University College London
Division of Surgery and Interventional Science
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Project title: **Can antibiotic modulate the immune system in sepsis?**
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Module: **SURG0161**

ABSTRACT

Sepsis is the dysregulated host response to an infection. Alongside supportive care, the sepsis treatment are antibiotics and managing the source of infection. It is shown that antibiotics influence the immune system, but their specific effects during sepsis remain unclear. I hypothesise that antibiotics directly impact immune cell function, potentially worsening immunosuppression in sepsis, particularly with broad-spectrum antibiotics.

We examine how both narrow- and broad-spectrum beta-lactam antibiotics affect immune function *in vivo* using peripheral blood mononuclear cells (PBMCs) isolated from patients attending the emergency department (ED) with infections.

PBMCs were treated *ex vivo* with Lipopolysaccharide (LPS) to evaluate monocyte function over 24 hours, and CD3/CD8 beads to evaluate CD4 lymphocyte function over 72 hours. These stimuli were applied both with and without the antibiotics Cefuroxime and Meropenem at clinically relevant low and high concentrations. The expression of standard functional markers on classical monocytes and CD4+ T lymphocytes was analysed using flow cytometry. Cell populations were identified using a Boolean gating strategy, selecting lymphocytes or PBMCs, single cells, viable cells, and specific cell markers. Fluorescence Minus One (FMO) controls were used to guide cell population gating for all fluorophores.

Cefuroxime and Meropenem are associated with anti-inflammatory changes including downregulation of monocyte class II-associated invariant chain peptides (CLIP), and CD14. Additionally, Meropenem resulted in a reduction in monocyte HLA-DR and NOX-2. CD4 lymphocyte CTLA-4 expression was increased with an associated reduction in cell proliferation. Additionally, Cefuroxime was associated with an increase in anti-inflammatory cytokines IL-4 and IL-10. In conclusion, beta-lactam antibiotics exacerbated features of sepsis-induced immunosuppression in monocytes and CD4 lymphocytes *ex vivo*. At higher doses, this might become more profound. Further research is required to determine the clinical implications.

Table of Contents

LIST OF ABBREVIATIONS

Areg - Amphiregulin

CCR2 - C-C chemokine receptor type 2

CD127 - Cluster of Differentiation 127 (Interleukin-7 receptor alpha)

CD152 - Cluster of Differentiation 152 (Cytotoxic T-Lymphocyte-Associated Protein 4, CTLA-4)

CD194 - Cluster of Differentiation 194 (Chemokine receptor, CCR4)

CD196 - Cluster of Differentiation 196 (Chemokine receptor, CCR6)

CD274 - Cluster of Differentiation 274 (Programmed death-ligand 1, PD-L1)

CD274 - Cluster of Differentiation 274 (Programmed death-ligand 1, PD-L1)

CD279 - Cluster of Differentiation 279 (Programmed cell death protein 1, PD-1)

CD28 - Cluster of Differentiation 28

CD284 - Cluster of Differentiation 284

CD3 - Cluster of Differentiation 3

CD4 - Cluster of Differentiation 4

CD64 - Cluster of Differentiation 64

CD74 - Cluster of Differentiation 74 (Invariant chain, Ii)

CD80 - Cluster of Differentiation 80 (B7-1)

CD95 - Cluster of Differentiation 95 (Fas receptor)

CIITA - Class II Major Histocompatibility Complex Transactivator

EGFR - Epidermal Growth Factor Receptor

FOX-3 - Forkhead box P3 (commonly known as FOXP3)

HLA-DM - Human Leukocyte Antigen - DM isotype

HLA-DP - Human Leukocyte Antigen - DP isotype

HLA-DR - Human Leukocyte Antigen - DR isotype

IFN-y - Interferon gamma

IL-10 - Interleukin-10

IL-17a - Interleukin-17a

IL-1b - Interleukin-1 beta

IL-2 - Interleukin-2

IL-4 - Interleukin-4

MHC I - Major Histocompatibility Complex class I

MHC II - Major Histocompatibility Complex class II

NF-kB - Nuclear Factor kappa-light-chain-enhancer of activated B cells

NLRP3 - NOD-like receptor thermal protein domain associated protein 3

NOX-2 - NADPH oxidase 2

STAT5 - Signal Transducer and Activator of Transcription 5

T-bet - T-box expressed in T cells (officially known as TBX21)

Th1 - T helper type 1 cells

Th1 - T helper type 1 cells

TLR4 - Toll-like receptor 4

TNF-a - Tumour Necrosis Factor alpha

1. INTRODUCTION

Sepsis is a major health care burden with the mortality rate is high (between 15% and 20%), and >40% increase in the risk of death especially with gram-negative bacteria (Breijyeh, Jubeh and Karaman, 2020). The infection triggers both innate and adaptive response. This affects the level of immunoregulatory molecules, and effector immune cells, resulting in tissue damage. Although one of the main treatments of sepsis are antibiotics, it was revealed to cause side effects, and worsen the condition of sepsis-induced immunosuppression. In parallel, The World Health Organisation (WHO) has declared antimicrobial resistance (AMR) a global health emergency, making medications for treating infection less effective, which could result in 10 million deaths per year.

1. 1 Immune System

The immune system consists of innate and adaptive components that work together to provide defence against pathogens. Innate immunity, the initial line of defence, is rapid and non-specific. The adaptive immune response is a more specific and long defence against pathogens.

The innate immune system is activated when pattern recognition receptors (PRRs) on immune cells detect pathogen-associated molecular patterns (PAMPs) from microbes and damage-associated molecular patterns (DAMPs) released during tissue damage (Christophides et al., 2002). This activation triggers the production of pro-inflammatory cytokines and chemokines, leading to the recruitment of neutrophils and macrophages. Proteins like kinases and transcriptional regulators are subsequently activated, contributing to signal transduction pathways (García-Patiño, García-Contreras and Licona-Limón, 2017)(Christophides et al., 2002). After immune recognition, the effector response leads to pathogen elimination through phagocytosis. This induces the activation of antigen-presenting cells (APCs), such as macrophages, which present antigens via HLA class II molecules to helper T cells, initiating adaptive immune responses. Activated innate immune cells contribute to tissue inflammation by releasing pro-inflammatory mediators like TNF-a, IL-1, and IL-6, which can lead to the production of reactive oxygen species.

Alongside innate immune responses, tissue inflammation involves the infiltration of adaptive immune cells (Yi et al., 2019). Adaptive immune cells, including T and B lymphocytes, convey antigen-specific immune responses. T cells differentiate into effector T cells, producing pro- or anti-inflammatory cytokines, while B cells differentiate into plasma cells, generating specific antibodies (Sage et al., 2019)(Saigusa, Winkels and Ley, 2020). Adaptive immunity is highly specific, mediated by T and B cells. T cells engage in a multi-step response beginning with the interaction of

T cell receptors (TCRs) with antigens presented by MHC complexes, followed by balancing signals from immunological checkpoints, which involve in stimulatory and inhibitory molecular pairs such as CD28/CD80-CD86 for stimulation and PD-1/PD-L1 for inhibition (Dai et al., 2017). Cytokine stimulation then promotes T cell proliferation and differentiation, enhancing cell activation (Dai et al., 2017) (Gerner et al., 2013). B cells works together with T cells with their ability to differentiate between self and non-self, ensuring recognition of foreign antigens over self-antigens. In collaboration with CD4+ T cells, B cells respond to various signals, including antigen recognition, immune checkpoints, and cytokine stimulation. Subsequently, B cells develop a memory capacity that allows for the recollection of past antigen encounters. This memory attribute significantly enhances future interactions, facilitating a more effective and rapid immune response (Althuwaiqeb and Bordoni, 2022).

1. 2 Antibiotics and immunity

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock is when profound circulatory and cellular abnormalities result in multiorgan failure (Singer, Deutschman and Seymour, 2016). Sepsis results in a failure to establish a balance between excessive and inadequate inflammation, causing severe organ damage and significant immunosuppression, which is an extreme inflammatory reaction to infection (Kovach and Standiford, 2012).

Initially, pathogen associated molecular pattern (PAMPs) from microorganisms is recognised by pattern recognition receptors (PRRs). PRR activation induces the gene transcription, and increases the synthesis and release of pro- and anti-inflammatory mediators, including cytokines by innate immune cells (neutrophils and monocytes) (van der Poll et al., 2017). The proinflammatory cytokines can result in an excessive amount of inflammation known as a cytokine storm, which may cause hypotension and organ failure (Boekstegers et al., 1991). This triggers a simultaneous anti-inflammatory reaction, releasing cytokines such as IL-10. This appears to cause a decrease in chemotaxis, and an increase in apoptosis of peripheral blood mononuclear cells (PBMCs) and splenocytes, resulting in immunosuppression. This leads to a decrease in chemotaxis, and an increase in apoptosis PBMCs and splenocytes, causing immunosuppression that affect both innate and adaptive responses (Boomer et al., 2011b). Additionally, CD4+ and CD8+ T-cell, B-cell and natural killer cells undergo increased apoptosis or exhaustion, further impairing effector and memory responses (Brady, Horie and Laffey, 2020). This immunological impairment is characterised by the reduction of IFN-y and TNF-a by T cells and a rise in checkpoint regulators like PD-1, leading to diminished pro-inflammatory cytokine production (Hotchkiss et al., 2002). T-helper cell polarisation from a pro-inflammatory Th1 phenotype towards an anti-inflammatory Th2 phenotype also leads to immunosuppression (Clinical Laboratory int., 2020).

Furthermore, decreased expression of HLA-DR, an important molecule for antigen presentation, along with diminished expression of co-stimulatory molecules such as CD80, contributes to impaired release of proinflammatory cytokines and reduced antigen-presenting capabilities. This results in lymphocyte anergy or death, thereby increasing the risk of nosocomial infections and mortality (Asalluah et al., 1995).

1.3 Antibiotics and immunity

The most commonly prescribed antimicrobials in the critically ill population include broad spectrum beta-lactams, particularly Meropenem, narrow-spectrum beta-lactams, Cephalosporins (particularly newer generations e.g. Cefortamine), Macrolides and Tetracyclines (Gonçalves-Pereira and Póvoa, 2011). Early and timely administration of appropriate antibiotics is crucial for improving the clinical outcomes of septic patients (Kumar et al., 2006)(Puskarich et al., 2011). In cases of septic shock, despite some conflicting evidence (Ryoo et al., 2015), any delay in treatment is linked to higher mortality rates and negative outcomes, including longer ICU stays and severe organ injuries, as measured by the Sepsis-Related Organ Assessment score (Rhodes et al., 2017). Furthermore, the correct dosage regimen are crucial for achieving clinical and microbiological success (Bloos et al., 2017).

There is a growing body of evidence supporting the immunomodulating effects of antibiotics, which may be either beneficial or harmful (Anderson et al., 2010). However, data on the immunomodulatory effect of beta-lactams in sepsis is limited, despite these being the most commonly used antibiotics and the highest risk population.

Our study focus particularly on the pathways involved in immunosuppression during sepsis as seen in figure 1. This includes monocyte antigen presentation and co-stimulation, such as CD80, CD86, and HLA-DR as well as immune checkpoint inhibition, including PD1 on lymphocytes and PD-L1 on monocytes. Additionally, we examined receptors related to lymphocyte proliferation and maturation, specifically the IL-2 and IL-7 receptors. Other areas of interest included lymphocyte activation markers CTLA-4 and CD28, and overall lymphocyte viability. Our comprehensive analysis also covered monocyte chemokine receptors, specifically CCR2 and CXCR4, and intracellular cytokines to provide a thorough understanding of the immune response in sepsis.

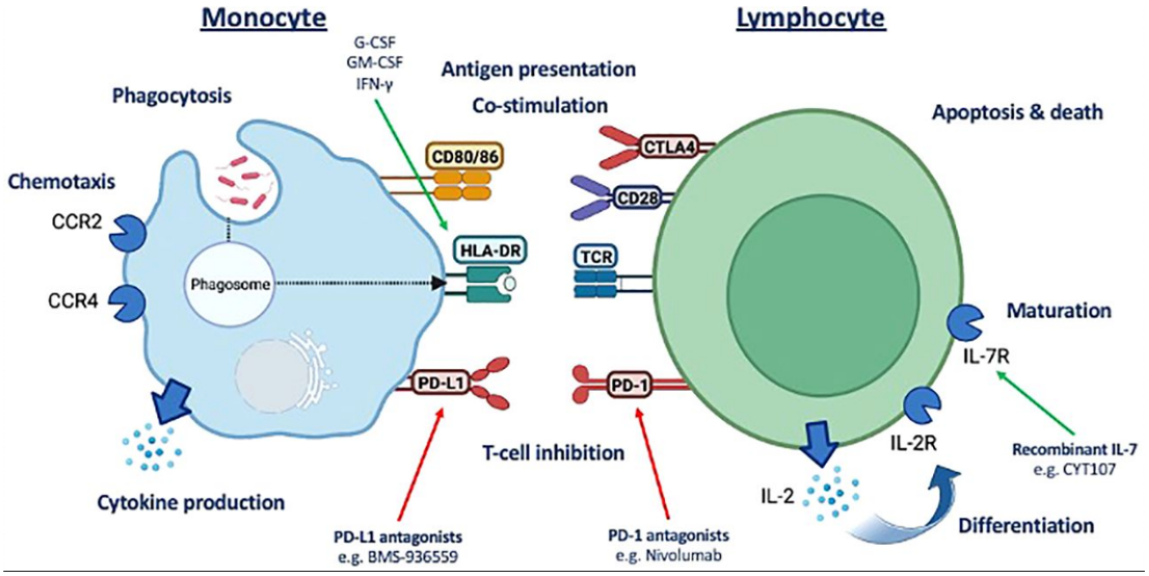


Figure 1: Monocyte and T-Lymphocyte Pathways (Arthur et al., 2024).
This figure illustrates key immune pathway targets in monocytes and T-lymphocytes that can be modulated through pharmacological intervention, particularly in contexts like sepsis and surgical immune responses. It details functions like phagocytosis in monocytes and co-stimulation in T-lymphocytes, highlighting targets such as CD (cluster of differentiation), HLA-DR, and immune checkpoints like PD-1 and CTLA-4. These markers represent potential interventions to modulate immune suppression seen in sepsis.

1.4 Aims and Hypothesis

I hypothesise that antibiotics have an off-target effect on immune cells, exacerbating immune dysfunction associated with sepsis; with broad-spectrum antibiotics having a greater effect.

My objective is to examine the *ex vivo* immunomodulatory effects of narrow and broad-spectrum beta-lactam antibiotics on peripheral blood mononuclear cells obtained from patients who attend the ER. We focused on assessing characteristics associated with sepsis- induced immune dysfunction.

2. MATERIALS AND METHODS

Our study, conducted at University College London Hospital, included adults admitted to the emergency department with infections and healthy volunteers working in the university.

2.1 Ethics

The acquisition of clinical samples and data was obtained from Queen Square Research Ethics Committee provided ethical approval (REC reference 20/LO/1024). The ethics for obtaining healthy volunteer samples and data was provided by The University College London Research Ethics Committee (REC reference 19181/001).

2.2 Product

Product	Catalogue No
LPS	Millipore LPS25
CD2-3-28 beads	MB 130-091-441
Cefuroxime	Flynn PL13621/0019
Meropenem	Milpharm 16363/0444
BL True-Nuclear™ kit	BL 424401
BL Cell staining buffer	BL 420201

Table 1 outlines the products used in the experiment, including crucial reagents and tool. This includes LPS for immune stimulation, CD2-3-28 beads for cell activation, studied antibiotics (Cefuroxime and Meropenem), buffer and BL True-Nuclear kits for nuclear staining and cell preparation in flow cytometry.

2.3 Methods

2.3.1 Defrosting stored PBMCs

PBMCs are taken out of liquid nitrogen storage. At room temperature, 1 mL of culture Roswell Park Memorial Institute medium (RPMI) was transferred to the cryopreserved PBMCs and pipetted to suspend the PBMCs. Upon partial thawing, the liquid was transferred into 10mL medium in a falcon tube mixed until samples were completely thawed. The cells were then centrifuged at 400 g for 5 minutes. After centrifugation, supernatant was discarded. Cells pellets were resuspended in 2.5 ml media to remove dimethyl sulfoxide found in the cryopreserved liquid/solution (Honge et al., 2017). Cells were centrifuged again and resuspended with 2 ml of

RPMI media before resting at room temperature for 1 hour. Cells were then plated into a 96- well plate at 200 µL per well.

2.3.2 Stimulation Of PBMC

Meropenem and Cefuroxime were dissolved in water to create stock solutions before diluting with phosphate-buffered saline (PBS) to achieve working concentrations. Antibiotics were diluted to working concentrations in media and added to 96- well plates to achieve final desired concentrations. Lipopolysaccharide (100ng/mL) was used for monocyte stimulation (24hrs) and CD3-CD28 beads used for lymphocyte stimulation (72 hrs) in separate wells. The antibiotic-containing media (± LPS) was replaced every 24 hours.

2.3.3 Cell Staining

For cell staining following stimulation, cells were centrifuged (400 rcf, 5 min) and the supernatant is collected for ELISA. Cells are then resuspended in a 30µl/well cell staining buffer and labelled with a panel of antibodies tagged with various fluorochromes to detect specific surface markers on monocytes and lymphocytes. After incubation at room temperature for 30 minutes, cells were centrifuged and washed with a staining buffer, followed by resuspended in 50µl/well with Nuclearfix, stopping cell process. Next, cells are incubated at 4°C for 45 mins, followed by centrifugation. They are then resuspended in 30µl/well with Nuclearperm, a permeabilization solution, allowing antibodies to stain for cytokines and pass through the cell. After a final incubation at 4°C for 40 minutes and subsequent centrifugation, the cells are resuspended in a staining buffer (Rodig, 2022). This method ensures both surface and intracellular markers are adequately labelled for subsequent detection and quantification by flow cytometry. Details of products and concentrations used are provided in table 2.

Monocytes				Lymphocytes			
Antibody	**Fluorochrome**	**Catalogue no**	**Dilution**	**Antibody**	**Fluorochrome**	**Catalogue no**	**Dilution**
CD11b	SBUV445	MCA711SBUV445	1:250	CD3	SBUV445	BR MCA463SBUV445	1:250
CD14	SBB580	MCA1568SBB580	1:250	CD4	BUV805	TF 368-0047-42	1:250
CD16	SB702	67-0168-42	1:250	CD8	APC-Fire 750	BL 301066	1:250
HLA-DR	BUV805	748338	1:250	CD19	BUV395	TF 363-0198-42	1:250
CD64	BUV737	612776	1:250	CD25	SBV570	BR MCA2127SBV570	1:250
CD66a	BUV563	741410	1:250	CD28	BUV496	BD 741168	1:250
CD66b	BB515	564679	1:250	CD95	BUV615	BD 752346	1:250
CD74	BV650	743734	1:250	CD127	PerCP-eFluor 710	TF 46-1278-42	1:250
CD80	BV480	751735	1:250	CD152	AF532	Nov NBP2-50286AF532	1:250
CD86	BUV496	749895	1:250	CD194	BUV563	BD 752566	1:250
CD192	BUV395	747854	1:250	CD196	BV786	BD 563704	1:250
CD274	RB545	756359	1:250	CD274	FITC	BL 393606	1:250
CD284	SB600	63-9917-42	1:250	CD279	BV480	BD 566112	1:250
EGFR	BUV661	750827	1:250	EGFR	BUV661	BD 750827	1:250
HLA-DM	APC	130-124-252	1:250	HLA-DR	BV711	BD 563696	1:250
HLA-DP	RB780	755757	1:250	Live-Dead	Zombie NIR	BL 423106	1:1000
Live/Dead	Zombie NIR	423106	1:1000				

Monocytes				Lymphocytes			
Antibody	**Fluorochrome**	**Catalogue no**	**Dilution**	**Antibody**	**Fluorochrome**	**Catalogue no**	**Dilution**
IL-1b	AF750	FAB10349S-100UG	1:100	IL-2	BV650	BL 500334	1:100
IL-10	BB700	566567	1:100	IL-4	PE-Cy7	BD 560672	1:100
IFN-g	BV750	566357	1:100	IL-10	BUV737	TF 367-7108-42	1:100
TNF-a	BV785	502948	1:100	IL-17A	APC-R700	BD 565163	1:100
Amphiregulin	PE	12-5370-42	1:100	IFN-g	BV750	BD 566357	1:100
CIITA	DY680	NBP2-59072FR	1:100	Amphiregulin	PE	TF 12-5370-42	1:100
NF-kb p65	PE-CF594	565447	1:100	Fox-p3	PE-Cy5	TF 15-4776-42	1:100
NLRP3	AF405	IC7578V-100UG	1:100	NF-kb	PE-CF594	BD 565447	1:100
Nox-2	PE-Cy7	NBP1-41012PECY7	1:100	STAT5	RB780	BD 568759	1:100
				T-bet	BV605	BL 644817	1:100

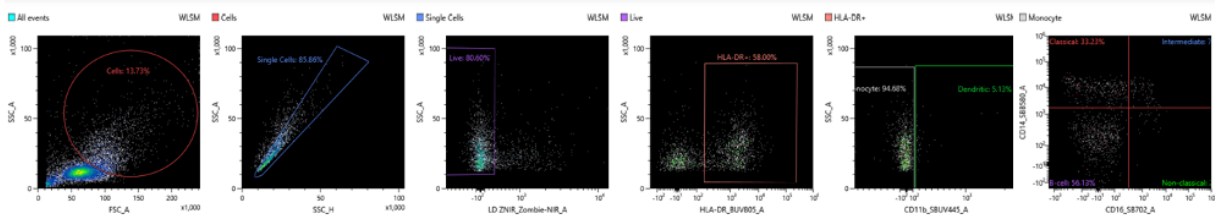
Table 2 provides a detailed list of antibodies used for staining monocytes and lymphocytes, indicating the specific fluorochrome, catalog number, and dilution for each. The antibodies target various cell surface markers, antigen presentation markers, and functional proteins such as cytokines and transcription factors, essential for identifying and analyzing different immune cell subsets via flow cytometry. The table is organized into separate sections for monocytes and lymphocytes, ensuring clarity and ease of reference for immunological assays.

2.3.4 Analysis

Cells were acquired on the Sony ID7000 spectral flow cytometer. Compensation controls were applied to all samples prior to analysis. Compensation was established using single-stained controls with BD Biosciences beads or cells stained with Live/Dead markers to adjust for spectral overlap between fluorochromes, ensuring channel-specific fluorescence detection. Fluorescence Minus One (FMO) samples for all fluorophores were run to guide gating of cell populations. This is to determine the boundary between negative and positive populations in multicolour flow cytometry panels. Cell populations of interest were identified using the following Boolean gating strategy which included the selection of lymphocytes or PBMCs, single cells, viable cells, and specific cell surface markers.

Differences in cytokine production and phenotypic expression between sepsis and non-sepsis were analysed using a two-tailed nonparametric Mann-Whitney U test with GraphPad Prism. Statistical significance was set at $p \leq 0.05$. The mean fluorescence intensity (MFI) of each marker is converted to MESF units using the Quantum Bead MESF standard curve. Running Quantum MESF beads simultaneously with subject samples on the same flow cytometer allows for direct comparison across different subjects and times. The data are shown as median interquartile ranges or percentages counts. Flow cytometry results are displayed in percentage of positive cells, including interquartile range, as illustrated in figure 1.

Monocytes:



Lymphocytes:

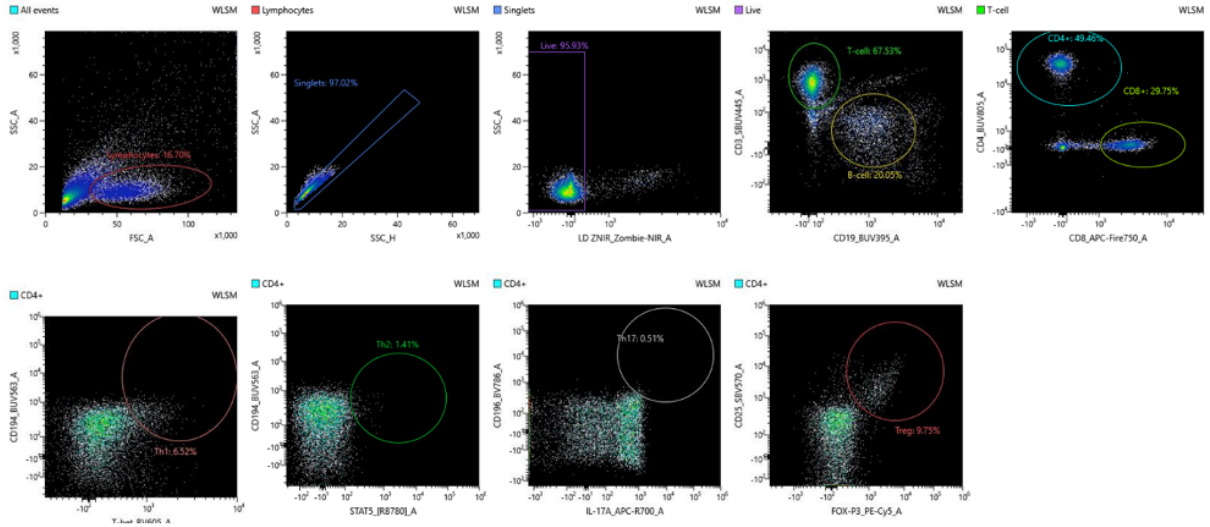


Figure 1 In the flow cytometry analysis of monocytes, gating strategy is applied to precisely identify and classify cells. Initially, monocytes are identified using a Forward Scatter (FSC) versus Side Scatter (SSC) plot that distinguishes them based on size and granularity. Then isolating single cells by excluding doublets, ensuring accuracy in the subsequent analysis. Following this, a live/dead discriminator, Zombie NIR, is used to separate live cells from dead ones, crucial for reliable results. Monocytes are then specifically gated based on the expression of HLA-DR. Finally, the analysis categories the monocytes into classical, intermediate, and non-classical subsets using the expression levels of CD14 and CD16, providing insights into functionalities of each immune responses.
Likewise, lymphocytes are identified on FSC) and SSC, plotted by their notably low side scatter and smaller size, characteristics typical of this cell type. Subsequent gating ensures that only single lymphocytes are analysed by excluding doublets, enhancing the precision of the data collected. The next step involves identifying live lymphocytes to ensure that the analysis includes only viable cells. In the later stages of gating, different T cells are distinguished based on their CD4 and CD8 expression. Various T cell subsets are identified based on the expression of CD4 and CD8. Additional panels show gating on B cells (CD19+), T regulatory cells (FoxP3+), and Th cells differentiated by cytokine profiles (IL-17A for Th17, STAT5 for activated T cells).

3. RESULTS

Twelve patients with sepsis and 4 healthy volunteers were examined. Percentage changes in cell phenotype percentages are presented in figures 3 and 4. These figures illustrate the expression patterns of specific cell markers, which have shown significant differences between groups.

3.1 Monocyte Stimulation

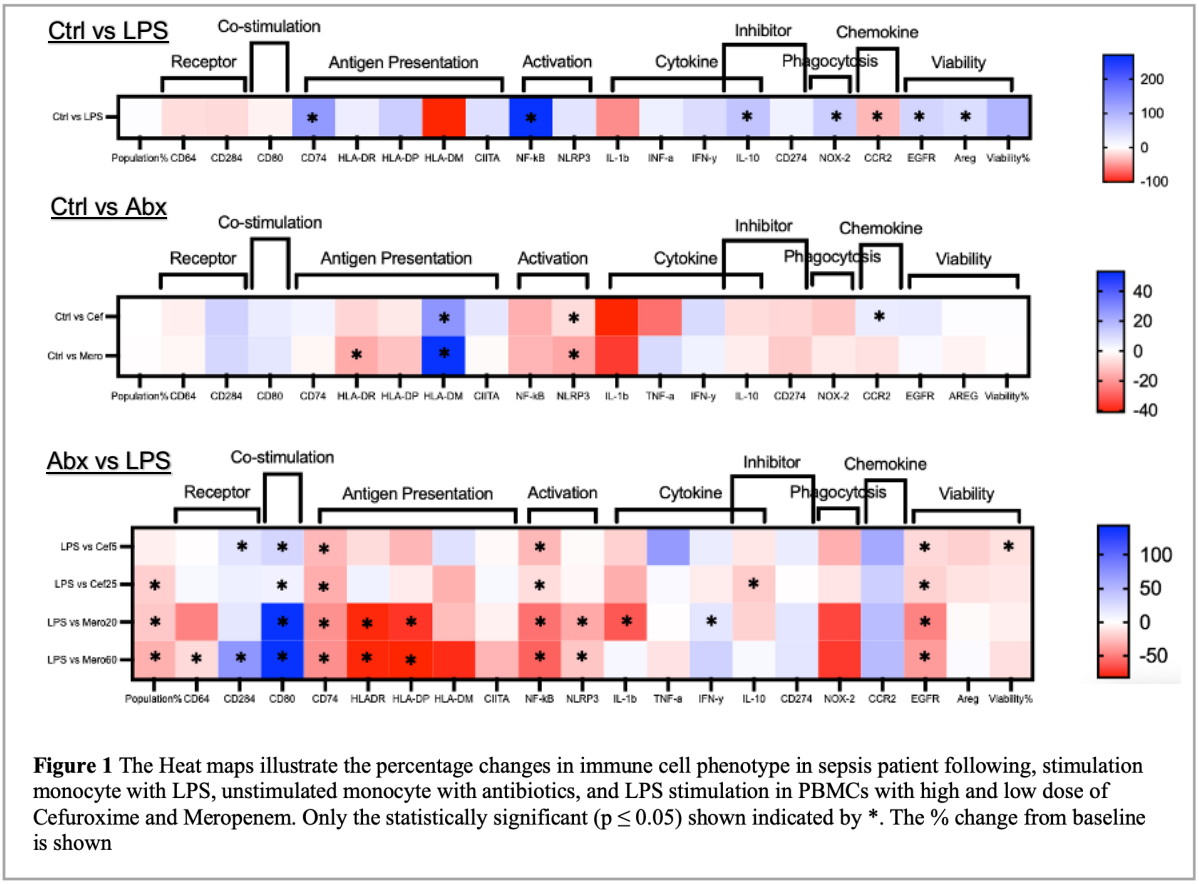


Figure 1 The Heat maps illustrate the percentage changes in immune cell phenotype in sepsis patient following, stimulation monocyte with LPS, unstimulated monocyte with antibiotics, and LPS stimulation in PBMCs with high and low dose of Cefuroxime and Meropenem. Only the statistically significant ($p \leq 0.05$) shown indicated by *. The % change from baseline is shown

3.1.1 Effect of LPS on Monocytes (Ctrl vs LPS)

Stimulation of monocyte with LPS resulted in a statistically significant increase in markers associated with antigen presentation (CD74; p=0.0078 and CIITA; p=0.0574). There was an upregulation in activation, NF-kB (p=0.0234). Likewise, there was an increase in intracellular cytokines, IFN-y (p=0.0574) and IL-10 p=0.0391), oxidative burst capacity (NOX-2; p=0.0391) and cell viability (p=0.0005) (Figure 3, Supplementary Figure 5).

3.1.2 Effect of Antibiotics on Unstimulated Monocytes (Ctrl vs Abx)

Co-incubation of unstimulated PBMCs with high dose Cefuroxime only resulted in an increase in monocyte markers associated with antigen presentation and cytokine, which were HLA-DM (p=0.0124) and IFN-y (p=0.0801), respectively. Likewise, there was an increase in chemokine, CCR2 (p=0.0455) (Figure 3, Supplementary Figure 5).

Co-incubation of unstimulated PBMCs with high dose Meropenem resulted in an increase in monocyte in monocyte antigen presentation markers, HLA-DM (p=0.0455) although HLA-DR was lower (p=0.0244). There was a reduction in activation markers, including NF-kB (p=0.0608) and NLRP3 (p=0.0005). However, Meropenem increased the cytokine level in TNF-a (p=0.0801) (Figure 3, Supplementary Figure 5).

3.1.3 Effect of Antibiotics on Stimulated Monocytes (LPS vs Abx)

Co-incubation of PBMCs with Cefuroxime resulted in lower in markers associated with cell viability in low and high dose (EGFR; p=0.0801, p=0.0175, respectively); however, percentage viability (p=0.0412) albeit only at low dose. At both the low and high doses, markers associated with antigen presentation, specifically CD74, were reduced (p=0.0027 and p=0.0244, respectively). Similarly, the activation marker NF-kB also showed reductions (p=0.0455 at the low dose and p=0.0601 at the high dose) (Figure 3, Supplementary Figure 6).

Co -stimulatory marker CD80 increased at low and high dose (p=0.0060 and p=0.0801 respectively). The cell surface receptor, CD284 (p=0.0455) also upregulated at low dose.

Co-incubation of PBMCs with Meropenem was associated with an increase in CD284 (p=0.0027 at high dose), co-stimulatory marker CD80 (p=0.0124 and p=0.0005 at low and high dose respectively), and IFN-y at low dose only (p=0.0801). CD64 (p=0.0080 at high dose), IL-1b (p=0.0801 at low dose), HLA-DR (p=0.0124 and p=0.0005 at low and high dose respectively), HLA-DP(p=0.0124 and p=0.0060 at low and high dose respectively), NF-kB (p=0.0064 and p=0.0124 at low and high dose respectively), NLRP3 (p=0.0610 and p=0.0450,at low and high dose respectively), and EGFR (p=0.0027 and p=0.024 at low and high dose respectively) were significantly reduced with meropenem co-incubation (Figure 3, Supplementary Figure 7).

3.2 CD4+ T Lymphocytes Stimulation

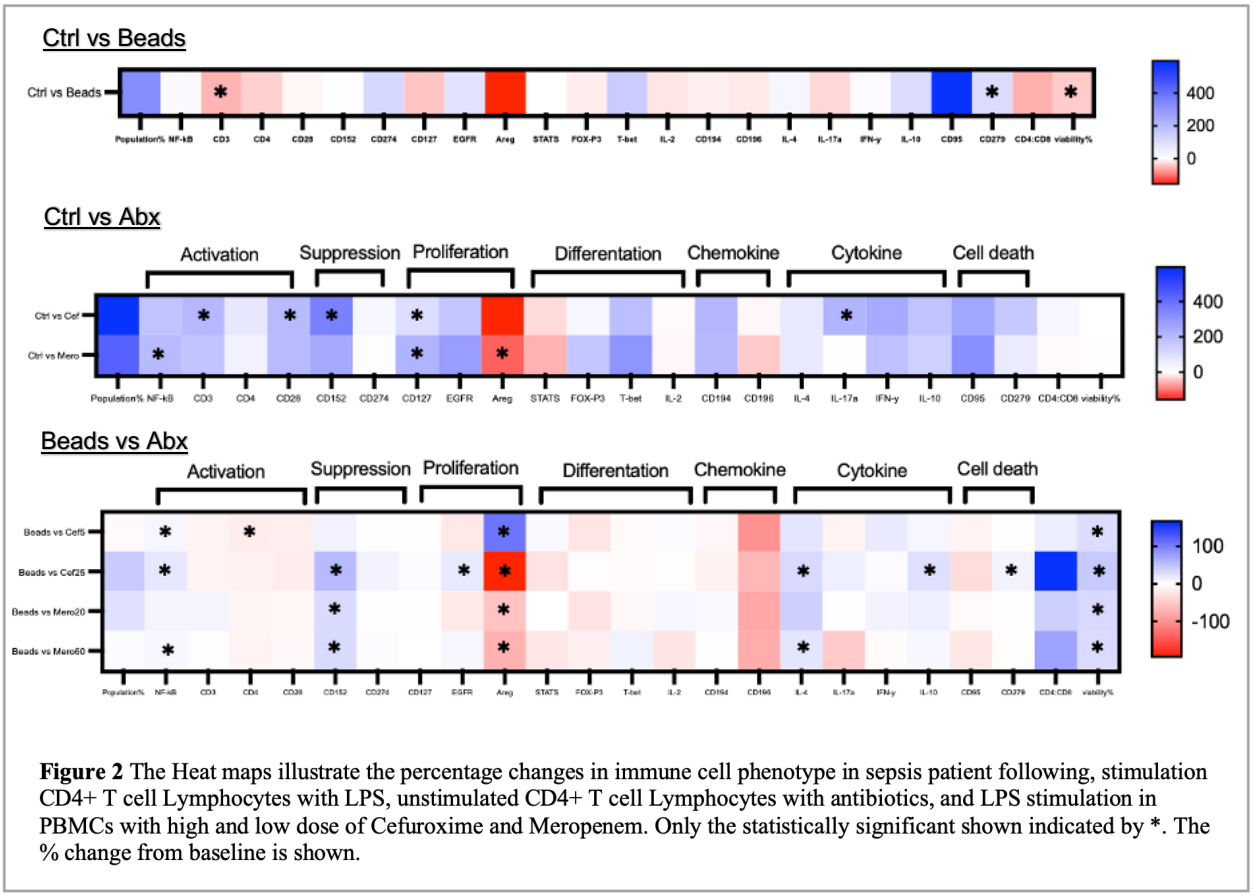


Figure 2 The Heat maps illustrate the percentage changes in immune cell phenotype in sepsis patient following, stimulation CD4+ T cell Lymphocytes with LPS, unstimulated CD4+ T cell Lymphocytes with antibiotics, and LPS stimulation in PBMCs with high and low dose of Cefuroxime and Meropenem. Only the statistically significant shown indicated by *. The % change from baseline is shown.

3.2.1 Effect of Beads on CD4+ T lymphocytes (Ctrl vs Beads)

Following beads stimulation of lymphocytes, the cell viability increased (p=0.0420). There is a downregulation in activation markers, including CD3 (p=0.0039) and CD4 (p=0.0645). However, the stimulation was seen to increase the marker associated with cell death, CD279 (p=0.0245) (Figure 4, Supplementary Figure 8).

3.2.2 Effect of Antibiotics on Unstimulated CD4+ T lymphocytes (Ctrl vs Abx)

Co-incubation of unstimulated PBMCs with high dose Cefuroxime upregulated monocyte markers associated with antigen presentation, NF-kB (p=0.0736), CD3 (p=0.0253) and CD28 (p=0.0073). The level of suppression associated marker, CD152 (p=0.0017) also increased. Proliferation markers generally showed an upsurge, including CD127 (p=0.0253) and EGFR (p=0.0736), except for Areg (p=0.0073) in both antibiotics, which are notably suppressed. Cefuroxime also augment cytokine production, particularly evident with a significant rise in IL-17a (p=0.0037) and IL-10 levels (p=0.0736) (Figure 4, Supplementary Figure 8).

Co-incubation of unstimulated PBMCs with high dose Meropenem, caused an increase in the expression of activation markers, including NF-kB (p=0.0253), CD4 (p=0.0736) and CD28 (p=0.0073). Meropenum also showed an effect on the makers associated with proliferation. High dose of Meropenem increased CD127 (p=0.0001) but decreased Areg (p=0.0442). However, the expression wasn't significant in suppression, differentiation, chemokine, cytokine and cell death (Figure 4, Supplementary Figure 8).

3.2.3 Effect of Antibiotics on Stimulated CD4+ T lymphocytes (Beads vs Abx)

Co-incubation of PBMCs with Cefuroxime resulted in an increase in viability in both low and high dose (p=0.003 and p=0.0330, respectively). Likewise, markers associated with activation NF-kB was upregulated in both dosages (p=0.0253 and p=0.0017 at low and high dose respectively). A dose-dependent increase in NF-kB was also observed. However, CD4 associated with activation was decreased in dose of Cefuroxime (p=0.0336). Additionally, there was a several changes in proliferation markers. At high dose, Cefuroxime decrease the expression of Areg (p=0.0017) but downregulated Areg at low dose (p=0.0253). There was also an increase in EGFR level (p=0.0442) with high dose of Cefuroxime. High dose of Cefuroxime was also seen to upregulate the expression of cytokine, IL-4 (p=0.0052) and IL-10 (p=0.0073). Lastly, CD279 (p=0.0588)associated with cell death was increased with high dose of of Cefuroxime (Figure 4, Supplementary Figure 9)..

Co-incubation of PBMCs with Meropenem was associated with an increase in viability in both low and high dose, similar to Cefuroxime (p=0.0003 and p=0.0330, respectively). Activation marker, NF-kB (p=0.0442) was increase in high dose Meropenum. Cytokine-related markers showed upregulation in high dose Meropenum, evidenced by raised levels of cytokines IL-4 (p=0.0442). Meropenum in both doses significantly increased CD152 expression. Additionally, Areg expression was lower with both low and high dose (p=0.0037, p=0.0017, respectively) (Figure 4, Supplementary Figure 10).

4. DISCUSSION

The excessive inflammatory response in sepsis is mediated by several cytokines and signalling molecules. My results suggest that antibiotics could modulate the immune response in septic patients. There are immunomodulatory properties of different types of beta-lactams at different dosage. To date, studies comparing the effect of beta-lactam antibiotics on the immune response in sepsis are limited. Our finding addresses a key area in understanding the immunophenotype in sepsis patients highlighting that antibiotics can alter immune function

Lipopolysaccharide (LPS), a main component of the outer wall of gram-negative bacteria, triggers an immune response through the interaction with the LPS-binding protein. The interaction between LPS and its binding protein facilitated in its transfer to cell surface markers, CD14 and CD284 on monocytes, causing the release of inflammatory cytokines and upregulation of co-stimulatory molecules on antigen presenting cells (McAleer and Vella, 2008). Our study corroborates the findings, demonstrating that LPS stimulation could regulate production of proliferation markers in monocytes. We observed that with monocytes, LPS can induce TFN-y and IL-10 in prototypical inflammatory cytokine, involving in acute phase protein and cytokine production, cell proliferation, differentiation and adhesion molecule expression. IFN-y induces cytotoxic activities of T cell and natural killer cells, promoting expression of other inflammatory cytokines and chemokines in an early stage of sepsis. IL-10 acts as an anti-inflammatory cytokine, and counterbalances aggressive inflammatory response. The downregulation of markers associated with inflammation and chemotaxis CCR2, thus enhanced oxidative burst capacity and cytotoxic T cell, highlighted by increases in Nox-2 and CD279. Despite there is no effect of LPS to intracellular cytokine levels, the release of cytokine, such as TFN-y, INF-a, and IL-10 can modulate the function of CD4+ T cells. The future work will involve measurement of released cytokines.

The immune response against microorganism is dependent on monocytes and lymphocytes, specifically through expression of HLA-DR on monocytes for the T-cell receptor to specifically activate T cells. Previous studies show a reduction of the monocyte HLA-DR expression and a corresponding to inflammatory stimuli (Volk et al., 1991b). These findings were interpreted with the reduction in antigen presentation cell simultaneously with signs of hyperinflammation seen in early phase of severe sepsis. Our study explored this further as we were able to tell the dose-dependent modulation of immune markers with Meropenem, including reduction in antigen presentation components like HLA-DR, HLA-DP. Our finding addresses the limitation in understanding HLA-DR level changes in sepsis patients within critical care, clarifying that critical care alone does not significantly alter HLA-DR levels. Previous studies also confirmed that HLA-DR levels normalise within a week in uncomplicated trauma recoveries (Hershman et al., 1990).

Sepsis treatments targeting proinflammatory cytokines like TNF-a and IL-6, have not resulted in decreased mortality (Franco, Chen and Pan, 2021). NF-kB plays a critical role in regulating apoptosis through oxidative stress, synthesising proapoptotic nitric oxide. In septic shock, higher NF-kB activity correlates with illness severity (Böhrer et al., 1997). Our findings demonstrate that antibiotics could increase the NF-kB expression in dose-dependent, potentially enhancing T cell response to inflammation. Sepsis activates the NLRP3 inflammasome, which mediates inflammation via cytokines like IL-1b and IL-18. Our findings reveal NLRP3 downregulation following antibiotic treatment. The upregulation of IFN-y and TNF-a in monocytes, potentially reversing immune paralysis in sepsis and enhancing macrophage intracellular killing (Flohé et al., 2008). These cytokines are key to fighting bacterial infections and initiating immune responses, with early TNF-a release activating immune cells and IL-1 from macrophages (Cohen et al., 2011). Furthermore, Meropenem was found to exhibit anti-inflammatory effects by decreasing IL-1b levels in monocytes, aligning with findings by Richard A (Zager, Johnson and Geballe, 2007). This suppression of pro-inflammatory cytokine responses suggests that beta-lactam antibiotics could modulate cytokine expression. The reduction in pro-inflammatory cytokines may be linked to impaired NF-kB phosphorylation, as indicated in stimulated monocytes and CD4+ T cells from sepsis patients (Cabrera-Perez et al., 2014).

T cell exhaustion during chronic infections, driven by persistent antigen exposure, results in functional decline and increased expression of co-inhibitory receptors like CTLA-4 (CD152) and PD-1 (CD279). In sepsis, overexpression of PD-1 and PD-L1 correlates with higher mortality and more hospital-acquired infections, while negatively impacting lymphocyte proliferation (Monneret and Venet, 2015). Our study found that CTLA-4 expression on lymphocytes rises during the first week of sepsis post-antibiotic treatment, highlighting the potential of anti-CTLA-4 blocking antibodies to enhance immune response in sepsis treatment. Accordingly, the antibiotics upregulated the expression of NF-kB, which is notably activated in sepsis (Liu and Malik, 2006). However, Cefuroxime suppressed CD4 expression, which is crucial for the formation of functional CD8 T cell memory and mediating adaptive immune responses (Muñoz-Ruiz et al., 2013b). This finding supports previous studies that highlight the direct role of CD4 T cells in mediating the host response to sepsis (Kim et al., 2008). We detected an upregulation of the anti-inflammatory cytokine IL-10 in both monocytes and CD4+ T cells during sepsis. Increased IL-10 production from CD4+ T cells correlates with enhanced survival rates in sepsis. This rise in IL-10, particularly from CD4+ T cells, aligns with benefits observed in sepsis-treated animals using CD28 agonists, and plays a critical role in modulating immune responses by down-regulating HLA-DR (Thibodeau et al., 2008). Conversely, Cefuroxime suppresses CD4 expression, crucial for developing functional CD8 T cell memory and mediating adaptive immune responses, underscoring the direct impact of CD4 T cells in sepsis response.

Additionally, The CD4+/CD8+ ratio in T cells is a reliable measure of immune function. During sepsis, this ratio often decreases, which is closely associated with immunosuppression. Although Xia et al. found that sepsis patients have a significantly lower CD4+/CD8+ ratio compared to healthy patients, our result doesn't show a significant change in ratio with antibiotics (Su Ji Jeong et al., 2014).

Monocytes

Functions	Antigen	Changes observed in non-survival sepsis patients	Variation observed in non-survival sepsis patient	References	Variation in effect of Cefuroxime	Variation in effect of Meropenem
Receptor	CD64	Found lower CD64 expression in non-survivors versus survivors of sepsis	↓	(Muzlovic, Ihan and Stubljar, 2016)	-	↓
	CD284	higher levels of CD28 activation are associated with better survival rates	↓	(Sun et al., 2020)	↑	↑
Co-stimulation	CD80	The expression level of CD80 and CD86 were reduced in response to sepsis	↓	(Venet et al., 2012)	↑	↑
Antigen Presenting Cell	CD74	Found a significant decrease in CD74 level in non-survival of sepsis	↓	(Cazalis et al., 2013)	↓	↓
	HLA-DR	The expression level of HLA-DR was reduced in sepsis non-survivors compared to survivor	↓	(Lekkou et al., 2004)	-	↓
	HLA-DP	-	-	-	-	↓
	HLA-DM	Found a significant decrease in HLA-DMA mRNA level in non-survival	↓	(Cazalis et al., 2013b)	-	-
	CIITA	-	-	-	-	-
Activation	NF-kB	Higher levels of NF-kB activity are associated with worse clinical outcome along with a higher rate of mortality	↑	(Liu and Malik, 2006)	↓	↓
	NLRP3	Higher expression of IL-1b was found in non-surviving septic patients	↑	(Wang et al., 2020)	-	↓

Cytokine	IL-1b	Higher expression of IL-1b was found in non-surviving septic patients	↑	(Wang et al., 2020b)	-	↓
	TNF-a	TNF-α levels have been measured during endotoxic shock and show a negative correlation with survival rates	↑	(Casey, 1993), (O'Riordain et al., 1996)	-	-
	IFN-y	T cell production of IFN-γ has been shown to enhance animal models of sepsis survival.	↓	(Döcke et al., 1997)	-	↑
	IL-10	The cell producing IL–10 production in sepsis shock patient may increase tumour necrosis	↑	(Fabri et al., 2021)	↓	↓
Inhibitor	CD274	monocyte CD274 expression was associated with risk of mortality in septic patients.	↑	(Shao et al., 2016)	-	-
Phagocytosis	NOX-2	NoX-2 level increase in patients with both sepsis and sepsis shocked, and increased in patients with infectious but not noninfectious SIRS	↑	(Mitaka et al., 2003)	-	-
Chemokine	CCR2	There are high levels of CCR2 in sepsis patients, which correlates positively with patient illness.	↑	(Souto et al., 2011)	-	-
Viability	EGFR	-	-	-	↓	↓
	Areg	-	-	-	-	-

The table 3 summarises key immunological markers in monocytes associated with sepsis outcomes, detailing variations in marker expression between non-survivors and survivors, and the effects of antibiotics Cefuroxime and Meropenem. It categorises markers into functional groups such as receptors, co-stimulation, cytokines, examining their roles in sepsis pathology and response to treatment. Each marker's correlation with sepsis survival, changes in expression, and response to antibiotics are noted, providing insight into potential therapeutic targets and the impact of antibiotic treatment on immune function in sepsis patients. An upward arrow indicates an upregulation of marker expression, while a downward arrow signifies downregulation. A dashed line indicates information that was not found.

CD4+ T Lymphocytes

Functions	Antigen	Changes in non-survival sepsis	Variation observed in non-survival sepsis patient	References	Variation in effect of Cefuroxime	Variation in effect of Meropenem
Activation	NF-kB	Higher levels of NF-kB activity are associated with worse clinical outcome along with a higher rate of mortality	↑	(Liu and Malik, 2006b)	↑	↑
	CD3	The expression of co-receptor, CD3, was significantly decreased in septic patients	↓	(Venet et al., 2012b)	-	-
	CD4	CD4 T cell loss in septic patients of all ages was found	↓	(Cabrera-Perez et al., 2014)	↓	-
	CD28	Higher expression of CD28 was associated with non-survivors	↑	(Monneret et al., 2003)	-	-
Suppression	CD152	They found a lower expression of CD152 in sepsis non-survivors	↓	(Monneret et al., 2003b)	↑	↑
	CD274	Increased expression of PD-L1 in septic patients was observed	↑	(Venet et al., 2012b)	↑(in high dose)/ ↓ (with low dose)	-
Proliferation	CD127	CD127 concentration was decreased in septic shock, however, higher CD127 level was found in non-survivors	↑	(Peronnet et al., 2016), (Mouillaux et al., 2019)	-	-
	EGFR	-	-	-	↑	-
	Areg	-	-	-		↓
Differentiation	STAT5	both septic patients and non-survivors were associated with decreased STAT5 expression	↓	(Demaret et al., 2015)	-	-
	FOX-3	A significantly reduced expression of FOX-3 in septic patients	↓	(Venet et al., 2010)	-	-
	T-bet	A significantly reduced expression of T-bet in septic patients	↓	(Venet et al., 2010b)	-	-
	IL-2	A study of 22 sepsis patient show a decrease in IL-2 synthesis 23	↓	(Roth et al., 2003b)	-	-
Chemokine	CD194	The increased level of CD194 was associated with higher mortality rate, however, the difference in the expression level was relatively small	↑	(Yang et al., 2023)	-	-
	CD196	-	-	-	-	-
Cytokine	IL-4	The expression level of IL-4 was significantly reduced in patients who had died	↓	(Wu et al., 2008)	↑	↑
	IL-17a	Increased level of IL-17a was associated with increased mortality	↑	(Venet et al., 2010b)	-	-
	IFN-y	Higher levels of IFN-y attenuate the clinical outcomes.	↑	(Romero et al., 2010)	-	-
	IL-10	Increased level of IL-10 was associated with increased mortality	↑	(Yang et al., 2023b)	↑	-

Cell Death	CD95	A lower level of CD95 may lead to more adverse clinical outcomes	↓	(Gao et al., 2016)	-	-
	CD279	Increased expression of PD-1 in septic patients was observed	↑	(Venet et al., 2012b)	↑	-
Viability	CD4:CD8	A normal CD4/CD8 ratio is greater than 1.0. Ratio ≤ 0.15 had overall higher morbidity rate	↓	(Xia et al., 2012)	-	-

The table 4 presents an analysis of various CD4+ T cell markers and their expression patterns in sepsis patients, differentiating between survivors and non-survivors. It categorises the markers into groups based on their function: activation, suppression, proliferation, differentiation, chemokine signalling, cytokine production, cell death, and overall cell viability. Each marker is evaluated for changes in expression during sepsis and how these changes correlate with patient outcomes. Additionally, the table includes the effects of two antibiotics, Cefuroxime and Meropenem, on these markers, providing insights into how these treatments might influence the immune response in septic conditions. This data is crucial for understanding the pathophysiology of sepsis and guiding targeted therapeutic strategies. An upward arrow indicates an upregulation of marker expression, while a downward arrow signifies downregulation. A dashed line indicates information that was not found.

In monocytes, as seen in table 3, lower CD64 expression is observed in sepsis non-survivors (Muzlovic, Ihan and Stubljar, 2016), while higher levels of CD284 activation are associated with better survival outcomes (Sun et al., 2020). Our study indicates that Meropenem tends to decrease CD64 expression further, while both Cefuroxime and Meropenem enhance CD284 activation. Co-stimulation marker CD80, is lower in sepsis non-survivors (Venet et al., 2012), with both antibiotics tested showing a capability to counteract this reduction. This restoration of CD80 expression could enhance the immune response during sepsis, possibly aiding in the overall recovery process.

NF-kB; a crucial transcription factor that regulates immune and inflammatory responses, is higher in sepsis non-survivors. Antibiotics decrease NF-kB activity, which might be beneficial as excessive NF-kB activation can lead to uncontrolled inflammation and subsequent tissue damage, exacerbating sepsis conditions

NLRP3 and IL-1b are integral to the inflammatory response (inflammasome), with IL-1b being a pro-inflammatory cytokine that plays a pivotal role in mediating systemic inflammation. Studies have shown that higher expression levels of IL-1b are found in non-surviving septic patients, suggesting that an overproduction of IL-1b may contribute to adverse outcomes. Antibiotics reduce IL-1b levels, potentially curtailing the harmful overstimulation of the immune system and improving survival prospects. Conversely, TNF-a, another critical cytokine in the inflammatory cascade, is higher in sepsis non-survivors. Our data does not demonstrate an impact of antibiotics on TNF-a levels.

Furthermore, IFN-y and IL-10 represent contrasting aspects of the immune response. IFN-y, increased with the use of antibiotic, possibly enhancing survival in sepsis and is crucial for the activation of macrophages and other immune cells. On the other hand, IL-10, an anti-inflammatory cytokine, tends to increase in septic patients and can lead to an immunosuppressive state, potentially detrimental in fighting infections. Antibiotics reduce IL-10 production, which might be used to prevent excessive immune suppression and facilitating a more balanced immune response.

With regards to CD4+ T cell lymphocytes as shown in table 4, NF-kB activation is observed at higher levels in sepsis non-survivors, suggesting that an overactive NF-kB pathway may correlate with poorer outcomes (Liu and Malik, 2006b). However, Cefuroxime and Meropenem appear to increase NF-kB activity, possibly exacerbating inflammation.

CD4+ T cell loss is prevalent across all ages in septic patients. Cefuroxime appears to exacerbate this suppression, suggesting a detrimental effect of this antibiotic on CD4+ T cell populations during sepsis. Additionally, CD152 expression is lower in non-survivors (Monneret et al., 2003b), and both Cefuroxime and Meropenem increase its levels, potentially contributing to a lymphocyte anergy. In contrast, increased expression of CD274 (PD-L1) was observed, which could suppress immune function excessively, although this effect varies with antibiotic dosage; higher doses increase PD-L1 expression, potentially leading to immunosuppression.

In patients who did not survive sepsis, there was a notable reduction in IL-4 levels (Wu et al., 2008). Both Cefuroxime and Meropenem are observed to increase IL-4 levels, which could indicate a beneficial role for these antibiotics in favourably modulating immune responses in septic patients. Regarding IL-10, although it is typically anti-inflammatory, its elevated levels in sepsis have been associated with increased mortality (Yang et al., 2023b), likely due to excessive immunosuppression. The administration of Cefuroxime and Meropenem, which both increase IL-10 levels, could potentially be detrimental by impairing effective immune responses during critical phases of sepsis. For CD279, higher expression of this exhaustion marker has been observed in sepsis, potentially leading to T cell dysfunction (Venet et al., 2012b). Antibiotics have been noted to increase the expression of PD-1, which could further contribute to immune cell exhaustion. Understanding how antibiotics influence the modulation of PD-1 is crucial for managing T cell viability and function in sepsis. This highlights the need for careful monitoring and management of immune exhaustion markers to improve treatment outcomes in septic patients.

4.1 Limitations and Future work

The limited number of participants and diversity among both the sepsis patients and controls, encompassing variables such as duration of illness, nutritional status, and level of comorbidity, affected the study's outcomes. A longer-term study monitoring the progression of immune phenotypes would yield deeper insights. Furthermore, all in vitro tests were conducted using only one concentration and strain of HKB or CD3-CD28 beads. The study did not explore neutrophil function, which is commonly compromised in sepsis, nor did it examine the function of B cells. Data on intermediate and non-classical monocyte subsets were not included due to insufficient cell counts from the patients. Additionally, the measurement of cell surface markers on monocyte subsets to determine immune status in critically ill patients is not a common practice.

While we analyzed the levels of ligands and receptors like PD-L1 and PD-1 using flow cytometry, we couldn't investigate their interactions or related pathways. Moreover, the in vitro response to stimuli such as LPS or CD3-CD28 beads might not accurately reflect the real-time changes in patients with infections.

An analysis using a broader array of markers could have identified additional potential therapeutic targets. Many studies have explored the transcriptomic profile of immune cells during the perioperative period, but changes in transcription may not be reflected those in cell surface protein/receptor expression, and bulk transcriptomics does not specifically evaluate the phenotype of individual cell subsets.

CONCLUSION

The interaction between immune function and antibiotic treatment in sepsis reveals a nuanced landscape where antibiotics can simultaneously enhance and impair immune function. This underscores the necessity for careful clinical evaluation and approaches in the management of sepsis. By tailoring antibiotic use based on specific immune profiles, clinicians may improve outcomes for sepsis patients, leveraging beneficial effects while minimizing potential harms.

Our results highlighted the detrimental effects of antibiotics on patients in an *ex vivo* setting. Beta-lactam antibiotics exacerbated features of sepsis-induced immune dysfunction in monocytes and CD4 lymphocytes, particularly at higher dosages. The clinical significance of these findings warrant further investigation.

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APPENDIX A

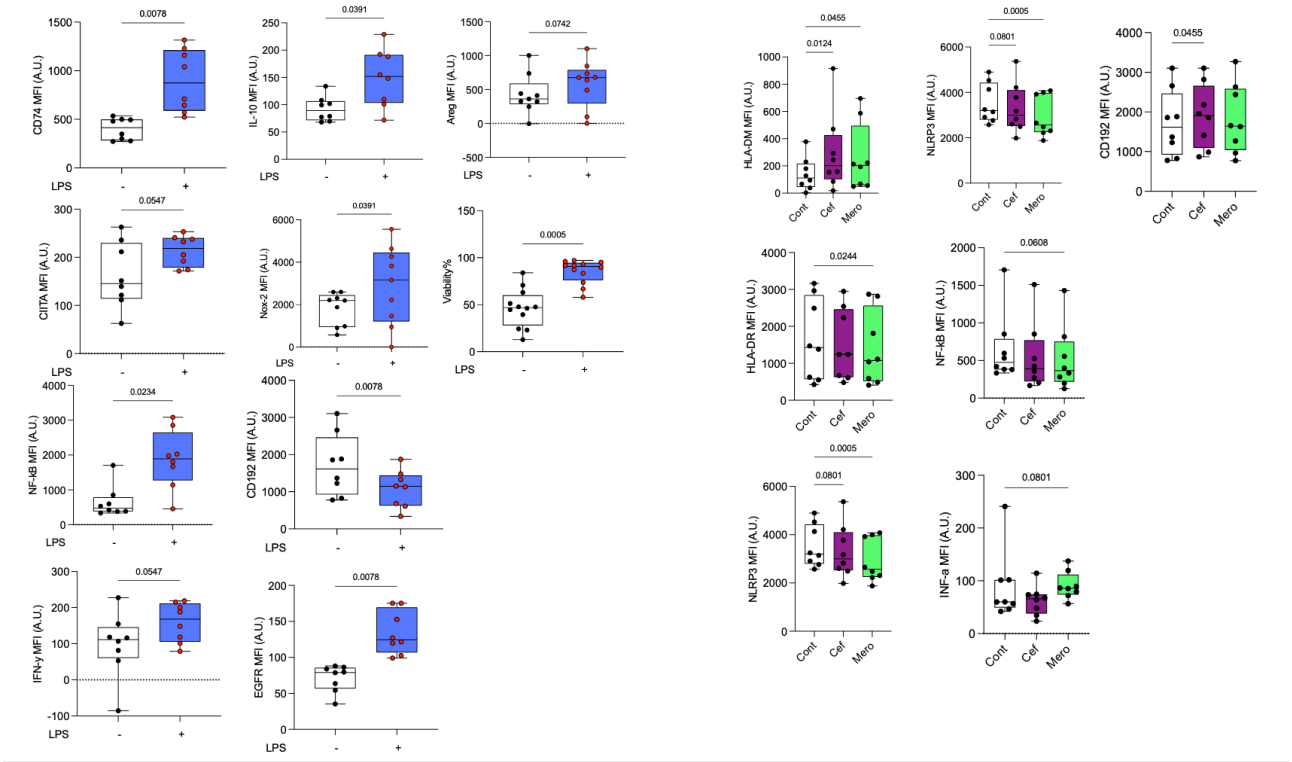

figure 5 is divided into two main sections: the left side illustrates the effect of LPS on monocytes, showing significant changes in immune markers. The right side illustrates the impact of different antibiotics (Control, Cefuroxime, Meropenem) on unstimulated monocytes, highlighting how each antibiotic affects markers. Each panel uses boxplots to display mean fluorescence intensity (MFI) values, with significant p-values shown, demonstrating the influence of LPS and antibiotics on monocyte immune responses.

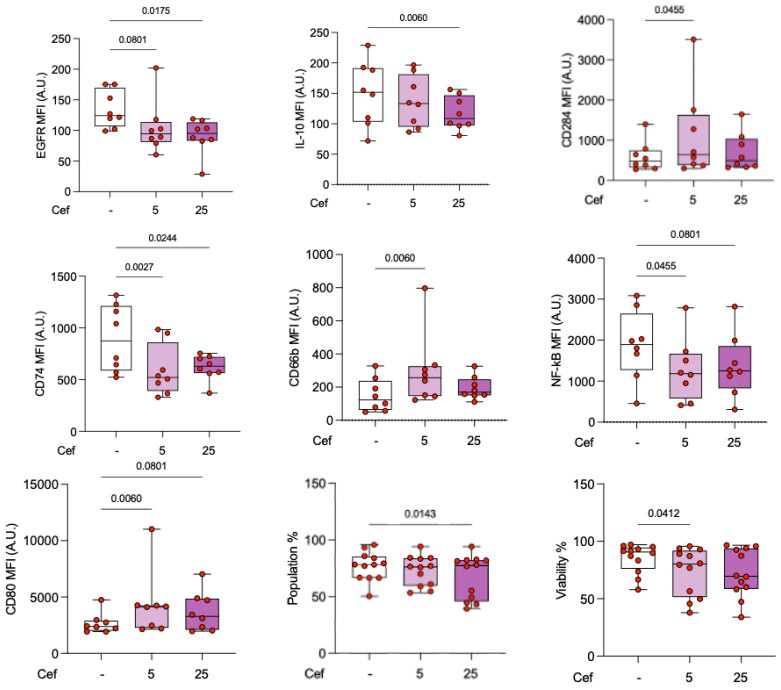

Figure 6 displays the effects of Cefuroxime (Cef) at different concentrations (0, 5, 25 mg/mL) on various monocyte markers in a controlled experiment. The data includes measurements of mean fluorescence intensity (MFI), as well as the overall population percentage and cell viability. Statistical significance is indicated by P value for each marker.

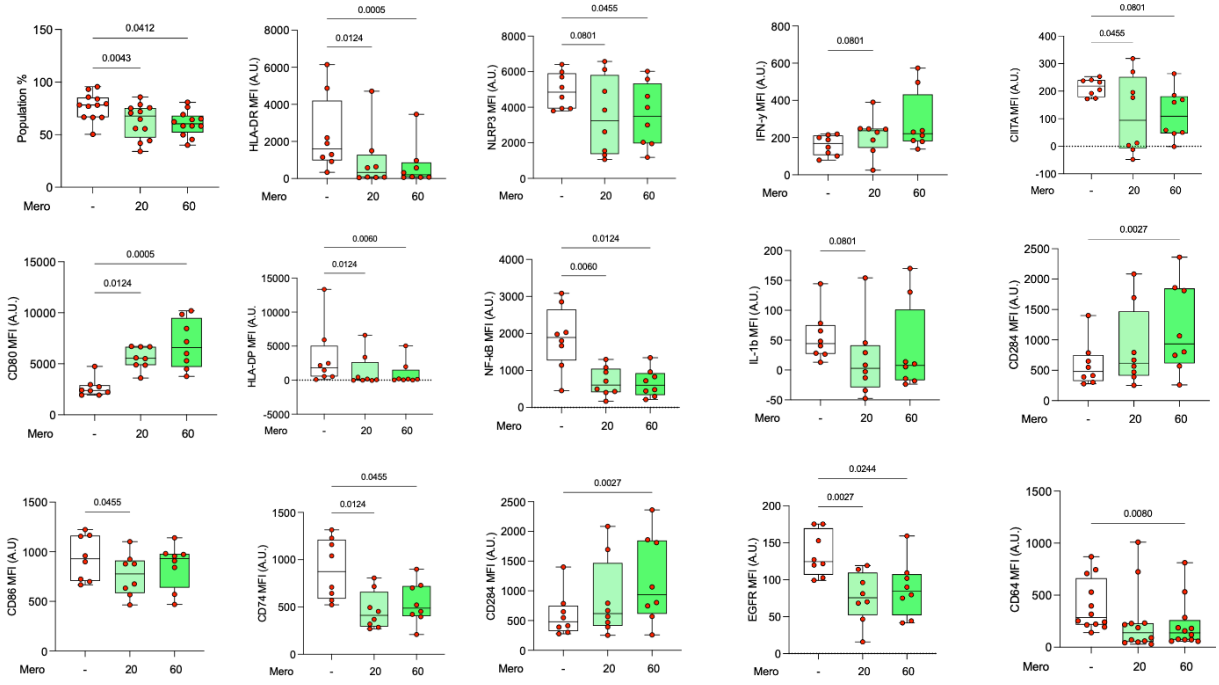

Figure 7 illustrates the impact of varying doses of Meropenem (Mero; 0, 20, 60 mg/mL) on different immune markers in monocytes. Key observations include significant changes in expression levels across multiple markers, and across the dosing spectrum.. Each panel provides mean fluorescence intensity (MFI) values for markers. Statistical significance between groups is indicated by p-values.

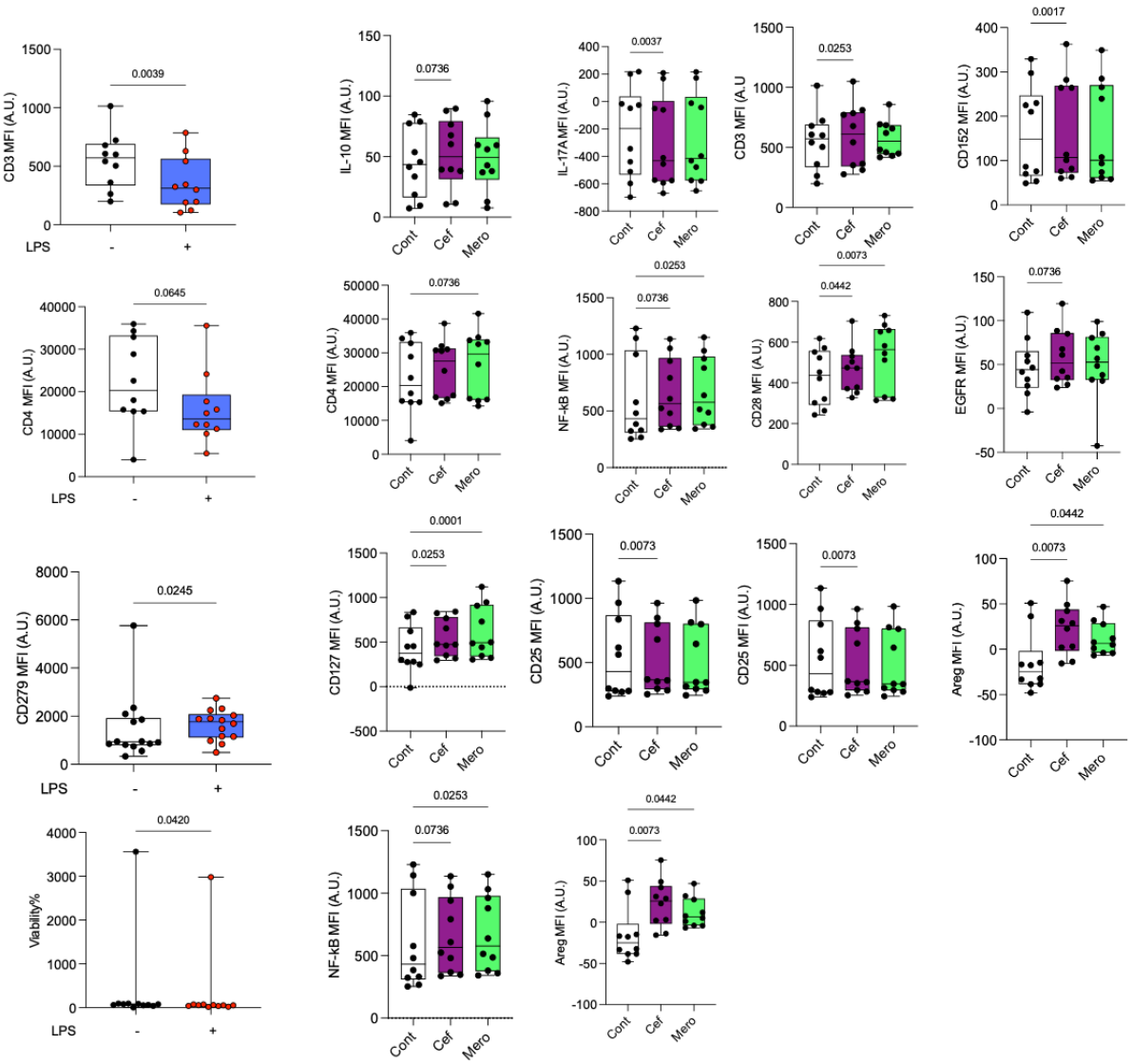

figure 8 is divided into two main sections: the left side illustrates the effect of beads on CD4+ T Lymphocytes, showing significant changes in immune markers. The right side illustrates the impact of different antibiotics (Control, Cefuroxime, Meropenem) on unstimulated CD4+ T Lymphocytes, highlighting how each antibiotic affects markers. Each panel uses boxplots to display mean fluorescence intensity (MFI) values, with significant p-values shown, demonstrating the influence of LPS and antibiotics on CD4+ T Lymphocytes immune responses.

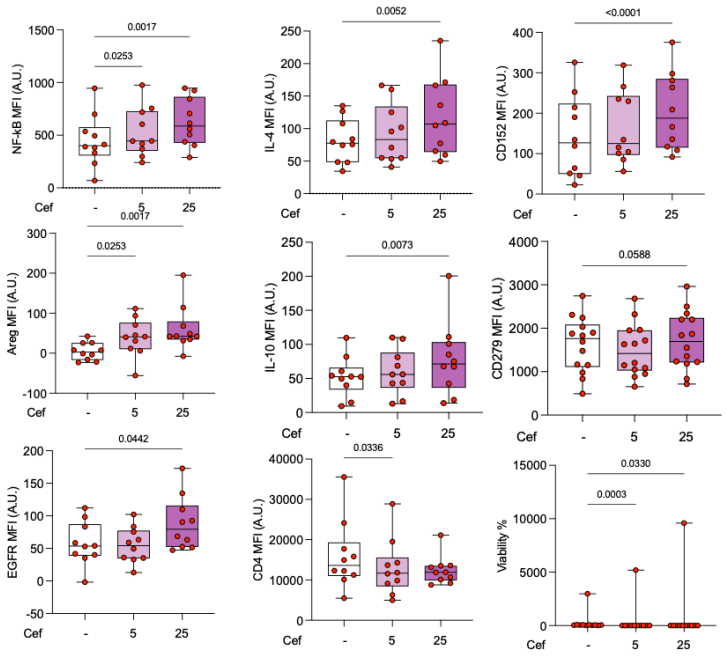

Figure 9 displays the effects of Cefuroxime (Cef) at different concentrations (0, 5, 25 mg/mL) on various CD4+ T Lymphocytes markers in a controlled experiment. The data includes measurements of mean fluorescence intensity (MFI), as well as the overall population percentage and cell viability. Statistical significance is indicated by P value for each marker.

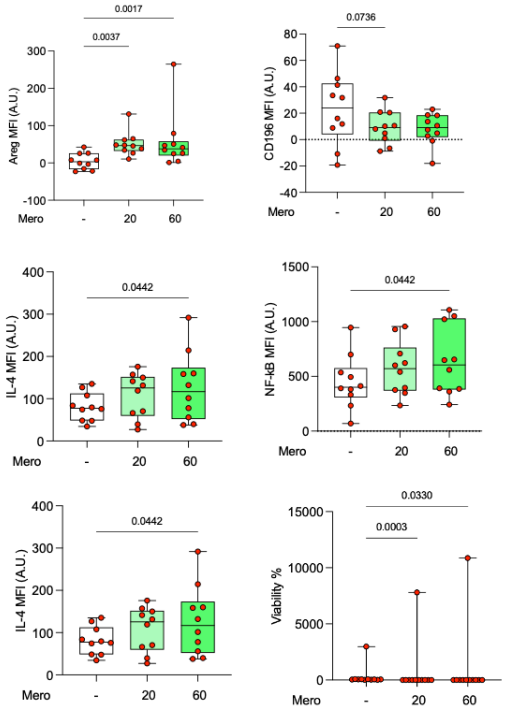

Figure 10 demonstrates the effect of varying doses of Meropenem (Mero; 0, 20, 60 mg/mL) on different immune markers in monocytes. The data include significant changes in expression levels across multiple markers, and across the dosing spectrum. The data includes measurements of mean fluorescence intensity (MFI), as well as the overall population percentage and cell viability. Statistical significance is indicated by P value for each marker.