranskriptionsfaktorer av potentiell betydelse vid sepsis. Den funktionella rollen för tidigare outforskade gener samt möjligheten att använda några av dessa gener som tidiga markörer för lungskada är föremål för framtida experimentella och kliniska studier.

Sammanfattningsvis visade denna avhandling flera nya mekanismer involverade i neutrofilberoende septisk lungskada. Flera potentiella terapeutiska mål, såsom c-Abl, Arp2/3-komplex och S100A9 visade lovande resultat. Sålunda kan inriktning på några av dessa molekyler vara ett användbart sätt att lindra sepsisinducerad vävnadsskada.

Acknowledgements

I would like to take this opportunity to acknowledge all my supervisors, collaborators, colleagues, and friends who have helped me to make this thesis possible.

First of all, I would like to express my sincerest gratitude to my main supervisor **Henrik Thorlacius**. Thank you for offering me the opportunity to study here as a PhD student, for introducing me to this wonderful research area, and sharing your brilliant inspirations with me. Your insightful advice, continuous guidance and constant encouragement always helped me correct false starts, solve problems, and overcome any setbacks I encountered. I also appreciate your heartfelt concern about my health after I came back to Sweden from the lockdown, which showed me that I have strong support whenever I need it. I am incredibly lucky to have you as my main supervisor.

I am deeply grateful to my co-supervisor **Milladur Rahman**. I will never be able to express how much you have helped me from the very beginning until the end of my study. You always helped me go through all the detailed parts of a project, and I would have definitely made many more mistakes without your patient reminders. Your boundless optimism and comfort was a consistent positive force on my life, and I am so glad to have been your student.

Many thanks to my co-supervisor **Yongzhi Wang** for not only taking care of my studies, but also for helping me solve many problems in life. From looking for an apartment to going to the hospital, you helped me so much when everything was new to me. It's been a delightful journey to work with you.

I am truly thankful to my co-supervisor **Carl-Fredrik Rönnow**. Thanks for helping me with the revision work of my papers, I learned a lot from these experiences. Your patience and kindness whenever I needed your help has been a huge support during my time here.

My special thanks to **Anne-Marie Rohrstock**. You are so kind and your consideration and thoughtfulness comforted me a lot when I was new to our lab. You take great care in what you do and meticulously make sure everything works properly. You also take care of me and protect me a lot, just like my mom. It's been a great pleasure to share an office with you.

There are no words to describe my gratitude to **Cheng Luan** for showing me the Western Blot technique, answering all my questions about research, talking with me when I feel confused and encouraging me when I want to give up. I've drawn energy from your kindness

and thoughtfulness. I will always respect you.

My deepest appreciation to **Helen Sjögren** for your understanding, encouragement and genuineness. Thanks for sharing your insights, vision, and professional experience with me, and for listening to my complaints, offering practical suggestions and helping me open my mind. I am indebted to you so much for being my mentor, and it is an experience I will never forget.

I am grateful to my collaborators, **Alexandru Schiopu** and **Gabriel Jakobsson**, thank you for your trust, inspired discussions and valuable advice.

Special thanks to all my colleagues,, **Avin Hawez**, **Raed Madhi**, **Erik Wetterholm**, **Johan Linders**, **Nader Algethami**, **Anwar Algaber**, **Dler Taha**, **Amr Al-Haidari**; thank you all for helping me get familiar with lab techniques and for the incredible discussions of all things science. Particularly to **Feifei Du**, for being a professional colleague and helping me finish several projects. **Richard Garland Averitt V**, thank you for helping me on my projects and being my friend. I will always remember the joyful chats with you, even if we sometimes had differing opinions.

Fortunately I have met a lot of friends during my study time in Sweden. **Yanni Li** and **Naiqi Zhang**, it's been amazing to stay with you by the sea, to travel with you over Sweden, and to spend the Chinese New Year together. Thanks for offering me selfless help in my toughest times. **Wuqing Huang**, and **Ming Sun**, thank you for accompanying me when I feel lonely. I can't help but laugh when I remember those days we spent together. **Mi Huang** and **Jingxue Pan**, I have been lucky to live in the same building with you, and you've helped me as if we were family. **Yingying Ye**, thanks for all your help. I had a delightful time with your little daughter, she is like an angel. **Qing Liu** and **Yusheng Wang**, thanks for all your help. From the first day I came to Sweden, you always made me feel at home. Qing, I love the snacks you have cooked for me and enjoy playing the piano with you. Thank you all for those lovely lunch talks and happy times.

My sincere thanks to the **China Scholarship Council**. Your funding allowed me to finish my work without worries, and for that I will be eternally grateful.

Countless thanks to all my teachers, who have enriched and enlightened my life.

Many thanks to my parents and grandparents, I am lucky to be the apple of your eye.

To all my dearest friends, no names are mentioned here but you know you are an invaluable treasure to me forever. Particularly **Xiaoling Zhou**, we haven't seen each other for a long time, but I know nothing can break our friendship. **Tianyu Zhao**, our exchange of thoughts on books, poems, and films has been one of the most fantastic parts of my life. **Mengni Peng**, thanks for your endless love and unconditional support, you have always been there for me.

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Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

About the author

Zhiyi Ding is a medical graduate from China. She completed her medical degree at Xiamen University in 2017. The focus of this doctoral thesis is to investigate new mechanisms involved in neutrophil extracellular trap formation and pulmonary endothelial cell activation in septic mice, and explore the potential therapeutic strategies to alleviate pathogenic inflammation and pulmonary tissue damage in abdominal sepsis.

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FACULTY OF MEDICINE

Department of Clinical Sciences, Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:60
ISBN 978-91-8021-221-2
ISSN 1652-8220