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YKL-40 as a biomarker for long-term changes in innate immunity after COVID-19 onset

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ABBREVIATION LIST

°C	degrees centigrade
µg	microgram
µl	microliter
µm	micrometer
ACE2	angiotensin-converting enzyme 2
ALI	air-liquid interface
AMCase	acidic mammalian chitinase
ANOVA	analysis of variance
APMA	4-aminophenylmercuric acetate
CCL	chemokine (C-C motif) ligand
CCR	C-C chemokine receptor
CD	cluster of differentiation
CD14	CD14 positive macrophages
cDNA	complementary deoxyribonucleic acid
CHI3L1	chitinase-3-like protein 1
CHIT1	chitotriosidase
CLPs	chitinase-like proteins
COVID-19	coronavirus disease 2019
Ct	threshold cycle
CXCL	C-X-C motif chemokine ligand
ddH_2O	double distilled water
DEPC	diethyl pyrocarbonate
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotides
ECM	extracellular matrix,
EDTA	ethylene diamine tetra acetic acid

EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EMEM	Eagle's minimal essential medium
EU	European Union
EX	excitation
FACS	fluorescent activated cell sorting
Fc	fragment crystallisable
FCS	fetal calf serum
FcγR	Fc gamma receptor
g	gram / relative centrifugal force
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GM-CSF	granulocyte-macrophage colony-stimulating factor
GPa	gigapascal
h	hour(s)
Hb	hemoglobin
hBECs	human bronchial epithelial cells;
HBV	Hepatitis B
Hct	hematocrit
HCV	Hepatitis C
HIV	Human Immunodeficiency Virus
HSAECs	primary human small airway epithelial cells
HSP	heat shock protein
IAV	Influenza A virus
IFNs	interferons
IFNγ	interferon gamma
IgG	immunoglobulin G
IL	interleukin
IL-1Ra	Interleukin 1 receptor antagonist

iNOS	inducible nitric oxide synthase
JMFI	juxtacrine macrophage-fibroblast interaction
L	liter
LIF	leukemia inhibitory factor
LPS	lipopolysaccharide
LTA	lymphotoxin-alpha
M	molar concentration
M(IFNγ)	macrophages activated with IFNγ
M(IL-4)	macrophages activated with IL-4
M(NS)	non-stimulated macrophages
M1	classically activated macrophages
M2	alternatively activated macrophages
MACS	magnetic-activated cell sorting
MARCO	macrophage receptor with collagenous structure
M-CSF	macrophage colony-stimulating factor
MIF	macrophage migration inhibitory factor
min	minute(s)
miRNAs	microRNAs
ml	milliliter
mM	millimolar concentration
mm	millimeter
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
mRNA	messenger RNA
N	equivalent concentration
N/A	Not applicable
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
ng	nanogram

NLRP3	NLR family pyrin domain containing 3
nm	nanometer
OPG	osteoprotegerin
PAMPs	pathogen-associated molecular patterns
PAR	polyarginine
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PEG	poly(ethylene-glycol)
PELA	ethylene oxide/lactic acid copolymer
PET	polyethylene terephthalate
PFA	paraformaldehyde
pg	picogram
PLGA	poly(lactide-co-glycolide)
PLT	platelets
PMFI	paracrine macrophage-fibroblast interaction
PMMA	polymethylmethacrylate
pmol	picomolar
PPR	multiple pattern recognition receptors
PUUV	Puumala orthohantavirus
PVA	poly(vinyl-alcohol)
RBC	red blood cells
RBC	red blood cells
RMA	Robust Multi-Array Average
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species

rpm	revolutions per minute
RSV	Respiratory Syncytial Virus
RT	room temperature
RT-qPCR	reverse transcription real-time polymerase chain reaction
s	second(s)
SA	heat killed Staphylococcus aureus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SR	scavenger receptor
Std	standard
TBEV	Tick-borne encephalitis virus
TGF	transforming growth factor
TGFβR1	transforming growth factor beta receptor I
TLR	toll-like receptor
TNFRSF	tumor necrosis factor receptor superfamily, member
TNFSF	tumor necrosis factor (ligand) superfamily, member
TNFα	tumor necrosis factor alpha
U	conventional units
WBC	white blood cells

1 INTRODUCTION

1.1 SARS-CoV2

1.1.1 Epidemiology

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), an emergent enveloped RNA β coronavirus, was identified in December 2019 in Wuhan, China, leading to the global outbreak of coronavirus disease 2019 (COVID-19) (Merad & Martin, 2020). As of early 2023, the World Health Organization reported over 762 million confirmed infections and nearly 7 million deaths due to COVID-19 complications, underscoring the pandemic's devastating impact (https://covid19.who.int/). A comprehensive analysis of closed cases by April 13, 2023, revealed a recovery rate of 99% among patients, highlighting the variable outcomes of the disease (https://www.worldometers.info/coronavirus/) (Li et al., 2020). Research indicates that the mean incubation period for COVID-19 is approximately 6.57 days, with a range from 1.8 to 18.87 days, suggesting significant variability in disease onset (Wu et al., 2022). Transmission primarily occurs via respiratory droplets during close contact activities, such as talking, coughing, or sneezing (Wiersinga et al., 2020). Individuals over 60 years of age, particularly those with pre-existing health conditions, are at an elevated risk of severe respiratory complications necessitating hospitalization (Hu, Guo, et al., 2021; Wu & McGoogan, 2020).

1.1.2 Symptoms

It had been confirmed that most of the infected patients (80%) had a mild disease (with either no or only mild pneumonia). Meanwhile, 14% developed severe disease (with dyspnea, hypoxia or greater than 50% lung involvement on imaging tests) and 5% developed the critical disease (marked by respiratory failure, systemic shock or multi-organ failure) (Merad & Martin, 2020; Wu et al., 2020). Initial symptoms commonly include fever, cough, shortness of breath, fatigue, body pain, headache, disturbance of smell or taste, sore throat, runny nose, nausea, vomiting and diarrhea (Huang et al., 2020; Marshall, 2021; Vetter et al., 2020).

Although SARS-COV-2 is considered a respiratory pathogen, neurological symptoms and conditions may precede typical respiratory symptoms by several days (Harapan & Yoo, 2021; Spudich & Nath, 2022). Furthermore, neurological symptoms-including fatigue, headache, and neurocognitive difficulties can persist for months after infection as part of Long COVID symptoms have been reported in 31%–69% of recovery individuals, which has become an emerging health crisis (Peluso & Deeks, 2022; Ryan et al., 2022; Spudich & Nath, 2022). Earlier hypotheses suggested that the virus might directly infect the central nervous system. However, it is rarely detected in cerebrospinal fluid of patients with neurological symptoms (Spudich & Nath, 2022). Current studies showed that aberrant neuroimmune responses (Franke et al., 2021; Groff et al., 2021) and widespread vascular dysfunction(Merkler et al., 2020) could be the main contribution to nervous system complications of COVID-19 (de Paula et al., 2023; Spudich & Nath, 2022).

1.1.3 Long- COVID

Despite the fact that over 99% of patients with SARS-COV-2 infection are able to survive acute COVID, a significant portion of survivors may experience persistent health issues, which is termed as long COVID (Lai et al., 2023). The incidence of long COVID is estimated at 10–30% of non-hospitalized cases, 50–70% of hospitalized cases (Davis et al., 2023; Lai et al., 2023). Long COVID is associated with all ages and a severity of the acute phase, with the highest percentage of diagnoses between the ages of 36 and 50 years. Notably, the majority of long COVID cases are in non-hospitalized patients with a mild acute illness(Davis et al., 2023). Consistent fatigue is the most common symptoms, followed by consistent breathlessness, cough and muscle ache (Brightling & Evans, 2022; Davis et al., 2023; Evans et al., 2021; Huang et al., 2022; Whitaker et al., 2022). It is still unclear what causes long COVID. Several hypotheses for its pathogenesis have been suggested, including persistent reservoirs of SARS-COV 2 in tissues(Proal & VanElzakker, 2021; Swank et al., 2023); immune dysregulation with or without reactivation of underlying pathogens(Proal & VanElzakker, 2021; Zubchenko et al., 2022); impacts of SARS-COV-2 on microbiota(Liu et al., 2022; Su et al., 2022; Yeoh et al., 2021); macrovascular blood clotting with endothelial dysfunction(Charfeddine et al., 2021; Haffke et al., 2022; Pretorius et al., 2022; Proal & VanElzakker, 2021); and dysfunction signaling in the brain stem and/or vagus nerve(Proal & VanElzakker, 2021; Spudich & Nath, 2022). It is still uncertain whether unforeseen consequences may develop years after the initial infection. Considering the millions of individuals infected with COVID-19, long-term infection poses a public health challenge for rehabilitation and recovery, as well as disruptions in the workplace due to reduced functional abilities(Spudich & Nath, 2022). Understanding the pathophysiology of these disorders is urgent to develop therapies that can modify their course.

1.2 Monocyte and macrophages in COVID-19

1.2.1 COVID-19 and innate immunity

SARS-CoV-2 infection and the destruction of lung cells trigger a local immune response, recruiting macrophages and monocytes. These cells respond to the infection, release cytokines and prime adaptive T and B cell immune responses (Tay et al., 2020). Serving as the human immune system's first line of defense, innate immunity plays a pivotal role in combating this novel virus (Schultze & Aschenbrenner, 2021). However, COVID-19 can provoke severe disease marked by an overly vigorous yet uncoordinated innate immune response, alongside an insufficient adaptive response, leading to significant immunopathology (Lowery et al., 2021). Infection with SARS-COV-2 and the pathogenesis of COVID-19 activate diverse innate immune cells, including macrophages (Schultze & Aschenbrenner, 2021). The innate immune response to SARS-CoV-2 is mediated by multiple pattern recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMPs) and release cytokines, such as interferons (IFNs) and chemokines. (Karki & Kanneganti, 2022). Despite the importance of early inflammatory responses in limiting viral replication (Park & Iwasaki, 2020), escaping from immune sensing (Vabret et al., 2020) and excessive inflammatory response in both lungs and bloodstream (Karki & Kanneganti, 2021; Karki et al., 2021) can lead to cytokine storm which will cause the tissue damage and organ failure (Karki & Kanneganti, 2022; Karki et al., 2022).

Monocyte- derived macrophages (MDM), as an important part of innate immunity, play an important role in driving inflammatory response to SARS-COV-2 (Bohnacker et al., 2022a). It has been reported that, compared to mild COVID-19 convalescent individuals, the increase monocyte activation marker can be seen in severe one (Rajamanickam et al., 2021). This observation underscores the importance of understanding the nuanced roles of immune cells in COVID-19's pathology and the potential for targeted interventions to mitigate severe outcomes.

1.2.2 Trained innate immunity in virus infections.

Changes in innate immunity, especially the monocyte-macrophages system, are crucial during the recovery period after a virus infection. A growing body of literature indicates that innate immune cells can show immunological memory in certain infections, a process termed 'immune training.' Previously, this was thought to be an exclusive hallmark of the adaptive immune response. (Netea et al., 2020). After brief stimulation, innate immune cells can develop a persistent hyperresponsive phenotype (Netea et al., 2011). Maintained by distinct epigenetic (Arts et al., 2016) and metabolic mechanisms (Bekkering et al., 2018). Recent studies showed this can persist for at least several months due to reprogramming of myeloid progenitor cells (Kleinnijenhuis, Quintin, Preijers, Benn, et al., 2014; Kleinnijenhuis, Quintin, Preijers, Joosten, et al., 2014).

Monocytes, key players in innate immunity with a typical lifespan of 3 to 5 days in circulation (Auffray et al., 2009) , have demonstrated the capacity for memory formation in response to pathogenic stimuli (Kleinnijenhuis et al., 2012; Tercan et al., 2021). Remarkably, trained monocytes remain detectable in the bloodstream a year after Bacillus Calmette-Guérin (BCG) vaccination (Kleinnijenhuis, Quintin, Preijers, Benn, et al., 2014), suggesting that monocyte reprogramming may occur on the level of hematopoietic stem and progenitor cells in the bone marrow (Brueggeman et al., 2022; Netea et al., 2020).

The potential of COVID-19 to initiate this process is still being explored. Notably, severe COVID-19 convalescent individuals exhibit increased monocyte activation markers compared to those with mild cases (Rajamanickam et al., 2021). Furthermore, patients after recovery displayed enhanced monocyte polyfunctionality compared to the healthy controls, hinting at various degrees of sustained, primed immune activation during convalescence (Su et al., 2022). Given that previous infections can alter epigenomic programming in monocytes (Netea et al., 2016) , such epigenetic modifications in circulating monocytes and a lasting inflammatory signature in monocyte-derived macrophages (MDM) could be crucial to understanding long-COVID (Bohnacker et al., 2022b). Single-cell transposase-accessible chromatin sequencing showed that, compared with healthy donors, COVID-19 convalescent individuals contained abundant TBET-enriched CD16+ and IRF1-enriched CD14+ monocytes with sequential trained and activated epigenomic states (M. You et al., 2021).

1.2.3 Macrophage–derived mediators of inflammation and cytokine storm

The severity of COVID-19 is influenced by multiple factors, and growing clinical evidence indicates that patients with severe COVID-19 exhibit elevated levels of pro-inflammatory cytokines (Carlos et al., 2020; Remuzzi & Remuzzi, 2020; B. Wang et al., 2020), such as IL-1β, IL-7, IL-8, TNF-α, and CXCL10 (IP10). The rapid increase in inflammatory molecules observed in COVID-19 is commonly known as the "cytokine

storm (Hu, Huang, et al., 2021; Mehta et al., 2020; Ramasamy & Subbian, 2021). The sudden increase in inflammatory factors can cause widespread microvascular clotting, potentially leading to conditions such as myocarditis, heart failure, arrhythmias, acute coronary syndrome, rapid health decline, and in some cases, sudden death. (Chen et al., 2021; Liu et al., 2020).

Impaired acquired immune response and uncontrolled inflammatory innate response to SARS-COV-2 may be the cause of the cytokine storm of COVID-19 (Hu, Huang, et al., 2021). After entering the respiratory epithelial cells by binding cell surface receptor ACE2 (Hoffmann et al., 2020; Soy et al., 2020), SARS-CoV-2 initiates an immune reaction that includes the production of inflammatory cytokines (Table 1) accompanied by a weak IFN response. The proinflammatory immune responses of pathogenic Th1 cells and intermediate CD14+CD16+monocytes are mediated by membrane‐bound immune receptors and downstream signaling pathways. The sequence of events leads to macrophages and neutrophils being drawn into the lung tissue, causing a cytokine storm (Hu, Huang, et al., 2021; Hussman, 2020).

In humans, both monocyte and macrophages express ACE2, rendering them susceptible to infection by SARS-CoV-2 (Y. Zhou et al., 2020). This infection leads to the activation and subsequent transcription of genes associated with proinflammatory responses (J. Wang et al., 2020). Single-cell sequencing of PBMC from COVID-19 patients showed that classical monocytes were the main source of major COVID-19-mediating cytokines (Vanderbeke et al., 2021). SARS-COV-2 can rapidly activate pathogenic Th1 cells to secrete proinflammatory cytokines, such as GM-CSF, IL-6. GM-CSF further activates CD14+CD16+ inflammatory monocyte to produce large quantities of IL-6, TNF-α and other cytokines (Hussman, 2020). Notably, these inflammatory monocytes have been found to secrete significantly higher levels of IL-6, particularly in ICU patients, exacerbating the severity of the inflammatory storm (Y. Zhou et al., 2020). Additionally, monocytes from COVID-19 patients have also been shown to have the ability to secrete GM-CSF (Y. Zhou et al., 2020). The rapid increase in GM-CSF+ and IL-6+ monocytes indicates a potentially high risk of monocyte-driven inflammatory cytokine storms (Y. Zhou et al., 2020).

Table 1. Role of monocytes and macrophages in cytokine storm.

Monocyte/ macrophage type	**Produced cytokine**	**Mechanism of induction**	**Target cells, response**	**Refs**
Circulating monocytes	CCL2	STAT3, NFκB, IL-6; TLR2, IFNγ, TNFα	Chemotactic agent of monocytes. Chemotactic agent of DCs. Chemotactic agent of basophils. Chemotactic agent of Th2 cells.	(Attiq et al., 2021; Murakami et al., 2019) (Qudus et al., 2023; Zhang et al., 2021)
Circulating monocytes; bronchoalveolar macrophages	CCL3	IFNγ, TNFα	Chemotactic agent of macrophages. Chemotactic agent of DCs. Chemotactic agent of eosinophils. Chemotactic agent of NK. Chemotactic agent of Th1-cell.	(Attiq et al., 2021; Gibaldi et al., 2020; Park et al., 2023; Ramasamy & Subbian, 2021)

Circulating monocytes; bronchoalveolar macrophages	CCL4	LPS, IL-7	Recruitment of macrophages; Recruitment of DCs; Recruitment of NK; Recruitment of Th1 cells.	(Attiq et al., 2021; Ramasamy & Subbian, 2021)
Circulating monocyte; bronchoalveolar macrophages	CXCL10	TLR2, IFNγ, TNFα	Recruitment of macrophages; Recruitment of NK; Recruitment of Th1 cells.	(Attiq et al., 2021; Ramasamy & Subbian, 2021; Zhang et al., 2021)
Circulating monocyte	GM-CSF		B cells, NK cells, CD8+ T cells, and CD4+ T cells	(Ramasamy & Subbian, 2021)
Circulating monocyte; bronchoalveolar macrophages	IFNγ	IL-18	Stimulate macrophages Chemotactic agent of Th1-cell.	(Gu et al., 2021; Han et al., 2020; Ombrello & Schulert, 2021; Pandolfi et al., 2020; Qudus et al., 2023)
Circulating monocyte; lung and liver macrophages	IL-1β	IFNγ, LPS, TLR2, NLRP3	increases the transport of neutrophils to the site of infection, activates macrophage. activates Th17 cell. induces epithelial and endothelial cells to produce a second wave of cytokines.	(Attiq et al., 2021; Fawzy et al., 2022; Gu et al., 2021; Ombrello & Schulert, 2021; Pandolfi et al., 2020; Qudus et al., 2023; Zhu & Kanneganti, 2017)
Circulating monocytes; bronchoalveolar macrophages	IL-2	NFκB,	Effector T-cell and regulatory T-cell growth factor	(Fawzy et al., 2022; Han et al., 2020; Hu, Huang, et al., 2021; Zhu & Kanneganti, 2017)
Circulating monocytes; lung, liver, and kidney macrophages	IL-6	GM-CSF, IL-1β; TLR2,	Monocytes, ECs. Pyrogenic cytokine with pro-inflammatory activity, stimulates acute-phase reactions and antibody production.	(Attiq et al., 2021; Hirano & Murakami, 2020; Hu, Huang, et al., 2021; Hussman, 2020; Ombrello & Schulert, 2021; Qudus et al., 2023) PMID: 34866574

Circulating monocytes, lung macrophages,	IL-8	STAT3, NFκB, IL-6	Chemotactic agent of neutrophils	(Attiq et al., 2021; Fawzy et al., 2022; Murakami et al., 2019; Pandolfi et al., 2020; Qudus et al., 2023)
Circulating monocytes; lung liver macrophages	IL-10	NFκB,	Promote M2 macrophage polarization. inhibit Th1 and cytokine release	(Han et al., 2020; Qudus et al., 2023)
Lung macrophages, liver macrophages	IL-18	NFκB,	Th1, Th2, neutrophils activation of Th1 pathway, exhibit synergism with IL-12 mediates IFN-γ production in T and NK cells	(Attiq et al., 2021; Gu et al., 2021; Qudus et al., 2023)
Lung macrophages	IL-33	NFκB,	potentiates Th1 and Th2 cells, NK cells, CTLs, and mast cells	(Attiq et al., 2021)
Circulating monocyte, Lung macrophages, liver macrophages	TNF-α	GM-CSF, IL6, IL-1β; TLR2	Monocyte. increase vascular permeability	(Gu et al., 2021; Hirano & Murakami, 2020; Hussman, 2020; Ombrello & Schulert, 2021) PMID: 34866574
Lung macrophages	VEGF	STAT3, NFκB, IL-6	Promotes angiogenesis	(Attiq et al., 2021; Murakami et al., 2019)
Lung macrophages	YKL-40	IFNγ	Regulating Th1 and Th2 differentiation; Chemotactic effect on vascular endothelium and smooth-muscle cells	(Attiq et al., 2021; Julia Kzhyshkowska et al., 2006)

1.3 Chitinase-like proteins and COVID-19

1.3.1 Family of chitinases and chitinase-like proteins

Glycoside hydrolase family 18 includes chitinases and non-enzymatic chitinase-like proteins (CLPs)(J. Kzhyshkowska et al., 2006), both of which bind chitin, a polysaccharide chain composed of N-acetylglucosamine repeats and present in arthropods and other taxa as a major structural polymer(Ting Zhao et al., 2020). The production of chitinase is a necessary component of chitin-containing fungi and parasites; however, mammals do not produce this substance. Chitinases are evolutionary conserved functions in lower life forms that protect organisms containing chitin (Kzhyshkowska et al., 2007). While chitinases cleave chitin, CLPs do not possess this enzymatic activity(Ting Zhao et al., 2020). In humans, six Glyco_18domain-containing proteins are identified as follows: chitotriosidase, acidic mammalian chitinase (AMCase), oviductin specific glycoprotein, YKL-40 (CHI3L1), YKL-39 (CHI3L2) and stabilin-1-interacting chitinase-like protein (SI-CLP). Additionally, two proteins closely related to the Glyco_18 domain, known as YM1 and YM2, have

been identified in rodents but are not present in humans. (Jin et al., 1998)(Ting Zhao et al., 2020).

Commonly, CLPs possess an enzymatically silent Glyco_18 domain due to the substitution of glutamic acid for leucine, isoleucine, or tryptophan at the end of the conserved motif (DxxDxDxE) (Kzhyshkowska et al., 2006; Ranok et al., 2015). While CLP's Glyco_18 domain lacks enzymatic activity, it exhibits lectin properties with specific sugar binding preferences (Fusetti et al., 2003). In the extracellular compartments, mammalian CLPs may bind carbohydrates polymer components (e.g. extracellular matrix and cell surface glycoproteins) (Jin et al., 1998). In glioblastoma, YKL-40's ability to bind heparan sulfates results in its interaction with syndecan-1, resulting in VEGF production and enhanced angiogenesis (Francescone et al., 2011). (Francescone et al., 2011).

CLPs are produced by multiple cell types and combine properties of cytokines and growth factors. The major cell types which produce CLPs includes macrophages, neutrophils, fibroblast-like cells, endothelial cells, and cancer cells (Kzhyshkowska et al., 2016; Ting Zhao et al., 2020). These CLPs can bind chitin by inducing conformational changes that depend on the length of the oligosaccharides in a similar fashion as catalytically active family 18 chitinases (Fusetti et al., 2003; Lee et al., 2011). However, due to mutations in their active domains, CLPs do not have chitinolytic enzyme activity. (Kzhyshkowska et al., 2016; Lee et al., 2011). Our outstanding of the roles CLPs in human disease is still quite limited (Lee et al., 2011). However, several articles have demonstrated the importance of CLPs in regulating innate(Reese et al., 2007) and adaptive Th2 inflammatory(Lee et al., 2009; Zhu et al., 2004) and remodeling responses(Lee et al., 2012; Matsuura et al., 2011).

1.3.2 Chitinase-like proteins as biomarkers

Several studies have examined the role of CLPs as biomarkers of pathology or disease severity in recent years (Chen et al., 2014; Sutherland, 2018; Zhu et al., 2012) (Table 2). Higher expression of YKL-39 was found in osteoarthritic joint (Knorr et al., 2003). As the most investigated CLPs, elevated serum levels of YKL-40 have been found to be associated with the severity of asthma, liver fibrosis, COVID-19 infection and poor prognosis in patients with cancer. At the same time, genetic variation in YKL-40 is closely associated with the incidence and prognosis of multiple inflammatory and neoplastic diseases (Berres et al., 2009; Kruit et al., 2007; T. Zhao et al., 2020).

Table 2. YKL-40 in pathology.

Disease Cohort (number of patients, source)	**Concentration in serum (ng/ml),**	**Genetic variants**	**Correlation with symptoms**	**Refs**
Renal cell cancer. (222 patients vs 35 controls, the departments of urology of University Essen and the Charité in Berlin, Germany)	103.7(59.3–242.0, with relapse)	N/A	Shorter overall survival	(Väänänen et al., 2017; Vom Dorp et al., 2016)

Renal cell cancer. (82 patients followed up in 2 years, Tampere University Hospital, Finland)	50.6 (33.8–97.1, without relapse)	N/A	Shorter overall survival	(Väänänen et al., 2017; Vom Dorp et al., 2016)
juvenile idiopathic arthritis (96 patients vs 45 healthy controls, The John Paul II Pediatric Center in Sosnowiec, Poland)	169.16 ± 41.25 (patients), 82.57 ± 19.41 (Healthy controls)	N/A	disease activity	(Winsz-Szczotka et al., 2020)
Stable coronary artery disease (87 poor collateral patients vs. 78 good collateral patients, Turkiye Yuksek Ihtisas Training and Research Hospital, Department of Cardiology, Ankara, Turkey)	177 ± 39 (poor collateral group); 151 ± 41 (good collateral group)	N/A	Poor collateral developments	(Akboga et al., 2016)
Idiopathic pulmonary arterial hypertension (82 patients vs. 54 healthy controls, Chinese Academy of Medical Sciences and Peking Union Medical College, China)	24.90 (17.68–39.78, IPAH), 16.58 (14.20–19.64, HC)	N/A	greater PAH severity;	(Chen et al., 2014)
Cardiovascular complications in patients with type 2 diabetes mellitus (119 patients, 21 reported cardiovascular events, Medical School, University of Ioannina, Ioannina, Greece)	105.1 (13.5 to 285.0, with event reported); 69.8 (5.0 to 301.0, no event reported)	N/A	subclinical atherosclerosis (arterial stiffness) and adverse clinical prognosis.	(Naka et al., 2018)
Acute myocardial infarction (47 patients with first AMI, 16 patients with CAD, Hvidovre Hospital, Denmark)	156 (40–3000, AMI); 106 (54–300, CAD)	N/A	increased in AMI patients	(Nøjgaard et al., 2008)

Myocardial infarction (5526 people without MI were followed, 1587 were reported MI in mean time 11.3 years, Norway)	42.0 (median, cases), 43.3 (median, controls)	N/A	lower risk of incident MI,	(Ueland et al., 2020)
Chronic myeloproliferative neoplasms (48 patients vs. 30 healthy controls, Copenhagen University Hospital, Denmark)	43 (patients), 28 (controls)	N/A	disease activity and the inflammatory state	(Bjørn et al., 2014)
Cystic fibrosis (188 patients, Institut de Recherches Cliniques de Montréal, Canada)	N/A	CHI3L1 rs4950928 SNP	potential biomarker of CF disease severity	(Coriati et al., 2021)
Cerebrovascular disease (123 patients with acute cerebral vascular disease, First Affiliated Hospital, Sun Yat-sen University, China)	ischemic stroke (178.58 ± 127.78), hemorrhagic stroke (105.32 ± 87.35) and TIA (148.09 ± 108 ng/ml)	N/A	diagnostic/prognostic biomarker for cerebrovascular disease	(Akboga & Sahinarslan, 2017; Xu et al., 2014)
Asthma (39 therapy-resistant asthma patients, 39 patients with controlled persistent asthma vs. 27 healthy controls, Karolinska University Hospital, Karolinska Institutet, Sweden)	19.2 (therapy-resistant), 16.3 (controlled patients), 13.8 (healthy control)	rs4950928	YKL-40 levels are increased in children with severe, therapy-resistant asthma compared to healthy children	(Konradsen et al., 2013)
COPD (52 patients with COPD, Recep Tayyip Erdoğan University School of Medicine, Turkey)	243.1 ± 129.2	N/A	severity of COPD	(Gumus et al., 2013; James et al., 2016)
COPD (61 patients with mild to moderate asthma, 76 patients with severe asthma, 45 patients with COPD, 48 healthy	33.3 (22.9–43.5, mild to moderate) 43.3 (31.1–75.9, severe asthma), 64.0 (37.4–142.20, COPD).	N/A	increased in asthma and more so in COPD.	(Gumus et al., 2013; James et al., 2016)

controls, Karolinska Institute, Sweden)	23.0 (17.1–26.5, healthy control)			
Endometriosis (33 patients vs 30 healthy control, University of Recep Tayyip Erdoğan School of Medicine Rize, Turkey)	106.0 ± 5.9 (patients), 52.2 ± 7.0 (Healthy control)	N/A	a novel biomarker for diagnosis and follow-up of endometriosis.	(Ural et al., 2015)
Hepatic fibrosis (129 patients with suspicion of liver disease, Department of Medicine, Hvidovre Hospital, University of Copenhagen, Denmark)	118 (105–165, Normal); 195 (50–408, fatty liver); 174 (111–380, m viral hepatitis); 330 (115–967, non-cirrhotic fibrosis); 425 (145–2070, post- hepatitic cirrhosis); 532 (82–4850, Alcoholic cirrhosis)	N/A	increased in patients with chronic liver disease.	(Johansen et al., 2000)
Cirrhosis (21646 individuals for plasma, 84738 for genotype, Herlev University Hospital, Denmark)	N/A	genotype: rs4950928	rs4950928 maker for alcoholic liver cirrhosis.	(Kjaergaard et al., 2014)
COVID-19 (103 patients, 58 healthy controls, University of Liege, Belgium)	186 (84–384, patients without ICU admission); 241 (172–827, patients with ICU admission);	N/A	1. COVID-19 patients had higher levels of YKL-40 2. Within the COVID-19 population, YKL-40 was an indicator of the seriousness of infection	(Schoneveld et al., 2021)

1.3.3 YKL40 as a marker of macrophage differentiation and activation

YKL-40 is an evolutionarily conversed member of 18 glycoside hydrolase gene family that is produced by a spectrum of cells in response to various injury and cytokine stimulation (Lee, 2009; Lee & Elias, 2010). YKL-40 is named based on its three N-terminal amino acids, which are tyrosine (Y), lysine (K), and leucine (L), and its molecular weight is 40 kDa (Mazur et al., 2021). YKL-40 are expressed in a variety of cells, including innate immune cells (activated macrophages and neutrophils), stromal cells (fibroblasts; vascular smooth muscle cells; endothelial cells; hepatic stellate cells) and epithelial cells (Hakala et al., 1993; Kzhyshkowska et al., 2016; Lee et al., 2009). YKL-40 is not produced in monocytes and is only minimally expressed in monocyte-derived dendritic cells. However, its expression is significantly induced during the late stages of human monocyte differentiation (Rehli et al., 1997; Rehli et al., 2003). Our

group's former studies illustrated that YKL-40, induced by IFN-γ, is expressed in human macrophages (Julia Kzhyshkowska et al., 2006). Thus, YKL-40 can be regarded as a macrophage differentiation marker (Lee et al., 2011; Rehli et al., 1997). Moreover, YKL-40 is overexpressed in many human cancer types and animal tumor modes, including glioblastoma(Pelloski et al., 2005), acute myeloid leukemia(Bergmann et al., 2005), prostate cancer(Bergmann et al., 2005), osteosarcoma, and malignant melanoma (Mazur et al., 2021).

To date, factors such as changes in the extracellular matrix (ECM), microRNAs (miRNAs), growth factors, cytokines, stress, and drugs have been identified as significant regulators of the synthesis and secretion of YKL-40.(Johansen et al., 2001; J. Kzhyshkowska et al., 2006; Millis et al., 1985; T. Zhao et al., 2020). The dysregulation of YKL-40 can be seen in acute and chronic inflammation and tissue remodeling (Lee et al., 2011). It is believed that YKL-40 plays a key role in activating critical pathway during injury and inflammation, regulating innate and adaptive immunity, and heals and protects (Dela Cruz et al., 2012; Lee & Elias, 2010; Sohn et al., 2010; Zhou et al., 2014). The level of YKL-40 in circulation is increased by inflammation, injury, remodeling and repair (Dela Cruz et al., 2012; Lee, 2009; Lee et al., 2011; Lee & Elias, 2010).

1.3.4 YKL-40 roles in virus infection

YKL-40 played a critical role in the pathogenesis of non- cancerous disease. A recent study of 94,665 individuals from the Danish general population found that baseline elevated plasma CHI3L1 is a strong indicator of future infection disease risk (Kjaergaard et al., 2020). In acute virus infection, such as COVID-19, levels of YKL-40 in serum correlated to the severity of the disease (Ge et al., 2023) and could be a prognostic biomarker (Ebihara et al., 2023). In chronic infections, such as HBV, the serum level of YKL-40 was reported to be a non- invasive biomarker for liver fibrosis which is associated with severity (Jin et al., 2020). In HIV-induced encephalitis, YKL-40 could play a critical role in neuroinflammation symptoms (Bonneh-Barkay et al., 2008).

Elevation of the YKL-40 level is induced by inflammations in the lungs. The causes include cigarette smoke (Bara et al., 2012), viruses (Kim et al., 2019), and bacterial infection (Dela Cruz et al., 2012). During RSV infection, YKL-40 was reported to be a biomarker of systemic inflammation in children (Sawatzky et al., 2019). In the airway, YKL-40 regulates M2 macrophage activation and Th2 immune response, which are key components of the body's defense mechanism (Kim et al., 2019). Knock- down YKL-40 in bronchial epithelial cells in vitro, RSV-induced production of IL-8 was inhibited (Lee et al., 2022). During SARS- Cov-2 infection, patients suffering from more severe disease had significantly higher YKL-40 expression levels in serum (De Lorenzo et al., 2022; Hawerkamp et al., 2023; Schoneveld et al., 2021). It suggested it could be a promising prognostic marker of severity in COVID-19 infection (Schoneveld et al., 2021). Kamle *et a*l proved that YKL-40 could be a potent stimulator of SARS-COV-2 receptor angiotensin-converting enzyme 2 (ACE2) and viral spike protein priming proteases (Kamle et al., 2021).

Table 3. YKL40 in virus infection.

Virus	Cohorts, animal model, cell model	Sample	Correlation with symptoms	Role of YKL40 in pathology	Refs.
HBV	97 patients with liver fibrosis (Third Affiliated Hospital of Zhejiang Chinese Medical University, China)	serum	severity of liver fibrosis	N/A.	(Bao et al., 2022)
HBV	50 patients with liver fibrosis (Sylhet MAG Osmani Medical College Hospital, Bangladesh)	serum	severity of liver fibrosis	N/A .	(Das et al., 2021)
HBV	98 chronic HBV patients (Zhejiang Provincial People's Hospital, China)	liver biopsies	stage of hepatic fibrosis	N/A	(Huang et al., 2015)
HBV	134 chronic HBV patients (Zhejiang Provincial People's Hospital, China)	serum	Biomarker of liver fibrosis	N/A	(Jin et al., 2020)
HBV, HCV	64 chronic liver patients, 54 HC (Rancho Los Amigos Hospital, University of Southern California, USA) Stimulate HepG2 and Huh7 cell line with rhYKL40 (National Institute of Allergy and Infectious Diseases, NIH, USA)	Serum, liver speciem nts	increased susceptibilit y of the aging liver to fibrosis progression	CHI3L1 promotes the proliferation and activation of primary human HSCs, major drivers of liver fibrosis.	(Nishimu ra et al., 2021)
HCV	102 patients with liver fibrosis (eking University First Hospital, China)	Serum	monitor changes in fibrosis in patients with chronic HCV patients.	N/A	(Kang et al., 2021)
HCV	105 chronic patients (46 sofosbuvir-based regimens treated patients, 34 pegylated interferon/ribavirin - experienced patients, and 25 untreated patients. Peking University First Hospital, China)	Serum	sensitive marker to monitor fibrosis variations in weeks during treatments and after achieving SVR.	N/A	(Kang et al., 2020)

HCV	10 chronic HCV patients, 10 alcoholic hepatitis patients, 10 non-alcoholic steatohepatitis patients and 10 normal donor livers (Washington University Medical Center/Barnes-Jewish Hospital, USA)	Liver biopsies	Upregulated in HCV Patients with Fibrosis	TNFα mediated YKL40 expression is regulated by miRNA-449a and its target NOTCH1 in human hepatocytes.	(Sarma et al., 2012)
HCV	222 patients with HCV infection. (National Hepatology and Tropical Medicine Egypt.)	serum	associated with liver steatosis	N/A	(Esmat et al., 2007)
HCV	149 patients, who underwent liver transplantation for HCV-induced liver disease (Charité, Campus Virchow, Germany)	Genomic DNA from peripheral blood leukocytes	acute cellular rejection - occurrence was associated with YKL-40-genotypes (rs4950928; G/C)	N/A	(Eurich et al., 2013)
HIV	105 Perinatally infected children living with HIV (University of Alberta, Canada)	Serum	associated with poor virologic control, immune dysregulation, and microbial translocation in CLWH on antiretroviral.	N/A	(Bernard et al., 2022)
HIV	19 Macaca nemestrina (University of Pittsburgh School of Medicine, USA)	CSF	a specific and sensitive biomarker for the presence of encephalitis and were highly correlated with CSF viral load	N/A	(Bissel et al., 2016)

HIV	1. Macaca nemestriny infected with SIVDeltaB670 viral. 2. CD14+ cells from buffy-coat cultured with presence of M-CSF for Macrophage differentiation. Astrocyte purified from Primary human embryonic mixed glial-neuronal cultures were obtained from human fetal tissue. (University of Pittsburgh, USA)	CSF	correlated with an increase in CSF viral load	YKL-40 expression and secretion by macrophages and microglia. YKL-40 can bind to the ECM and is capable of displacing ECM-bound bFGF as well as blocking biological activities of this growth factor in neurons.	(Bonneh-Barkay et al., 2008)
IAV	1.S. pneumoniae infected BEAS-2B YKL-40 knocked-down cell line 2.Co- infection of IAV and S. pneumoniae in BEAS-2B cell line and ALI cultured hBECs (Universities of Giessen and Marburg Lung Center,Germany)	N/A	Promotes the pneumococcal infection	IAV pre- infection decreases YKL-40 levels to promote pneumococcal infection.	(Karwelat et al., 2020)
PUUV	79 hemorrhagic fever ith renal syndrome patients (Tampere University Hospital, Finland)	Plasma	elevated during acute PUUV infection and correlated with the overall severity of the disease	N/A	(Outinen et al., 2019)
RSV	43 children with RSV infection, 12 patients with mild respiratory symptoms (Severance Children's Hospital, Seoul Korea)	nasopharyngeal aspirate	Promote the airway infection induced by RSV infection	YKL-40 regulated M2 macrophage activation in RSV infection.	(Kim et al., 2019)
RSV	RSV infected BEAS-2B cells line with or without YKL-40 knocked down (Yonsei University	N/A	contributes to airway inflammation	Infection with RSV increased CHI3L1 and IL-8 expression.	(Lee et al., 2022)

	College of Medicine, South Korea)				
RSV	58 children (37 with RSV, 10 with rhinovirus, 11 control, Jinja Regional Referral Hospital and Kambuga District Hospital. Canada)	plasma	biomarkers of Systemic Inflammation	N/A	(Sawatzky et al., 2019)
RSV	YKL-40 knockdown BALA/c mice infected RSV virus. (Zhongnan Hospital of Wuhan University, China)	N/A	N/A	promotes RSV induced immune imbalance and airway hyperresponsiveness by regulating the functional transformation of DCs.	(Ge et al., 2023)
SARS-Cov-2	103 patients with infection of COVID-19 (CHU de Liège, Domaine Universitaire du Sart-Tilman, Belgium)	Serum	prognostic marker of severity	N/A	(Schoneveld et al., 2021)
SARS-Cov-2	191 COVID-19 patients, 80 survivors, and 40 control (IRCCS San Raffaele Scientific Institute, Italy.)	Plasma	predicts COVID-19 outcome	N/A	(De Lorenzo et al., 2022)
SARS-Cov-2	First discovery cohorts, n= 306. (Massachusetts General Hospital, USA) Second n=53, validation cohorts, n=113 (Osaka University Hospital, Japan)	Plasma	associated with mortality and clinical outcome	N/A	(Ebihara et al., 2023)
SARS-Cov-2	60 COVID-19 patients, 40 control (Faculty of Medicine, Atatürk University, Turkey.)	Plasma	Correlation with disease severity	N/A	(Parlak & Laloğlu, 2022)
SARS-Cov-2	15 children hospitalized for Omicron-related neurological manifestations ((Queen Mary Hospital, Princess Margaret Hospital, and Pamela Youde	Plasma and CSF	Associated with patients' length of hospitalization	N/A	(Tso et al., 2023)

	Nethersole Eastern Hospital, China))				
SARS-Cov-2	18 COVID-19, 10 pre-pandemic patients who died of acute respiratory distress syndrome (ARDS), and 7–8 control patients with no ARDS or COVID-19 (Columbia University Irving Medical Center, USA.)	medulla oblongata	Contribute to the neuroinflammation	N/A	(Madden et al., 2023)
SARS-Cov-2	1.COVID-19+ (Rhode Island Hospital, Brown University, USA.) 2.Calu-3 cell, A549, HSAECs, stimulated by rhYKL-40, was infected by SC2 pseudovirus. 3.CHI3L1-overexpressing Tg mice and CHI3L1-null mutant mice. (Rhode Island Hospital, Brown University, USA.)	serum	Associated with age and patients with comorbid diseases	potent stimulator of ACE2 and SPP	(Kamle et al., 2021)
SARS-Cov-2	uptake of pseudovirus with ancestral and mutated S proteins by untreated and CHI3L1-treated Calu-3 cells. (Rhode Island Hospital, Brown University, USA.)	N/A	N/A	augments epithelial cell infection by pseudoviruses	(Kamle et al., 2022)
TBEV	16 patients with menigoencephalitis, 17 with meningitis, 17 control (Medical University in Białystok, Poland)	CSF	higher in meningitis than in meningoencephalitis	N/A	(Czupryna et al., 2022)

HBV: Hepatitis B; HCV: Hepatitis C; HIV: Human Immunodeficiency Virus; IAV: Influenza A virus; PUUV: Puumala orthohantavirus; ALI: air-liquid interface; hBECs: human bronchial epithelial cells; HSAECs: primary human small airway epithelial cells; RSV: Respiratory Syncytial Virus; TBEV: Tick-borne encephalitis virus. N/A: Not applicable.

1.4 Aim and objectives.

The aim of this study is to investigate the long- term changes of trained immunity after recovery from moderate SARS-Cov-2 infection.

The specific objectives were:

1. To compare the YKL-40 expression in MDM from both COVID-19 convalescent and healthy donors.
2. To investigate whether YKL-40 could be a potential marker for long-term changes in innate immunity after COVID-19 onset.
3. To find the correlation of YKL-40 expression in MDM from convalescent individuals with the clinical symptoms in the acute phase.

2 MATERIALS AND METHODS

This work was written under the operating system Windows 10 with the text processing program Microsoft Office Word 2021. The presented pictures, as well as numerous reports, have been processed by t GraphPad Prism 9 and Biorender (https://www.biorender.com/).

2.1 Chemicals, reagents and kits

Table 4. Chemicals and reagents.

Product	**Company**	**Cat. No**
Bovine Serum Albumin (BSA)	Sigma Aldrich	1193003
CD14 MicroBeads	Miltenyi Biotec	130-050-201
DEPC water	Thermo Scientific	
Dulbecco's PBS, sterile 1x, without $CaCl_2$, $MgCl_2$	Gibco	14190094
Ethanol	Roth	
Ficoll Paque Premium	Cytiva	GE17-1441-02
LPS	Sigma Aldrich	L4391
Recombinant Human IFN-γ	Peprotech	300-02
Recombinant Human IL-4	Peprotech	200-04
Substrate reagent	R&D systems	DY999
Tween20	Sigma Aldrich	9127.1
UltraPure™ 0.5M EDTA	Invitrogen	
X-VIVO™ 10	Lonza	BE04-380Q

Table 5. Kits.

Product	**Company**	**catalogue N**
E.Z.N.A. total RNA kit I	Omega bio-tek	
Human Chitinase 3-like 1 DuoSet ELISA kit	Bioline meridian	Cat#DY2599
SensiFAST cDNA synthesis kit	Fermentas	BIO-65054
RNeasy mini kit	Qiagen	

2.2 Consumables

Table 6. Consumables.

Product	**Company**
22µm filters	Fisherbrand
CASYcups	Omni Life Sciences
Cell culture 12 wells plates	Thermofisher
Elisa Plates	R&D systems
LS columns	Miltenyi Biotec
PCR tubes	Star Labs
Pipette tips (10/20µL, 200µL, 1000µL)	Starlab
qPCR plate sealers	Axon Labortechnik
qPCR plates	Axon Labortechnik

Product	**Company**
Safe-Lock Eppendorf Tubes, 1.5ml	Eppendorf
Falcon tubes (15ml, 50ml)	Greiner
LS column	Miltenyi Biotec

2.3 Equipment

Table 7. Equipment.

Product	**Company**
Autoclave VX-95	Systec
BD FACSCanto II	BD Biosciences
CASY Cell counter	Schärfe System
Centrifuge 5415C	Eppendorf
Centrifuge 5415R	Eppendorf
Deep freezer (-80°C)	Thermo
Freezer (-20°C)	Liebherr
HydroFlex ELISA microplate washer	Tecan
Ice machine AF 100	Scotsman
Incubator (37°C)	Edmund Bühler GmbH
Light cycler LC480	Roche
Mini plate centrifuge	Nippon genetics
NanoQuant Plate	Tecan
Roller shaker	IDL
Shaker	Neolab
Tecan Infinite 200 Pro	Tecan
Vortex	Scientific industries

2.4 Buffers and solutions

Table 8 Buffers

Wash buffer for ELISA	10% Tween 20 was prepared by adding 50 ml of Tween 20 to 450 ml of PBS. 1000 ml of 10x PBS was mixed with 9 l of ddH2O.
MACS buffer	500 ml of PBS, 2.5g of BSA and 2 ml of 0.5M EDTA

2.5 Patients and healthy donors

Patients who had recovered from COVID-19 were recruited as donors for convalescent plasma and invited to participate in the CORE (COVID-19 convalescent) trial as an accompanying research program (CORE study). Non-infected healthy donors were recruited as control donors at their regular plasma donation appointment. All donors had a negative SARS-CoV-2 antigen test at the time of blood donation. The CORE study was approved by the Ethical Committee of the Medical Faculty Mannheim (Ref: 2020-643N). All donors gave written informed consent to participate in the CORE study (Müller et al., 2022). The inclusion criteria for the convalescent donors were: 1)

SARS-CoV-2 infection documented by a positive RT-PCR (from nasal or pharyngeal swap) or a positive anti-SARS-CoV-2 antibody test; 2) at least two weeks since the end of symptoms; 3) no residual severe organ dysfunction; 4) no fever at the time of blood sampling; 5) age 18–68 years (Müller et al., 2022). The convalescent donors suffered from COVID-19 without disease or mild or moderate symptoms without hospitalization (Figure 1).

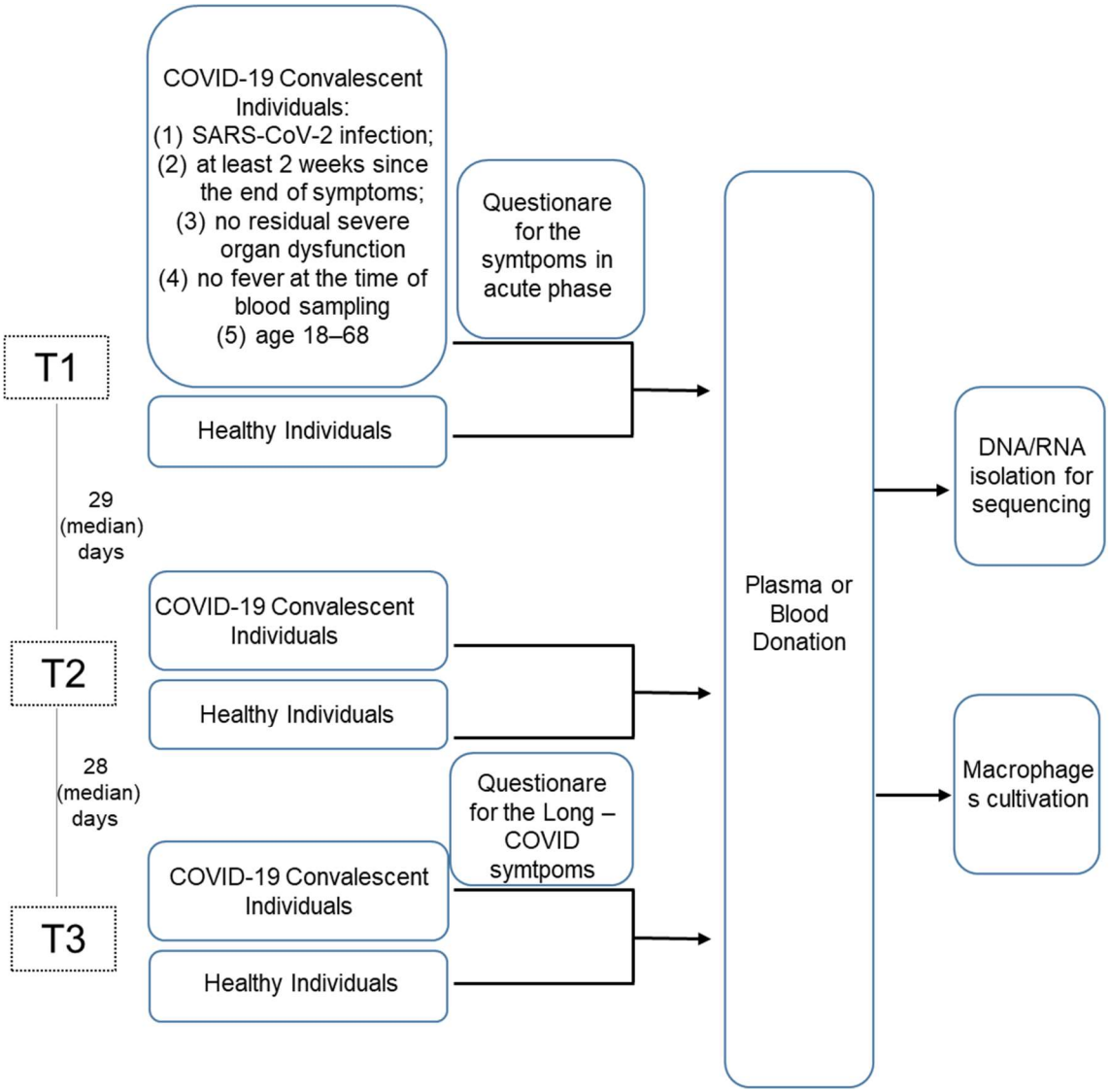


Figure 1. Schematic presentation of the cohort's collection

2.6 Molecular biology techniques

2.6.1 Primers

Table 9 List of primers designed in the lab

Target gene	Sequence (5'-3' direction)
18s	CATTCGAACGTCTGCCCTAT
18s	TCACCCGTGGTCACCATG

18s	ACTTTCGATGGTAGTCGCCGTGCCT
IL-1ra	GAAGATGTGCCTGTCCTGTGT
IL-1ra	CGCTCAGGTCAGTGATGTTAA
IL-1ra	TGGTGATGAGACCAGACTCCAGCTG
IL1β	ACAGATGAAGTGCTCCTTCCA
IL1β	GTCGGAGATTCGTAGCTGGAT
IL1β	CTCTGCCCTCTGGATGGCGG

F: forward, R: reverse, Pr: probe. All primers and probes were ordered from Eurofins MWG Operon.

List of ready-made mixes.

All ready-made mixes were ordered from Life Technologies.

Table 10. list of primer

Assay code	**Target Gene**
Hs00609691_m1	CHI3L1
Hs00961622_m1	IL-10

2.6.2 Isolation of total RNA

E.Z.N.A. total RNA kit I used for RNA isolation. The lysis buffer was prepared by adding 20µl of β-mercaptoethanol to each 1ml of the TRK buffer. RNA isolation performed according to the manufacturer's instructions.

1. For cells: 350µl of lysis buffer was added to 3-5x10^6 cells, which were completely disrupted by passing through a needle fitted to a syringe for 10-15 times.

2. 350µl of 70% ethanol were added to the lysate and vortexed briefly. The sample was added to a HiBind RNA spin column placed into a 2ml collection tube.

3. After centrifuging for 1min at 10000g the flow-through was discarded. The column was washed once with 500µl wash buffer I.

4. The column was centrifuged at 10000g followed by washing twice in 500µl wash buffer II (each wash was followed by a centrifugation step at 10000g).

5. The final flow-through was discarded and a new 2ml collection tube was placed under the column which was centrifuged for 2 min at maximum speed.

6. The column was placed in a fresh 1.5ml RNase free Eppendorf tube and RNA was eluted with 45µl of DEPC-treated water and incubated for 5min in room temperature. Samples were centrifuged at maximum speed for 1min.

7. The RNA concentration was determined by measuring the absorption peak at 260nm wavelength with Tecan Infinite 200 PRO.

8. RNA samples were stored at –80°C for later application.

2.6.3 cDNA synthesis

For cDNA synthesis Kit from Meridian Bioscience was used. The experiment performed according to the manufacturer's instructions.

1. For this the following components were used:

Total RNA	5µl (up to 1µg)
5x TransAmp Buffer	4µl
Reverse Transscriptase	1µl
RNase- free water	Up to 20 µl

2. Mix gently by pipetting.

3. The samples were incubated at 37°C for 10 minutes and 42°C for 10 minutes and 95°C for 5 minutes.

4. The cDNA samples were stored at -20°C for later use.

2.6.4 Real-time PCR with Taqman probe

Genes were amplified from cDNA templates using primers specific to the gene of interest.

1. A master mix for all the samples was prepared in a 1.5ml Eppendorf tube containing:

Reagent	**Amount (µl) / sample**
Sensimix II	5
Reference Primer	0.5
Target Primer	0.5
ddH2O	3

2. The master mix was divided among 1.5ml Eppendorf tubes. As each sample was run in triplicates, 3µl of cDNA template was added to each tube and mixed by pipetting.

3. Amplification was performed using LightCycler 480 from Roche and the following program was used:

95°C	10min	
95°C	15s	} 50 cycles
60°C	1min	
37°C	2min	

Dual-labeled probes for target genes contained FAM on 5' end and BHQ1 quencher at 3' end of sequence. In all experiments 18s was used as the reference gene.

2.7 Cell culture techniques

2.7.1 Isolation of CD14+ monocytes from human peripheral blood treated with EDTA

1. Each EDTA tube contained 9 ml of blood. EDTA tubes were centrifuged at 420g, for 10 minutes with break.

2. Plasma was placed to 1.5 ml tubes and stored at -80°C.

3. Blood was transferred from a 50 ml Falcon tube and filled up to 35 ml with PBS 1x in case of 2 EDTA tubes. When the EDTA tubes were 5, fill up to 50 ml with PBS 1x.

4. For each 35 ml of diluted blood, a 50 ml Falcon tube with 15 ml Ficoll-Paque separating solution was prepared.

5. 35 ml of diluted blood (from step 3) was carefully layered (against the tube wall) on top of Fiocoll. The tubes were centrifuged at 420g, at RT, for 30 min without brakes.

6. PBMC (the second layer, white ring) were collected and transferred into a new 50 ml Falcon tube.

7. The PBMC suspension was adjusted up to 50 ml with PBS, cell was sedimented by centrifugation at 420g for 10 min.

8. The supernatant was discarded and the cell pellets from each donor were resuspended with 5ml PBS and collected in a new 15 ml Falcon tube for each donor.

9. The cells were washed with 10 ml PBS and centrifuged at 420g for 10min.

10. After discarding the supernatant, the cells were resuspended in 750 µl MACS buffer with 75 µl of CD14+ microbeads, and incubated for 30 minutes on a roller shaker at 4°C.

11. The tubes were filled up to 10 ml with MACS buffer and centrifuged at 420g, for 10min.

12. During this time, LS columns were attached to the magnetic stand, and 15 ml Falcon tubes were placed under columns. The columns were calibrated with 3 ml of MACS buffer.

13. The cell pellet (from step 8) was resuspended in 1 ml MACS buffer and then added to each column. The column was washed 3 times with MACS buffer, each time 3 ml.

14. The column was removed from the magnetic separation unit and placed on top of a fresh 15 ml Falcon tube. CD14+ monocytes were eluted from the column with 10 ml MACS buffer.

15. Cells were counted and centrifuged at 420g, for 10min. The cell pellet was resuspended in 1 ml of X-VIVO medium and cultured according to the experimental plan. The purity was examined by FACS.

2.7.2 Isolation of CD14+ monocytes from human peripheral blood using filters of plasma isolation kits

To minimize additional blood uptake from the convalescent plasma donors, we used filters of plasma isolation kits to harvest monocytes in parallel with the isolation out of blood samples.

1. Blood in the plasma set was collected in 50 ml Falcon tubes. It was centrifuged at 1400 rcf, for 20 minutes, at RT.

2. Supernatants were discarded and the pellets were resuspended with PBS. All pellets from the same donor were gathered in one 50 ml Falcon tubes, and the tubes were filled up to 35 ml with PBS.

3. Cell pellets were resuspended in 5 ml of PBS and $20x10^6$ PBMC were taken out and placed on ice until cultured.

4. The remaining cells were washed with 50 ml PBS and centrifuged at 420g for 10min.

5. The cell pellet was resuspended in CD14+ microbeads and MACS buffer according to the formula: 10 µl CD14 beads and 90 µl MACS buffer per $1x10^7$ cells.

6. The cells were incubated for 20min on a rotator at 4°C.

7. The tubes were filled up to 10ml with MACS buffer and centrifuged at 420g, for 10min.

8. During this time, LS columns were attached to the magnetic stand (One column is maximal for $1x10^8$ cells) and 15 ml Falcon tubes were placed under columns. The columns were washed with 3 ml of MACS buffer.

9. The cell pellet was resuspended in 1ml MACS buffer and then added to each column. The column was washed 3 times with MACS buffer, each time 3ml.

10. The column was removed from the magnetic separation unit and placed on top of a fresh 15 ml Falcon tube. CD14+ monocytes were eluted from the column with 10ml MACS buffer.

11. Cells were counted and centrifuged at 420g, for 10 minutes. The cell pellet was resuspended in 1ml of X-VIVO medium and cultured according to the experimental plan. The purity was checked by FACS.

2.7.3 Cell counting

Monocyte and PBMC cell counting was performed on a CASY cell counter.

1. 10µl of sample was added to 10ml of CASYton solution in a CASYcup.

2. The CASYcup was placed under the CASY capillary and the appropriate program for PBMC was chosen.

3. After each measurement 3 cycles of cleaning procedure were performed.

4. Only the viable cell number was used for further calculations.

2.7.4 Cultivation conditions used for primary human monocytes.

Isolated monocytes were seeded into 12-well plates in serum-free medium (X-VIVO, Gibco) at a concentration of $1x10^6$ cells/mL. Monocytes were stimulated with M-CSF at 5 ng/ml for all phenotypes. IFN-γ, at a concentration of 100 ng/ml, was used for polarizing monocytes towards M1 phenotype; IL-4 at a concentration of 10 ng/ml was used for polarizing monocytes towards M2 phenotype. No cytokines were added for M0 differentiation. Cells were cultivated at 37°C with 5% carbon dioxide. After 24 h of cultivation, stimulation with LPS was performed.

2.8 Immunological methods

2.8.1 Antibodies

Table 11. List of primary antibodies.

Antibody	Company	Catalog number	Species	Application
Anti human CD14, FITC-labelled	Invitrogen	11-0149-42	Mouse	FACS
IgG1 isotype control, FITC-labelled	Invitrogen	11-4714-41	Mouse	FACS

2.8.2 Flow cytometry.

Flow cytometry was used to verify the purity of CD14+ monocytes after isolation (data not shown). All procedures were performed in FACS tubes and according to the manufacturer's instructions

After monocyte or PBMC isolation $1x10^6$ cells were taken for FACS staining.

1. The cells were divided into two fractions (0.5x106 cells) and possible contamination with erythrocytes was removed with addition of 2ml of RBC-lysis buffer. Cells were incubated for 10min at RT.

2. Cells were centrifuged for 4min at 400g with break, at RT.

3. Cells were resuspended in 100µl FACS buffer.

4. 10µl of FcR Blocking Reagent was added and incubated with cells for 5min at 4°C.

5. Antibodies (conjugated with fluorochrome) were added (0,2-1µg per tube according to manufacturer instructions) to each sample. The cells were briefly vortexed and incubated for 30min at 4°C.

6. Cells were washed 2 times with 500µl FACS buffer. Each wash was followed by a centrifugation step (4min at 400g with break, at RT)

7. Cells were resuspended in 200µl FACS buffer before measurement. Staining was analyzed using BD FACS Canto II.

2.8.3 Enzyme-linked Immunosorbent Assay (ELISA)

Protocol for ELISA kits from R&D systems.

All procedures were performed at RT unless stated otherwise. ELISA was performed according to the manufacturer's instructions.

1. After 48 hours cultured, the supernatant of monocyte-derived macrophages from both COVID-19 convalescent and healthy individuals was collected. Each sample was diluted at a ratio of 1:25.

First day：

2. The Capture Antibody was diluted to working concentration according to manufacturer instruction in PBS without carrier protein.

3. A 96-well micro plate was immediately coated with 100µl per well of Capture Antibody. The plate was sealed and incubated overnight.

Second day:

4. The plate was washed with HydroFlex ELISA microplate washer (350µl wash buffer 3 times).

5. Each well was filled with 300µl of Reagent Diluent (1% BSA in PBS) to block unspecific binding sites. Incubate 1 hour.

6. The Reagent Diluent was aspirated from the wells and 100µl of fresh Reagent Diluent was added to the wells designated for standards and 50µl of Reagent Diluent was added to the wells designated for samples.

7. 100µl of standards and samples diluted in Reagent Diluent was added per well. The plate was covered with an adhesive strip and incubated for 2 hours.

8. The plate was washed with HydroFlex ELISA microplate washer (350µl wash buffer 4 times).

9. The Detection Antibody was diluted to working concentration according to manufacturer instruction in Reagent Diluent. 100µl of Detection Antibodies were pipetted into each well and the plate was incubated for 2 hours.

10. The plate was washed with HydroFlex ELISA microplate washer (350µl wash buffer 4 times).

11. Streptavidin-HRP was diluted to working concentration according to manufacturer instruction in Reagent Diluent. 100µl of Streptavidin-HRP was added to each well and the plate was incubated for 20 minutes in the dark.

12. The plate was washed with HydroFlex ELISA microplate washer (350µl wash buffer 4 times).

13. Substrate Solution was prepared by mixing 5ml of Reagent A with 5ml of Reagent B. 100µl of Substrate Solution was added to each well and the plate was incubated for 20 minutes in the dark.

14. 50µl of Stop Solution (2N H_2SO_4) was added to each well. The absorbance at 450nm was read on a Tecan Infinite 200 PRO reader.

15. To determine each sample's concentration a standard curve was drawn by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. Individual sample absorbance values were then compared to the standards values and the concentration was calculated according to the polynomial equation that describes the curve. In diluted samples, the concentration read from the standard curve was multiplied by the dilution factor. The sample's concentration was extrapolated if the absorbance values were below the lowest standard and above blank.

2.9 Statistical analysis

The significance of the differential expression data was analyzed using ratio paired Student's t-test for normal distribution or Mann-Whitney test for non-normal distribution. The significance of the data of correlation was analyzed using the ratio paired Pearson test for normal distribution or spearman test for non-normal distribution. We considered a two-tailed p-value of less than 0.05 to indicate statistical significance (confidence level 95%). ns = non-significant, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ and * $P \leq 0.001$.

3 RESULTS

3.1 Clinical characteristics of the study cohorts and experiments design

3.1.1 Clinical characteristics of the study cohorts

In total, 39 COVID-19 convalescent individuals (19 females, 20 males, average age ±SD, 40.4±14.1) and 36 healthy individuals (16 females, 20 males, average age ±SD, 33.4±12.8) at T1 were included in the cohort. General information for healthy and COVID-19 convalescent individuals is summarized in Table 12. The healthy population was statistically significantly (*P*=0.0354) younger (33.4±12.8 years) than the COVID-19 convalescent populations (40.4±14.1).

Table. 12 General Information about the donors

	Healthy individuals (n=36)	**COVID-19 convalescent individuals (n=39)**	**P value**
Age (average ±SD years)	33.4±12.8	40.4±14.1	0.0354(*)
BMI (average ±SD)	26.78±15.39	26.73±5.04	0.9403
Gender			0.6496
Male	20 (55.56%)	19(50.00%)	
Female	16 (44.44%)	19(50.00%)	
Medical History			
High Blood Pressure			>0.9999
-	33(91.67%)	35(89.74%)	
+	3(8.33%)	4(10.26%)	
Allergy			0.7418
-	28 (77.78%)	28 (71.79%)	
+	8 (22.22%)	11 (28.21%)	
Medicine Take			0.0498(*)
-	32 (88.89%)	27 (69.23%)	
+	4 (11.11%)	12(30.77%)	

The routine clinical blood cell quantification test demonstrated a normal physiological range for all parameters for both groups and no statistically significant differences were identified for the cellular fractions in blood (Table 13). Donors from COVID-19 convalescent cohorts had a statistically significant higher concentration of anti -Spike IgG.

Table 13 Clinical Parameter for Healthy and COVID-19 Convalescent Individuals (T1)

Parameter (average ±SD)[1]	Healthy individuals (n=36)	COVID-19 Convalescent individuals (n=39)	*P* value
Hct	0.410±0.0376	0.412±0.0328	0.9749
Hb (g/L)	140.2±14.58	141.9±12.18	0.6046
WBC (*103/μL)	6.722±1.784	5.959±1.026	0.0651
RBC (*106/μL)	4.814±0.5270	4.875±0.4355	0.7499
MCV	85.57±3.726	85.34±3.821	0.9693
PLT (*103/μL)	264.1±11.72	252.9±9.358	0.5889
Anti- Spike IgG	892.3±342.0	2160±616.0	0.0002 (***)

[1]Blood counts at T1: Hct, hematocrit (normal range: 0.37–0.54); Hb, hemoglobin (normal range: 120–180 g/L); WBC, white blood cells (normal range: 4–11X10^3/μL); RBC, red blood cells (normal range: 4.2–6.3 X10^6/μL); PLT, platelets (normal range: 150–400X10^3/μL).

In convalescent cohorts, all donors were graded 2 or less on the 8-point WHO severity scale for COVID-19 acute disease (World Health Organization. COVID-19 Therapeutic Trial Synopsis. https://www.who.int/publications/i/item/covid-19-therapeutic-trial-synopsis Updated February 18, 2020. Accessed August 31, 2021). Furthermore, no comorbidities were reported among the donors. Clinical parameters in the acute phase of the COVID-19 convalescent cohort are summarized in the Table 14. The most reported symptoms in the acute phase were exhaustion (76.9%), disturbance of smell or taste (74.4%), headache (71.8%), body pain (64.1%) and fever (59.0%). Among all convalescent plasma donors, 28.2% of individuals reported Long-COVID symptoms.

Table 14. Clinical symptoms for COVID-19 convalescent individuals in phase

	Symptoms	COVID-19 Convalescent individuals (n=39)	
		-	+1
Respiratory symptoms	Cough	17 (43.6%)	22 (56.4%)
	Sniff	18 (46.2%)	21 (53.8%)
	Sore throat	18 (46.2%)	21(53.8%)
	Difficult in breath	21 (53.8%)	18 (46.2%)
	Pneumonia	32 (82.1%)	7 (17.9%)
Systemic symptoms	Eye inflammation	33 (84.6%)	6 (15.4%)
	Fever	16 (41.0 %)	23 (59.0%)
	Skin rash	37 (94.9%)	2 (5.1%)
	earache	35 (89.7%)	4 (10.3%)
	Exhaustion [2]	8 (20.5%)	31 (79.5%)
	Headache [2]	12 (30.8%)	27(69.2%)
Neurological symptoms	Exhaustion2	8 (20.5%)	31 (79.5%)
	Headache2	12 (30.8%)	27(69.2%)
	Disturbance of smell or taste	9 (23.1%)	30 (76.9%)
	Nausea	30 (76.9%)	9 (23.1%)
	Limb/ joint/ back pain	14 (16.5%)	25 (64.1%)

[1] - without event. + with event; [2] The symptoms of exhaustion and headache belong to systemic and neurological system.

3.1.2 Experiments design

To determine the imprint of monocytes in COVID-19 convalescent individuals, monocytes were isolated from the EDTA-treated whole blood or plasmaset donated by COVID-19 convalescent or healthy individuals. Monocytes were stimulated with M-CSF at all phenotypes. IFN-γ was used for polarizing monocytes towards M(IFNγ) phenotype; IL-4 was used for polarizing monocytes towards M(IL-4) phenotype. No cytokines were added for M(NS) differentiation. To analyze whether YKL-40 expression induced in monocytes-derived macrophages by strong pathogen-derived pro-inflammatory stimulation will also differ between COVID-19 convalescent and healthy plasma donors, monocytes differentiated towards M(NS), M(IFN-γ) and M(IL4) macrophage phenotypes for 24 hours were challenged with or without LPS for next 24 hours (Figure 2).

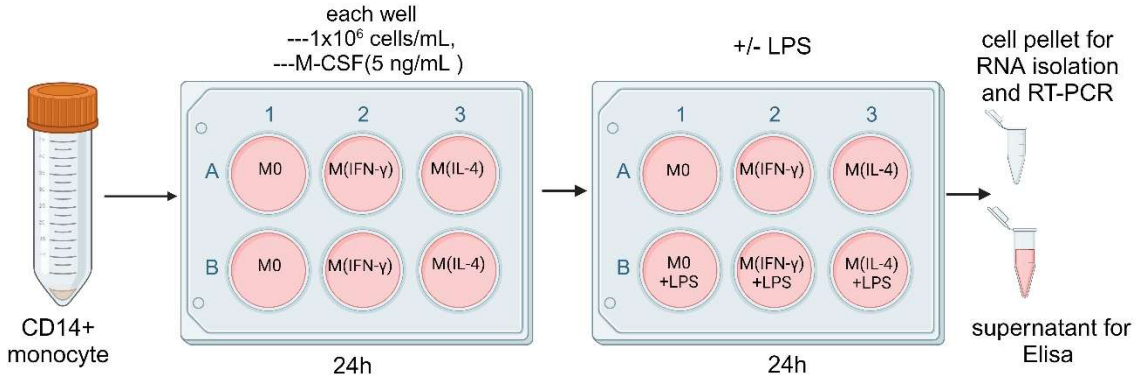


Figure 2. Schematic presentation of macrophage differentiation. Peripheral blood monocytes were isolated using CD14+ MACS sorting and cultivated in a serum-free medium at a concentration of $1x10^6$ cells/mL in the presence of M-CSF. IFN-γ was used for polarizing macrophages towards M(IFN-γ) phenotype; IL-4 was used for polarizing macrophages towards M(IL4) phenotype. No cytokines were added for M0 differentiation. After 24 h of cultivation, stimulation with LPS was performed for next 24 h. Cell pellets for RNA isolation and conditioned medium for ELSA were harvested after total 48 h in ex vivo conditions.

3.2 Expression of YKL-40 was statistically significantly increased in homeostatic and healing macrophages differentiated out of monocytes of COVID-19 convalescent plasma donors compared to healthy donors

To analyze the imprint of monocytes following SARS-CoV-2 infection, changes in 4 cytokines at T1 across all six macrophage types were selected: IL-1β for activation of M(IFNγ) macrophages, IL-10 and IL-1ra for activation of M(IL4) macrophages, and YKL-40 for macrophage differentiation (Fig. 3). There were 39 COVID-19 convalescent donors and 37 healthy donors for M(NS), M(NS)+LPS, M(IFNγ) + LPS and M(IL4) + LPS, 35 COVID-19 convalescent donors and 34 healthy donors for macrophages of M(IFN-γ) and 31 COVID-19 convalescent donors and 34 healthy donors for macrophages of M(IL4). RT-PCR was used to analyze the expression of the 4 genes. As the result showed, there is no statistically significant differences was found in IL-1β and IL-1ra in all 6 types of macrophages. A statistically higher expression of YKL-40 was found in M(NS) (P=0.0257), M(NS) + LPS (P=0.0181), and M(IL4) (P=0.0008)

from COVID-19 convalescents. Statistically significant higher expression of IL-10 can be detected in M(NS)+LPS (*P*=0.0176) and M(IFNγ) (*P*=0.0275, Fig.3).

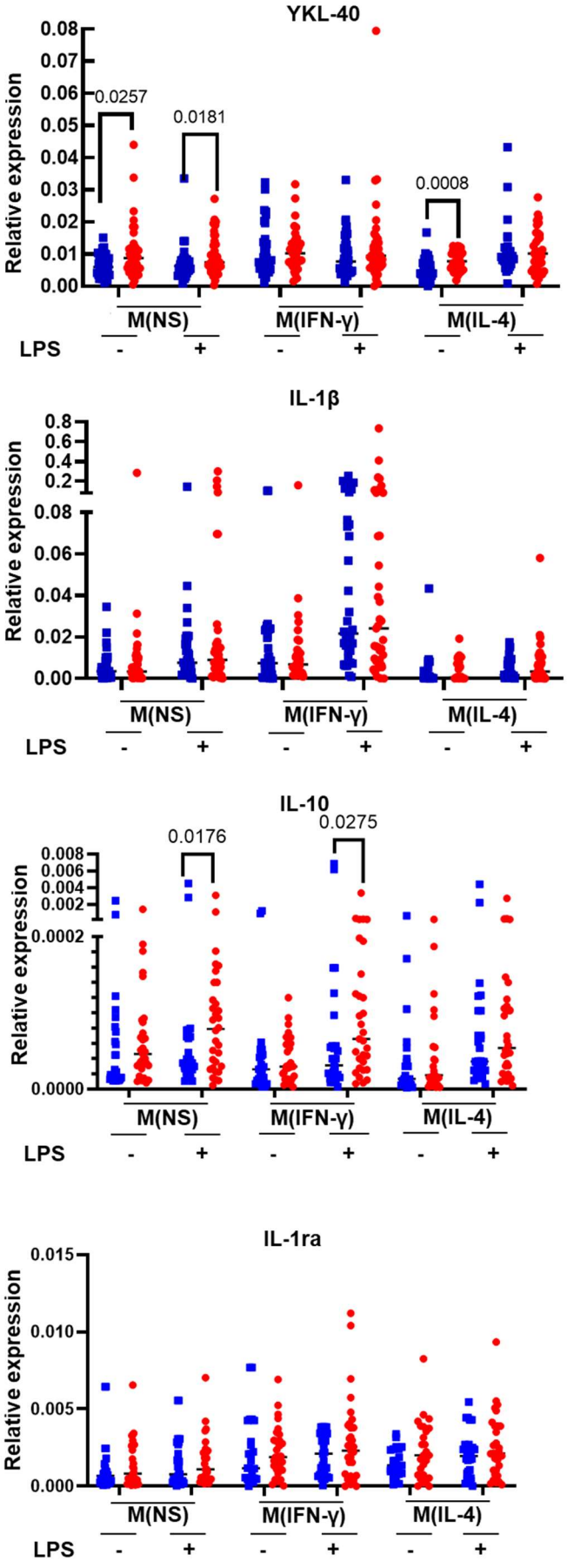


Figure 3. Expression of activation biomarkers in macrophages differentiated out of monocytes of COVID-19 convalescent plasma donors (red) and healthy individuals (blue). Macrophage stimulation was performed according to Fig. 2. Expression of YKL-40, IL-1β, IL-10, IL-1ra was analyzed by RT-PCR. Individual points present single donors n=39 for COVID-19 convalescent donors and n=37 healthy donors for M(NS), M(NS)+LPS, M(IFNγ) +LPS and M(IL4)+LPS; n=35 for COVID-19 convalescent donors and n=34 healthy donors for macrophages of M(IFN-γ); n=31 for COVID-19 convalescent donors and n=34 healthy donors for M(IL4). Mann-Whitney test was performed for statistical analysis. The diagram provides P values that indicate statistical significance.

Following the discovery of elevated YKL-40 expression in monocyte-derived macrophages from COVID-19 convalescent donors at T1, we extended our analysis to include YKL-40 expression at T2 and T3 (Fig. 4). In the graphs of M(NS), M(NS)+LPS, M(IFNγ) + LPS and M(IL4) + LPS, each point presents a single donor of 39 COVID-19 convalescent donors and 37 healthy donors for macrophages at T1, 28 COVID-19 convalescent plasma donors and 29 healthy donors at T2, 20 COVID-19 convalescent plasma donors and 16 healthy donors at T3. In the graph of M(IFN-γ), each point represents a single donor, 35 COVID-19 convalescent plasma donors and 34 healthy donors for macrophages in T1, 23 COVID-19 convalescent plasma donors and 23 healthy donors in T2, 18 COVID-19 convalescent donors and 13 healthy donors in T3. In the graph of M(IL4), each point represents a single donor of 35 COVID-19 convalescent donors and 34 healthy donors for macrophages in T1, 18 COVID-19 convalescent plasma donors and 19 healthy donors in T2, 15 COVID-19 convalescent donors and 13 healthy donors in T3. Higher expression of YKL-40 can be seen in all 6 types of macrophages at all 3 time points. In M(NS), the statistically significant higher YKL-40 expression can be found at T1 (P=0.0257) and T2 (P=0.0327). With LPS stimulation, the statistical significance can be still found in T1 (P=0.0181). In M(IL4), the statistically significant higher YKL-40 expression can be found at T1 (P=0.0008). With LPS stimulation, the statistical significance can be still found in T3 (P=0.0154). With or without LPS stimulation, M(IFN) does not show statistical significance. Both COVID-19 convalescents and healthy donors did not exhibit any statistically significant differences in YKL-40 expression of MDM at different time points.

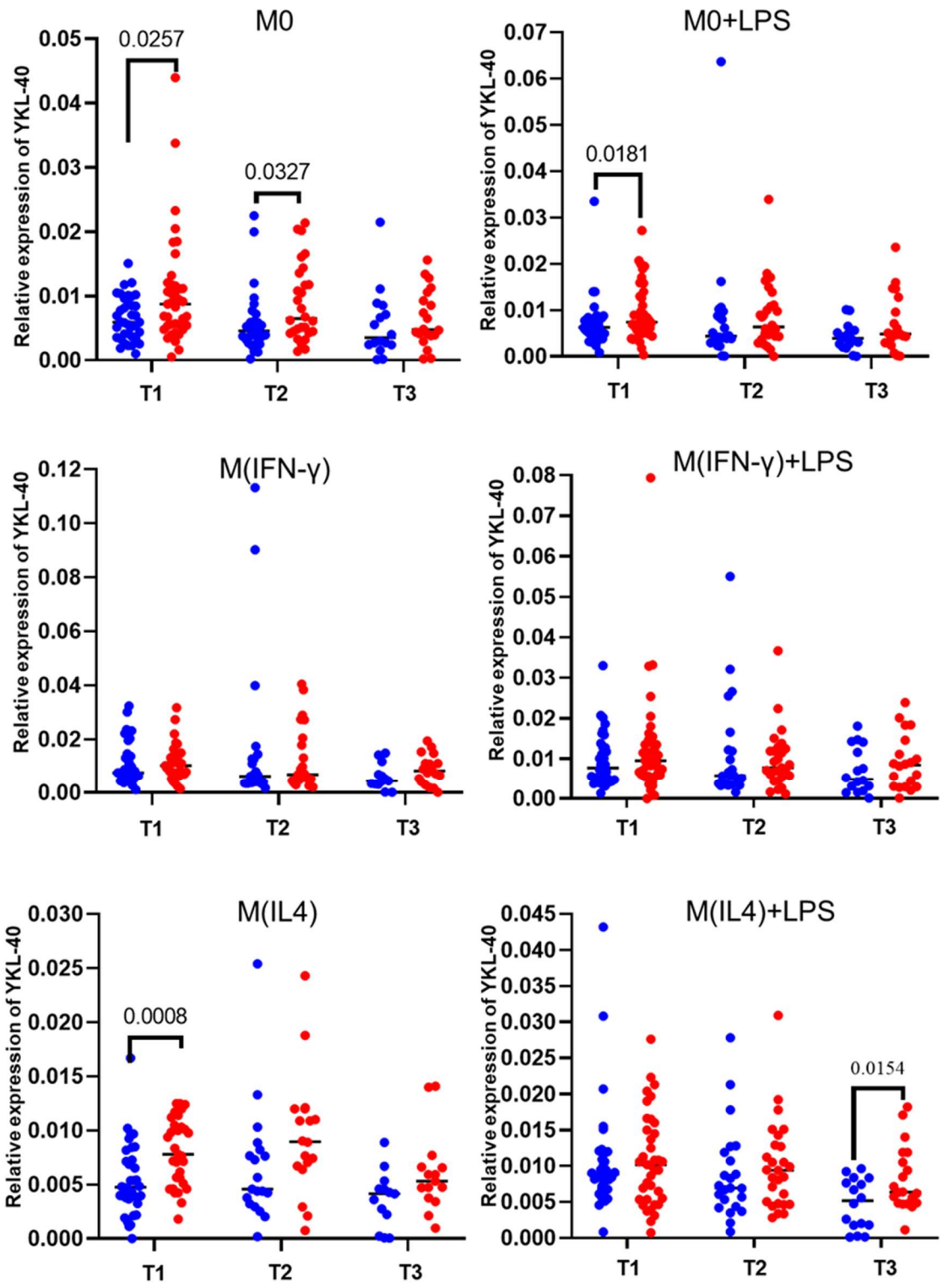


Figure 4. Comparison the expression of YKL-40 in MDM between COVID-19 convalescent plasma donors (red, C) and healthy individuals (blue, H) at time points T1, T2 and T3. In the graphs of M(NS), M(NS)+LPS, M(IFN-γ) + LPS and M(IL4) + LPS, each point presents single donor of n (C)=39 COVID-19 and n(H)=37 at T1, n (C)=28 and n (H)= 29 healthy donors at T2, n (C)=20 and n (H)=16 at T3. In the graph of M(IFN-γ), each point represents n (C)=35 and n (H)=34 at T1, n(C)=23 and n (H)= 23 at T2, n(C)=18 and n(H)=13 at T3. In the graph of M(IL4), each point represents a single donor of n(C)=35 and n(H)=4 at T1, n(C)=18 and n(H)=19 at T2, n(C)=15 and n(H)=13 at T3. Mann-Whitney test was performed for statistical analysis. The diagram provides *P* values that indicate statistical significance.

Since the expression of YKL-40 in M(NS) at T1 and T2 showed the statistical significance, we further analysis the secreted level of YKL-40 by Elisa for the M(NS) from both COVID-19 convalescent plasma donors and healthy donors. 39 COVID-19 convalescent donors and 37 healthy donors for macrophages at T1, 28 COVID-19 convalescent plasma donors and 29 healthy donors at T2, 20 COVID-19 convalescent plasma donors and 16 healthy donors at T3 were included in this analysis. The differential concentration of YKL-40 between 2 group was analyzed by Mann- Whitney analysis. The higher concentration of secreted YKL-40 in M(NS) can be found in COVID-19 convalescent plasma donors' group, but no statistical significance was found (Fig.5). We further analyzed the correlation between the relative expression and secreted concentration level of YKL-40 by Spearman correlation test. The positive significant statistical correlation between the relative expression and secreted concentration level of YKL-40 can be found at T1 (P=0.0017, r_s=-0.3565) and T3 (P=0.0007, r_s=-0.6123). There is still a positive correlation at T2, but it did not reach statistical significance (Figure 6).

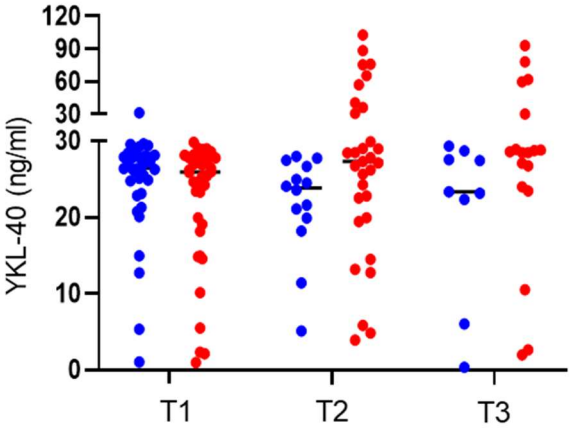


Figure 5 Concentration for secreted YKL-40 in M(NS) differentiated out of monocytes from COVID-19 convalescent plasma donors (red) and healthy individuals (blue) at T1, T2 and T3. ELISA was used to analyze the YKL40 concentration. The differential concentration of secreted YKL-40 between the 2 groups in T1, T2, T3.

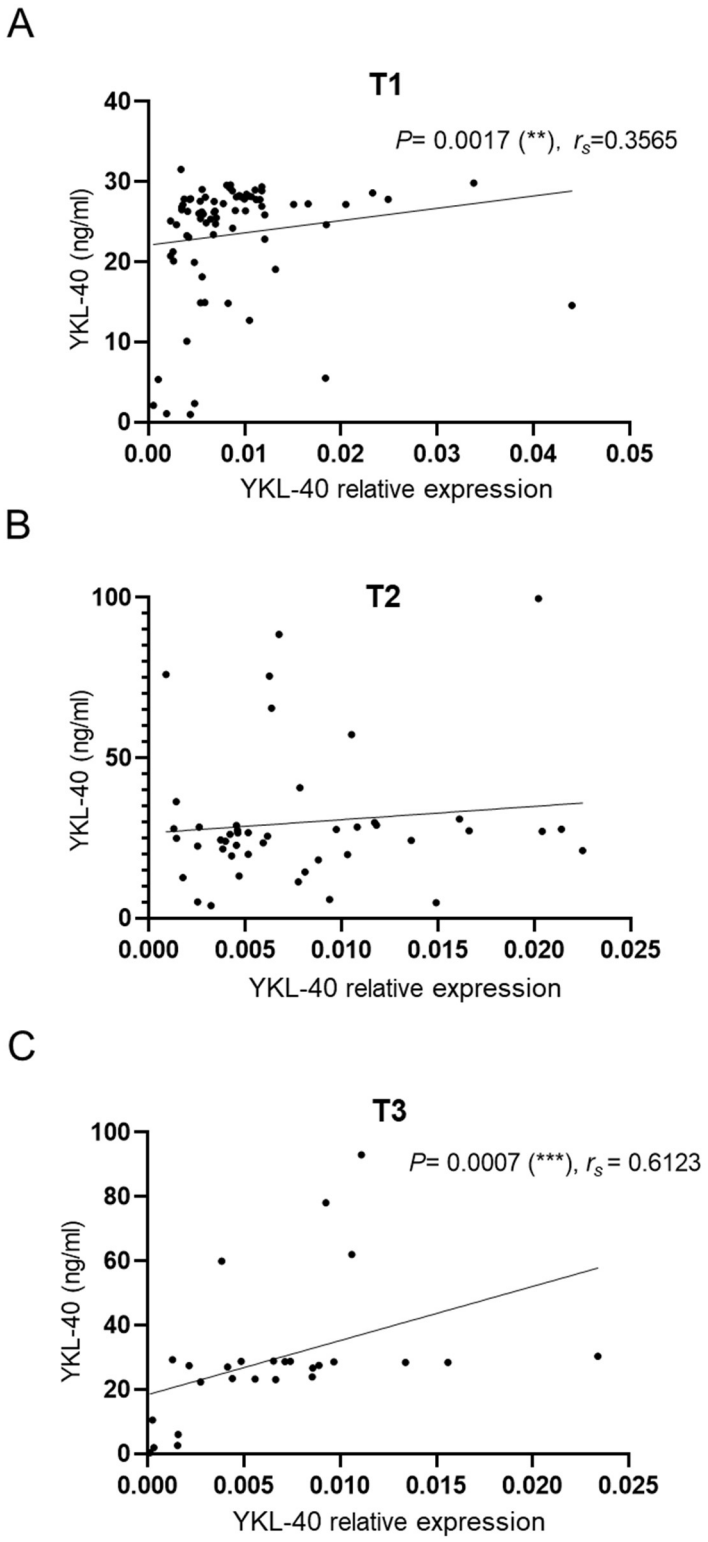


Figure 6 The correlation between the level of concentration for secreted YKL-40 and relative expression of YKL-40 in M(NS) differentiated out of monocytes from COVID-19 convalescent plasma donors and healthy individuals in T1 (A), T2 (B) and T3 (C). ELISA was used to analyze the concentration of secreted YKL-40. P value and rs value are provided in the diagram to indicate the statistical significance. There are 39 COVID-19 convalescent donors and 37 healthy donors for macrophages in T1, 28 COVID-19 convalescent plasma donors and 29 healthy donors in T2, and 20 COVID-19 convalescent plasma donors and 16 healthy donors in T3. The correlation was analyzed using Spearman correlation.

To further analysis the long-term pro- inflammatory function changes for monocyte differential macrophages in COVID-19 convalescent plasma donor, IL-1β changes in all 6 types of macrophages at all 3 time points were further analysis by RT-PCR. In the graphs of M(NS), M(NS)+LPS, M(IFNγ) + LPS and M(IL4) +LPS, each point presents single donors of 39 COVID-19 convalescent donors and 37 healthy donors for macrophages in T1, 28 COVID-19 convalescent plasma donors and 29 healthy donors in T2, 20 COVID-19 convalescent plasma donors and 16 healthy donors in T3. In the graph of M(IFNγ), each point represents a single donor, 35 COVID-19 convalescent donors and 34 healthy donors for macrophages in T1, 23 COVID-19 convalescent plasma donors and 23 healthy donors in T2, 18 COVID-19 convalescent donors and 13 healthy donors in T3. In the graph of M(IL4), each point represents a single donor of 35 COVID-19 convalescent donors and 34 healthy donors for macrophages in T1, 18 COVID-19 convalescent plasma donors and 19 healthy donors in T2, 15 COVID-19 convalescent donors and 13 healthy donors in T3. All data showed non- logical distribution. Mann-Whitney test was used for statistical analysis. There is no statistical significance found in all 6 types of macrophages at all 3-time point (Fig.7).

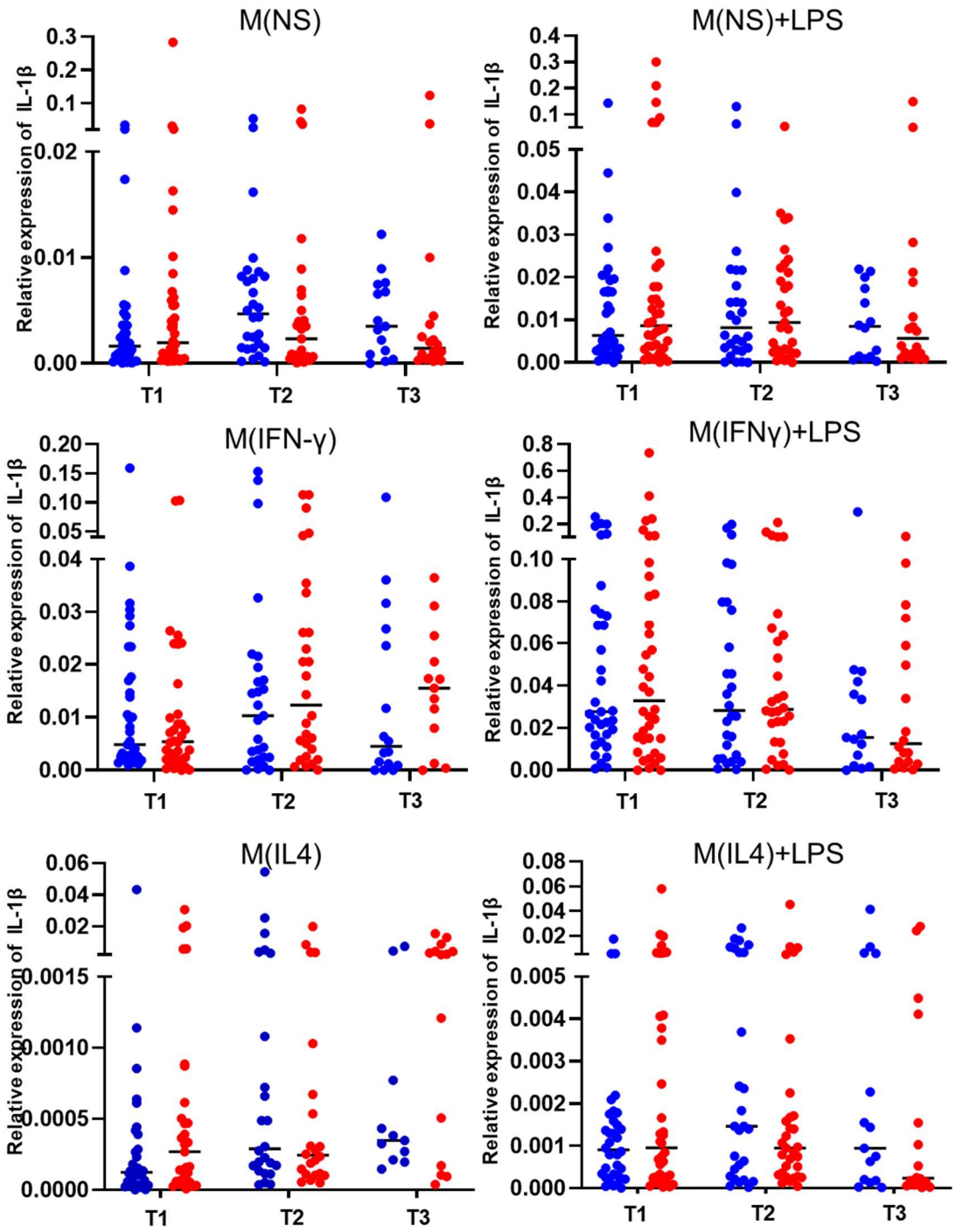


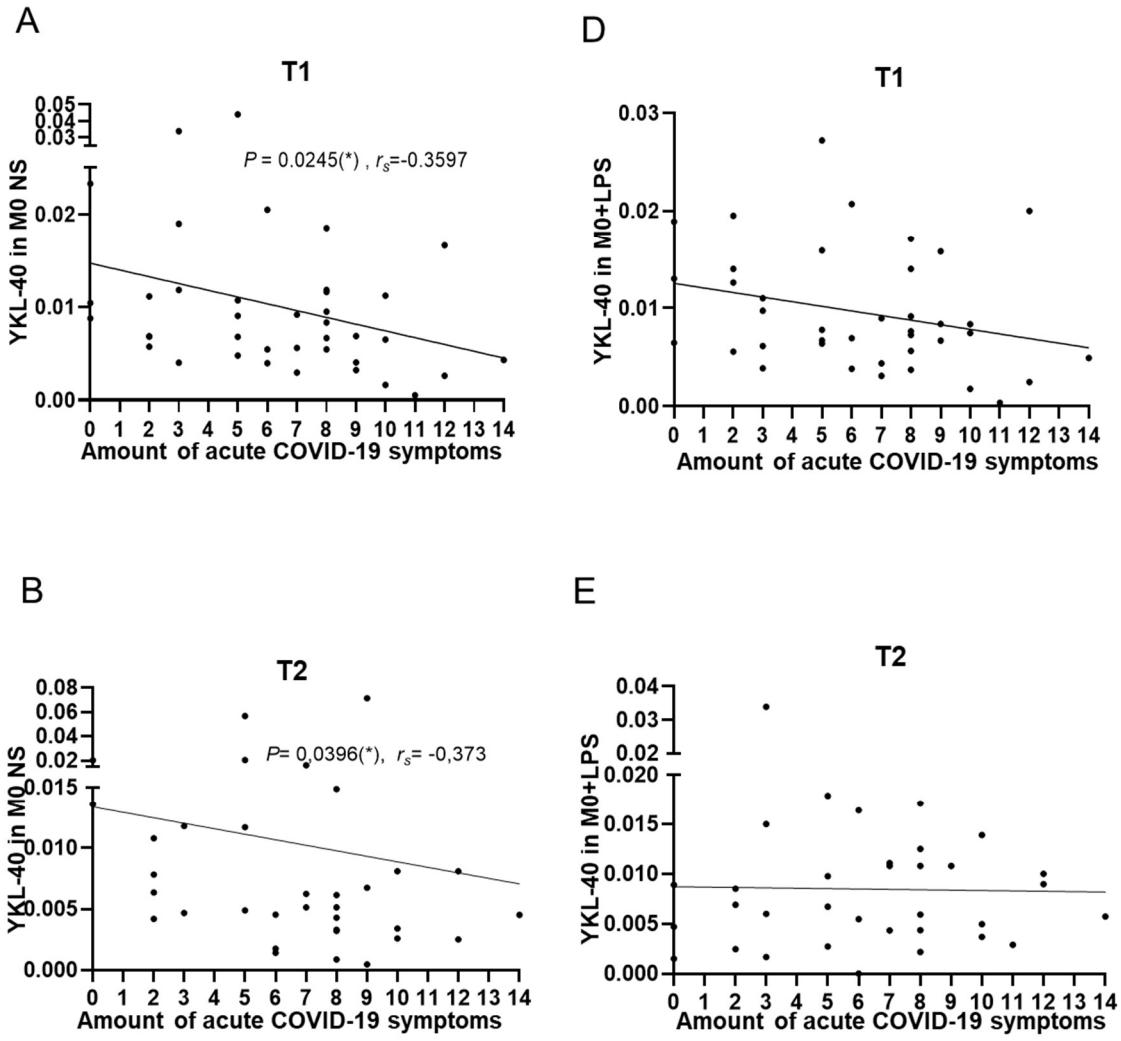
Figure 7 The expression of IL-1β in macrophages differentiated out of monocytes from COVID-19 convalescent plasma donors (red) and healthy individuals (blue) at time points T1, T2 and T3. Macrophage stimulation was performed according to Fig. 2. IL-1β expression was analyzed by RT-PCR. In the graphs of M(NS), M0+LPS, M(IFN-γ) + LPS and M(IL4) + LPS, each point presents single donor of n=39 COVID-19 convalescent (n (C)), n=37 healthy donors (n(H)) at T1, n (C)=28 and n (H)= 29 healthy donors at T2, n (C)=20 and n (H)=16 at T3. In the graph of M(IFN-γ), each point represents n (C)=35 and n (H)=34 healthy donors for at T1, n(C)=23 and n (H)= 23 healthy donors in T2, n(C)=18 and n(H)=13 at T3. In the graph of M(IL4), each point represents a single donor of n(C)=35 and n(H)=4 at T1, n(C)=18 and n(H)=19 healthy donors at T2, n(C)=15 and n(H)=13 healthy donors at T3. Mann-Whitney test was performed for statistical analysis. The diagram provides P values that indicate statistical significance.

3.3 Analysis the correlation between the clinical symptom in acute phase and YKL-40 expression in monocyte- differentiation macrophage

3.3.1 The expression of YKL-40 negative correlated to the total amount of acute symptoms in COVID-19 convalescent plasma donor.

Since the higher expression of YKL-40 in monocyte- differentiated macrophages was found in COVID-19 convalescent, its correlation to the clinical feathers was further analyzed. 14 symptoms information in acute phase was collected for each COVID-19 convalescent plasma donors. Due to characteristics of cohorts in this study, the correlation between the total amount of symptoms and YKL-40 expression in all 6 types macrophages at all 3 time points were analyzed.

In M(NS) and M(NS)+LPS, 39 donors in T1, 28 donors in T2, 20 donors in T3 were included. The correlation was analyzed by Spearman correlation test. The negative correlation was found in all 3 time points and showed statistical significance in T1 (P=0.0245, r_s=-0.3597) and T2 (P=0.0396, r_s=-0.373). With LPS stimulation, this negative correlation can still be found, but showed no statistical significance (Fig.8).



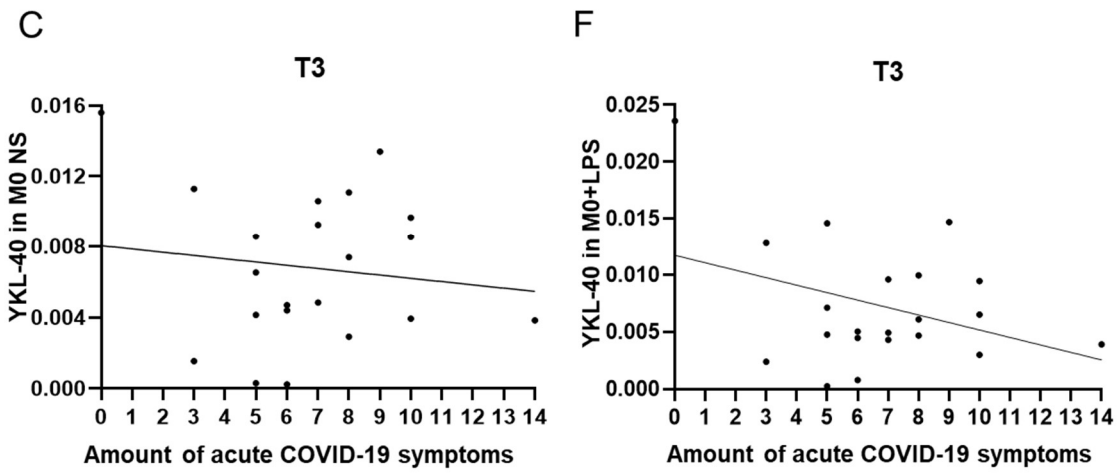


Figure 8. Correlation between the relative expression of YKL-40 M(NS) with or without LPS stimulation, and total amount of symptoms in acute phase for all 3 time points. The significant negative correlation between the total amount of COVID-19 symptoms in acute phase and YKL-40 relative expression can be found in M(NS) in T1 and T2. *P* value and r_s value are provided in the diagram to indicate the statistical significance. In the graphs of M(NS) and M(NS)+LPS each point presents single donors of COVID-19 convalescent plasma donors n=39 in T1, n=28 in T2, n=20 in T3. The correlation was analyzed using Spearman correlation.

In M(IFNγ), the correlation was analyzed among 35 COVID-19 convalescent plasma donors in T1, 23 in T2, 18 in T3 by Spearman correlation test. As the result shows, the negative correlation between the YKL-40 expression and the total amount of symptoms can be found at time point T1 and T2. At T3, however, the positive correlation was found (Fig. 9). With LPS stimulation, the correlation was analyzed among 30 COVID-19 convalescent plasma donors in T1, 28 in T2, 20 in T3 by Spearman correlation test. As the result shows, the negative correlation can be found at all 3 time points. All correlation showed no statistical significance (Fig. 9).

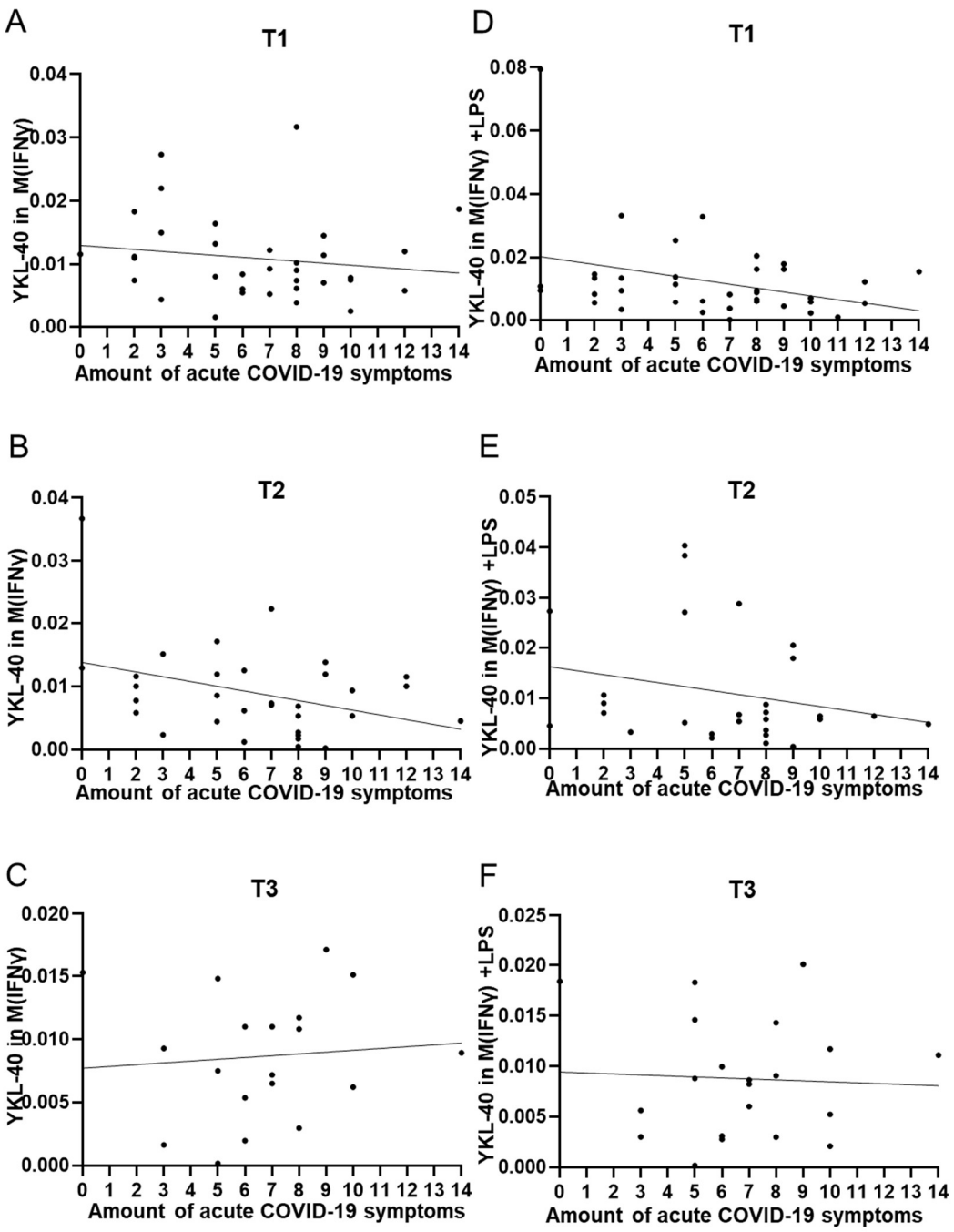


Figure 9. Correlation between the YKL-40 relative expression in M(IFNγ) with or without LPS stimulation and total amount of symptoms in acute phase. The negative correlation between the total amount of COVID-19 symptoms in acute phase and YKL-40 relative expression can be found in M(IFNγ) in T1 and T2, and M(IFNγ) in T1, T2 and T3. Positive correlation was found in M(IFNγ) in T3. *P* value and r_s value are provided in the diagram to indicate the statistical significance. In the graphs of M(IFN-γ) each point presents single donors of COVID-19 convalescent plasma donors for n=35 in T1(A), n=23 in T2 (B), n=18 in T3(C). In the graphs of M(IFNγ) +LPS each point presents single donors of COVID-19 convalescent plasma donors which contains n=39 in T1(D), n=28 in T2 (E), n=20 in T3 (F). The correlation was analyzed by Spearman correlation

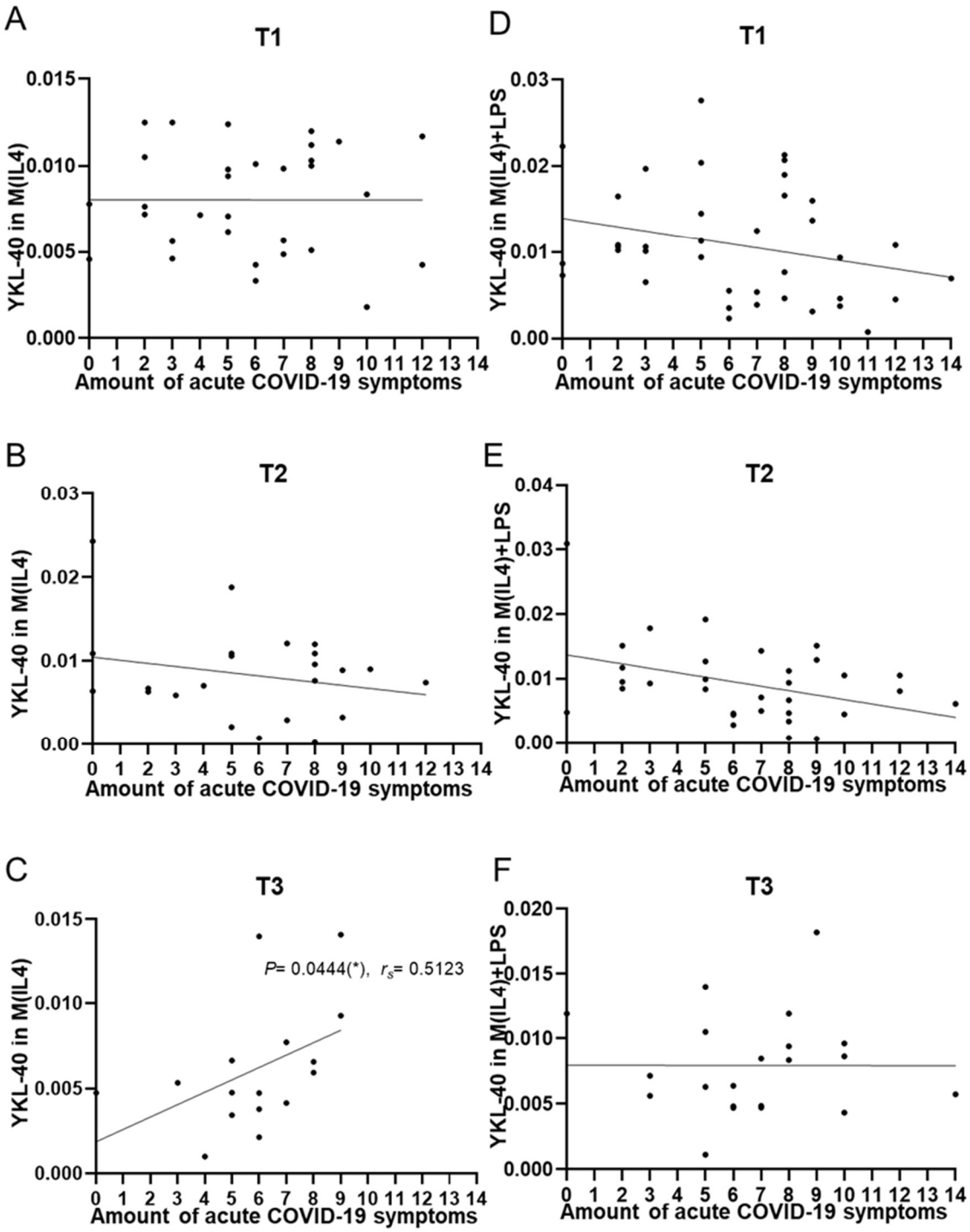


Figure 10. Correlation between the YKL-40 relative expression in M(IL4) with or without LPS stimulation and total amount of symptoms in acute phase. The negative correlation between the total amount of COVID-19 symptoms in acute phase and YKL-40 relative expression can be found in M(IL4) in T2, and M(IL4) in T1, T2 and T3. The positive correlation can be found in M(IIL4) in T1 and T3. In the graphs of M(IL4) each point presents single donors n=35 in T1 (A), n=18 in T2 (B), n=15 in T3 (C) of COVID-19 convalescent plasma donors. In the graphs of M(IFN-γ) +LPS each point presents single donors which contains n= 39 in T1(D), n= 28 in T2 (E), n=20 in T3 (F) for COVID-19 convalescent plasma donors. The correlation was analyzed by Spearman correlation.

In M(IL4), the correlation was analyzed among 35 COVID-19 convalescent plasma donors in T1, 18 in T2, 15 in T3 by Spearman correlation test. As the result shows, the

negative correlation between the YKL-40 expression and the total amount of symptoms can be found at time point T1 and T2. At T3, however, the statistically significant positive correlation was found (P=0.0444, r_s=0.5123, Fig. 10). With LPS stimulation, the correlation was analyzed among 30 COVID-19 convalescent plasma donors in T1, 28 in T2, 20 in T3 by Spearman correlation test. As the result shows, the negative correlation can be found at all 3 time points, but showed no statistical significance (Fig. 10).

3.3.2 The correlation of YKL-40 expression in monocyte- differential macrophages of COVID-19 convalescent donors and the number of symptoms in the different system symptom in acute phase

To further analysis the YKL-40 expression in monocyte- differential macrophages correlation with the clinical symptoms in different system. We further classified 14 symptoms into 3 systems, respiratory, systemic and neurological system (Table 15).

Table 15. Clinical symptoms for COVID-19 convalescent individuals in acute phase

	Symptoms	COVID-19 Convalescent individuals (n=39)	
		-	+[1]
Respiratory symptoms	Cough	17 (43.6%)	22 (56.4%)
	Sniff	18 (46.2%)	21 (53.8%)
	Sore throat	18 (46.2%)	21(53.8%)
	Difficult in breath	21 (53.8%)	18 (46.2%)
	Pneumonia	32 (82.1%)	7 (17.9%)
Systemic symptoms	Eye inflammation	33 (84.6%)	6 (15.4%)
	Fever	16 (41.0 %)	23 (59.0%)
	Skin rash	37 (94.9%)	2 (5.1%)
	earache	35 (89.7%)	4 (10.3%)
	Exhaustion [2]	8 (20.5%)	31 (79.5%)
	Headache [2]	12 (30.8%)	27(69.2%)
Neurological symptoms	Exhaustion[2]	8 (20.5%)	31 (79.5%)
	Headache[2]	12 (30.8%)	27(69.2%)
	Disturbance of smell or taste	9 (23.1%)	30 (76.9%)
	Nausea	30 (76.9%)	9 (23.1%)
	Limb/ joint/ back pain	14 (16.5%)	25 (64.1%)

[1] -: without event reported. + with event reported.
[2] The symptoms of exhaustion and headache belong to the systemic and neurological system.

The correlation between the amount of symptoms in each system and YKL-40 expression in 6 types of macrophages at 3 time points were analyzed by the Spearman correlation test (Fig.11). In the respiratory system, all negative correlations can be found in all 6 types of macrophages at all 3 time points. In respiratory symptoms, the statistically significant negative correlation can be found in M(NS) (P=0.0125, -0.424), M(NS)+LPS (P=0.0392, r_s=-0.355) and M(IFNγ) (P=0.0419, r_s=-0.351) at T1, in M(IL4) +LPS (P=0.0075, r_s=-0.457) at T2, M(IFNγ)+ LPS (P=0.0080, r_s=-0.454) and M(IL4)+LPS (P=0.0375, r_s=-0.364) at T3. In neurological symptoms, the statistically significant negative correlation can be found in M(NS) (P=0.0176, r_s=-0.378) and M(IFNγ)+LPS (P=0.0262, r_s=-0.356) at T1, M(IFNγ)+LPS (P=0.0194, r_s=-0.373) at T2, M(NS) (P=0.0378, r_s=-0.375), M(IFNγ) (P=0.0347, r_s=-0.380) and M(IFNγ)+LPS (P=0.0108, r_s=-0.409) at T3.

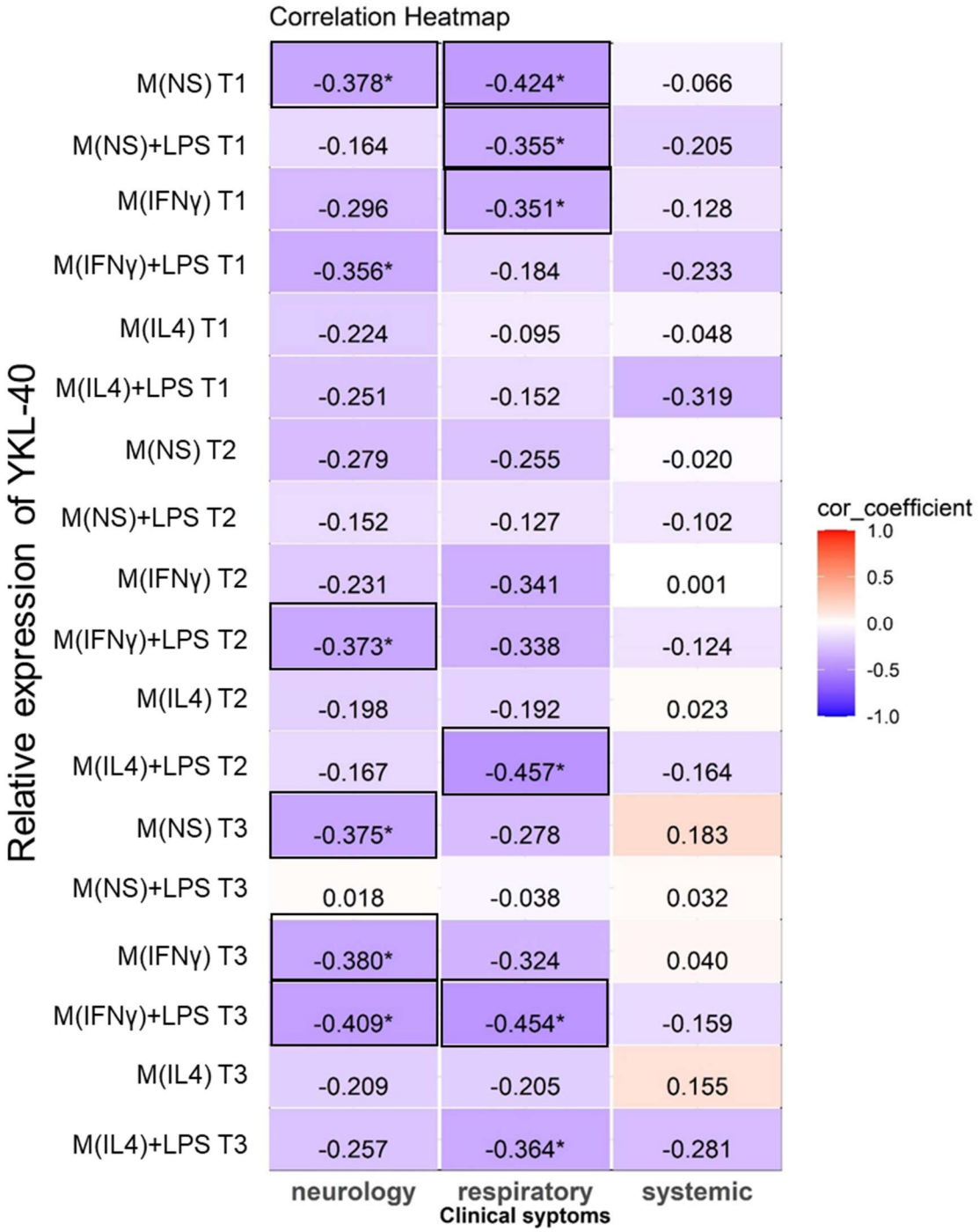


Figure 11. Correlation between the expression of YKL-40 in macrophages differentiated out of COVID-19 convalescent plasma donors and the amount of systemic, respiratory and neurological COVID-19 symptoms at T1, T2 and T3. Blue showed the negative correlation while red showed the positive correlation. Each value presents the r_s value, with * indicating statistical significance. The correlation was analyzed by Spearman correlation test by cor_test R package. The graph was made by ggplot2 R package.

3.3.3 Lower expression level of YKL-40 in monocyte- differential macrophages can be found in the COVID-19 convalescent donors reported the presence of respiratory, neurological or systemic symptoms

We then further analysis the expression of YKL-40 in MDM for patients with presence of certain system symptoms reported in acute phase. In respiratory system, in total of 10 donors reported no presence of respiratory symptom, while 29 donors reported the presence (Fig. 12). In the graphs of M(NS), M(NS)+LPS, M(IFNγ) + LPS and M(IL4) + LPS, each point presents single donor of 10 COVID-19 convalescent donors without the presence of respiratory symptoms and 29 donors with the presence of the symptoms for macrophages at T1, 9 without the presence of respiratory symptoms and 25 donors with the presence of the symptoms at T2, 3 without the presence of respiratory symptoms and 18 donors with the presence of the symptoms at T3. In the graph of M(IFN-γ), each point presents single donor of 8 COVID-19 convalescent donors without the presence of respiratory symptoms and 27 donors with the presence of the symptoms for macrophages at T1, 9 without the presence of respiratory symptoms and 20 donors with the presence of the symptoms at T2, 3 without the presence of respiratory symptoms and 16 donors with the presence of the symptoms at T3. In M(IL4), each point presents single donors of 8 COVID-19 convalescent donors without respiratory symptoms and 29 donors with respiratory symptoms in T1, 8 donors without respiratory symptoms and 16 with respiratory symptoms in T2. There is not enough sample for M(IL4) in T3. Compared to the donors without the symptoms, donors reported respiratory symptoms showed lower expression level of YKL-40 trend in all 6 types of macrophages at all 3 time points. The statistically significant lower level of expression in donors reported the presence of respiratory symptoms can be found in M(NS) at T2 (P=0.04) and T3 (P=0.0166), M(IFNγ) at T3 (P=0.0155).

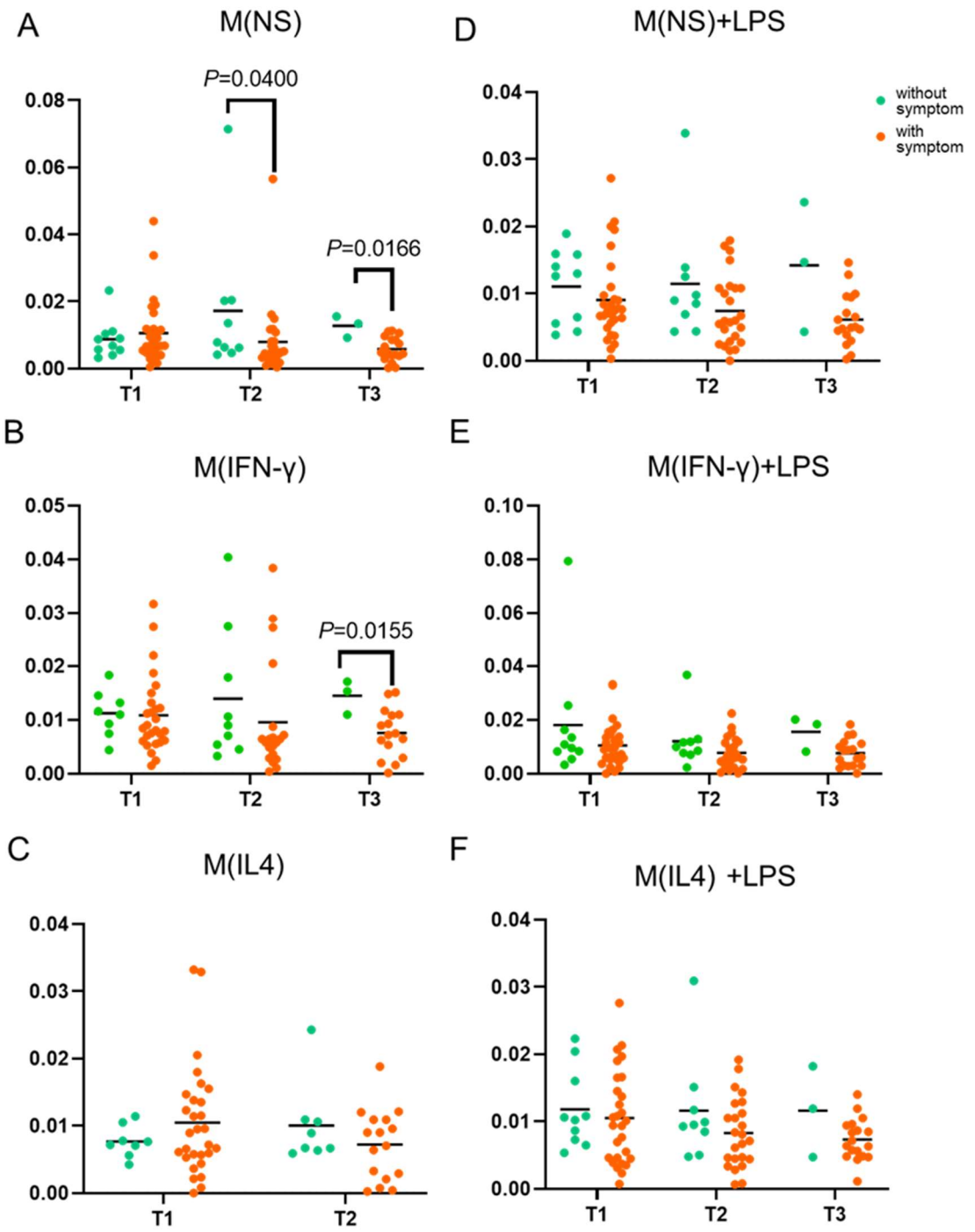


Figure 12. The expression of YKL-40 in macrophages differentiated out of monocytes from COVID-19 convalescent plasma donors reported the presence (orange) or absence (green) of respiratory symptoms in acute phase at T1, T2 and T3. Macrophage stimulation was performed according to Fig. 2. YKL-40 expression was analyzed by RT-PCR. In the graphs of M(NS), M(NS)+LPS, M(IFN-γ) + LPS and M(IL4) + LPS, each point represents a single donor of n=10 COVID-19 convalescent donors without respiratory symptoms (n(CR-)) and 29 donors with respiratory symptoms(n(CR+)) at T1, n(CR-) = 9 and n(CR+) = 25 with symptoms at T2, n(CR-) = 3 and n(CR+) = 18 at T3. In the graphs of M(IFNγ), n(CR-)= 8 and n(CR+) = 27 at T1, n(CR-)= 9 and n(CR+) = 20 at T2, n(CR-)= 3 and n(CR+)= 16 at T3. In M(IL4), n(CR-)= 8 and n(CR+) = 29 at T1, n(CR-)=8 and n(CR+) =16 at T2. There is not enough sample for M(IL4) at T3. The diagram provides *P* values that indicate statistical significance.

In neurological system, in total of 3 donors reported no the absence of neurological symptom, while 36 donors reported the presence (Fig. 13). In the graphs of M(NS), M(NS)+LPS, M(IFN-γ) + LPS and M(IL4) + LPS, each point represents a single donor of 3 COVID-19 convalescent donors without neurological symptoms and 36 donors with neurological symptoms at T1. Donors with the presence of neurological symptoms in acute phase showed lower expression level of YKL-40 trend in all 4 types of macrophages at all but there is no statistically significant was found.

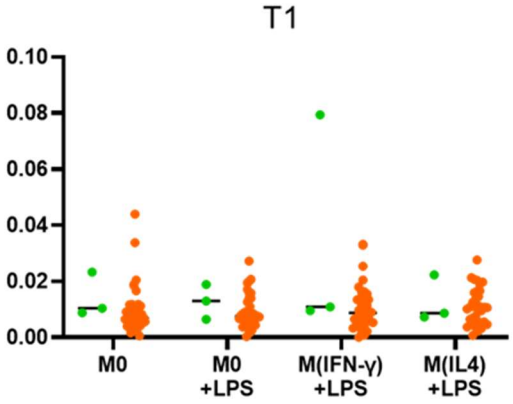
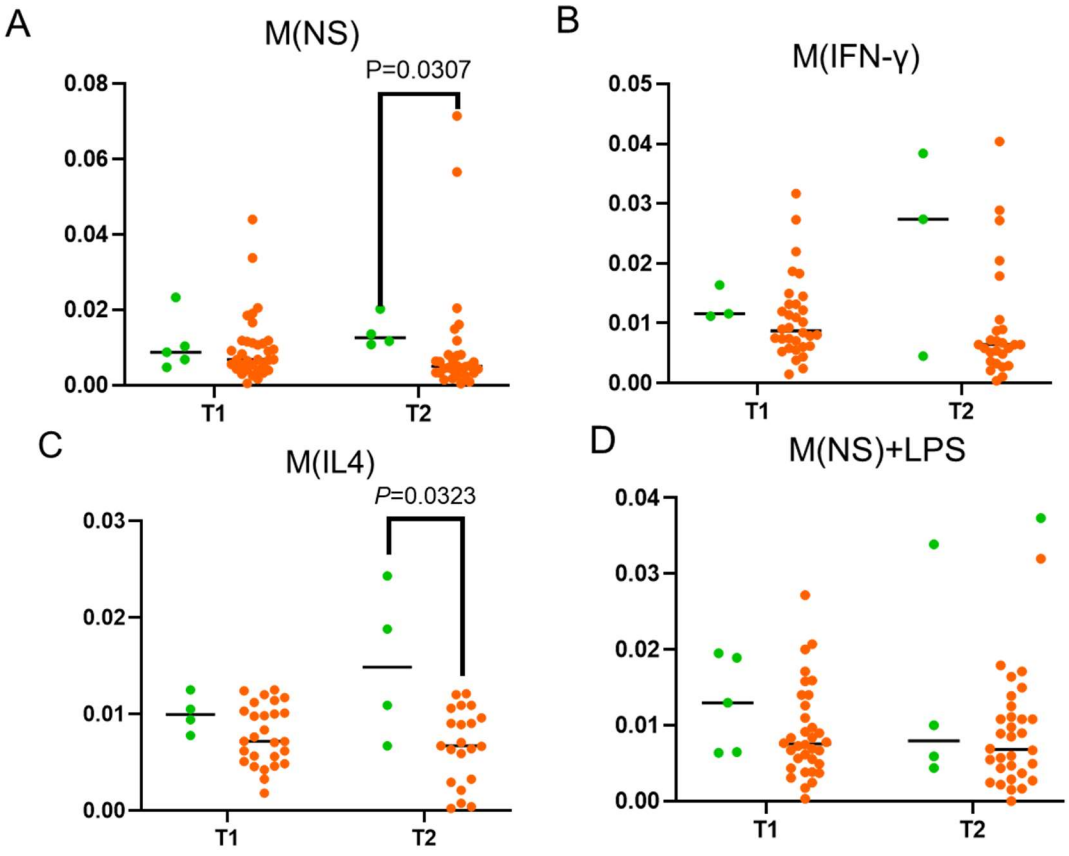


Figure 13. The expression of YKL-40 in macrophages differentiated out of monocytes from COVID-19 convalescent plasma donors reported the presence (orange) or absence (green) neurological symptoms in T1. Macrophage stimulation was performed according to Fig. 2. YKL-40 expression was analyzed by RT-PCR. In the graphs of M(NS), M(NS)+LPS, M(IFN-γ) + LPS and M(IL4) + LPS, each point represents a single donor of n=3 COVID-19 Convalescent donors without neurological symptoms and n=36 donors with neurological symptoms in T1.



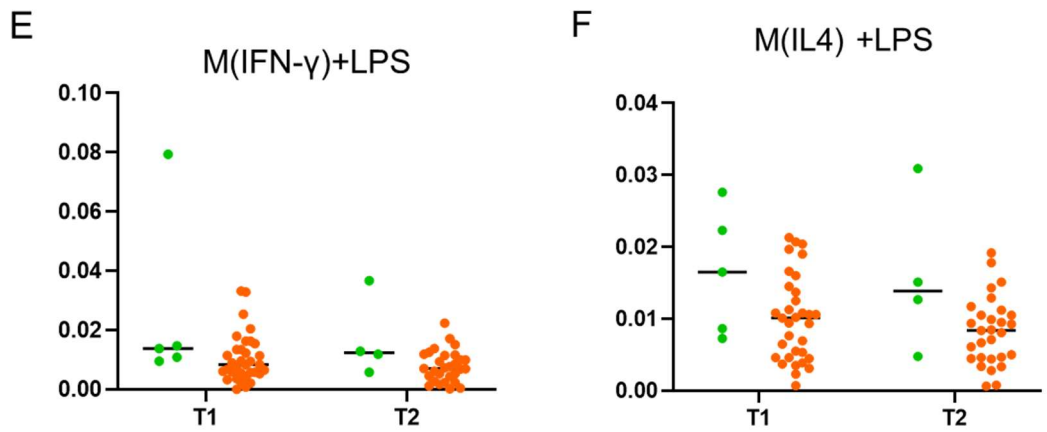


Figure 14. The expression of YKL-40 in macrophages differentiated out of monocytes from COVID-19 convalescent plasma donors reported the presence (orange) or absence (green) of systemic symptoms in acute phase at T1, T2 and T3. Macrophage stimulation was performed according to Fig. 2. YKL-40 expression was analyzed by RT-PCR. In the graphs of M(NS), M(NS)+LPS, M(IFN-γ) + LPS and M(IL4) + LPS, each point represents a single donor of n=5 COVID-19 convalescent donors without systemic symptoms (n(CS-)) and n=34 donors with systemic symptoms(n(CS+)) at T1, n(CS-) = 4 and n(CS+) = 30 at T2. In the graphs of M(IFNγ), n(CS-)= 3 and n(CS+) = 32 at T1, n(CS-)= 3 and n(CS+) = 20 at T2. In M(IL4), n(CS-)= 4 and n(CS+) = 27 at T1, n(CS-)=4 and n(CS+) =21 at T2. There is not enough sample for analysis at T3. The diagram provides P values that indicate statistical significance.

3.4 The correlation of expression of YKL-40 in monocyte differentiated macrophages in COVID-19 convalescent plasma donors and long-COVID symptoms

As millions of individuals are infected with COVID, long- COVID symptoms has been consider as a public health challenge. In our cohorts, among 39 COVID-19 convalescent donors, 11 reported at least one persistence symptoms for at least 100 days after documented SC2 infection. The most reported long- COVID symptoms was persistent difficulty in concentrating (63.6%), persistent smell and/or taste disorders (54.5%), persistent fatigue or reduced performance (45.5%), persistent headache (27.3%) and persistent body pain (27.3%, Table 16).

Table 16. Long-COVID symptoms for COVID-19 Convalescent Individuals

	Long- COVID symptom (n=11)	
	-	+[1]
Persistent difficulty concentrating	4 (36.4%)	7 (63.6%)
Persistent smell and/or taste disorders	5 (45.5%)	6 (54.5%)
Persistent fatigue or reduced performance	6 (54.5%)	5 (45.5%)
Persistent cough and/or breathing	7 (63.6%)	4 (36.4%)
Persistent headaches	8 (72.7%)	3 (27.3%)
Persistent limb/joint pain	8 (72.7%)	3 (27.3%)
Constant tachycardia or cardiac arrhythmias?	9 (81.8%)	2 (18.2%)
Persistent chest tightness	10 (90.9%)	1 (9.1%)
Persistent depression	10 (90.9%)	1 (9.1%)

- without event. + with event

The differential expression of YKL-40 in monocyte-differentiated macrophages in COVID-19 convalescent patients with the presence and absence of long- COVID

symptoms was then analyzed (Fig. 15). In the graphs of M(NS), M(NS)+LPS, M(IFNγ) + LPS and M(IL4) + LPS, each point represents a single donor of 28 COVID-19 convalescent donors without long-COVID symptoms and 11 donors with long-COVID symptoms in T1, 24 donors without long-COVID symptoms and 10 with symptoms in T2, 16 without long-COVID symptoms and 5 without long-COVID symptoms in T3. In the graphs of M(IFNγ), each point presents single donors of 23 COVID-19 convalescent donors without long-COVID symptoms and 8 donors with long-COVID symptoms in T1, 17 donors without long-COVID symptoms and 8 with long-COVID symptoms in T2, 12 donors without long-COVID symptoms and 3 donors without long-COVID symptoms in T3. In M(IL4), each point presents single donors of 22 COVID-19 convalescent donors without long-COVID symptoms and 9 donors with long-COVID symptoms in T1, 17 donors without long-COVID symptoms and 7 with long-COVID symptoms in T2, 12 donors without long-COVID symptoms and 4 with long-COVID symptoms in T3. Except for M(IFNγ) + LPS at T2 and M(IL4) at T2, donors reported long-COVID symptom showed higher expression level of YKL-40 trend in 6 types of macrophages at all 3 time points. The statistically significant higher expression in donors reported the presence of long- COVID symptoms can be found in M(NS) at T3 (P=0.0167).

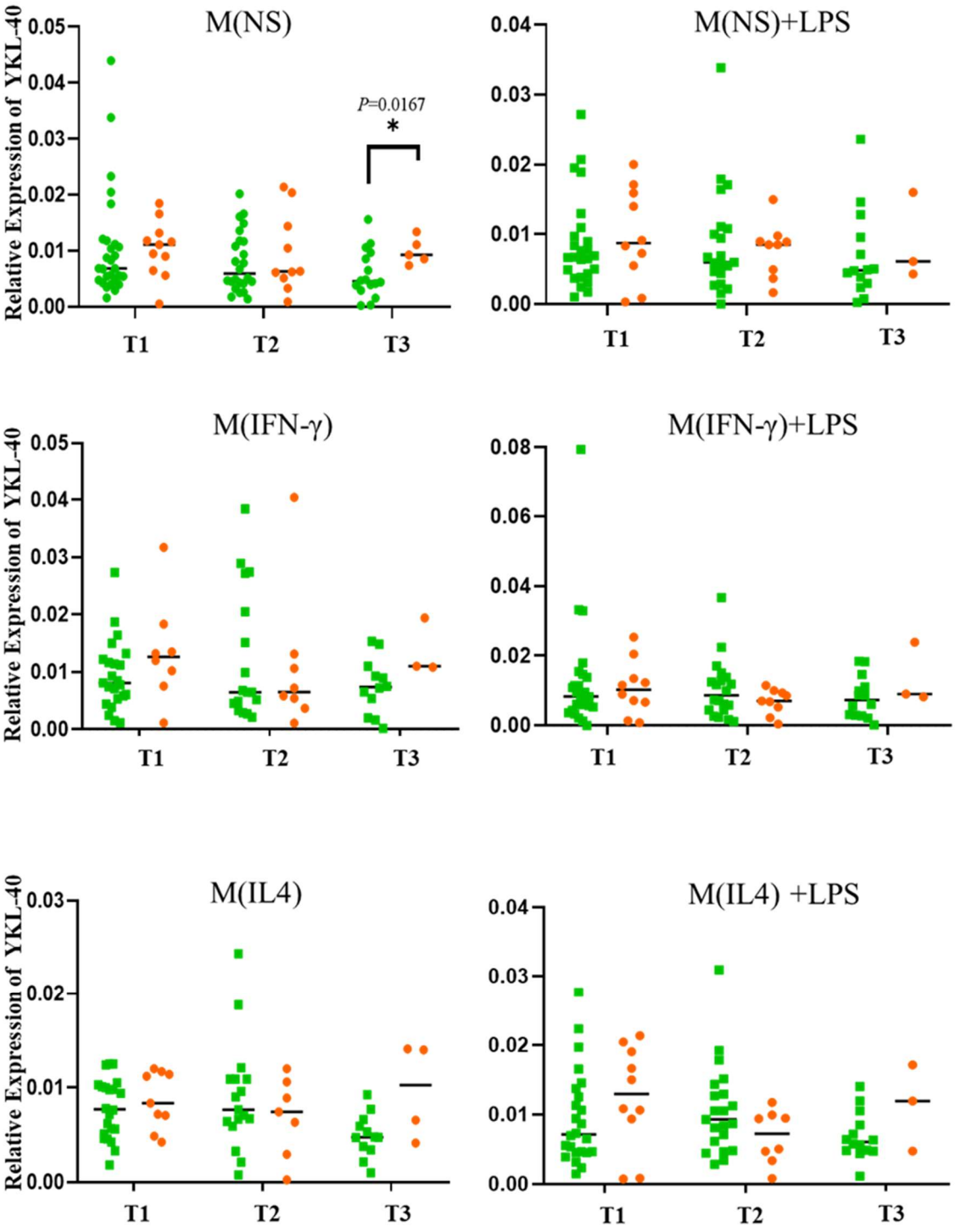


Figure 15. The expression of YKL-40 in macrophages differentiated out of monocytes from COVID-19 convalescent plasma donors with (red) or without (blue) long -COVID symptoms in T1, T2 and T3. Macrophage stimulation was performed according to Fig. 2. YKL-40 expression was analyzed by RT-PCR. In the graphs of M(NS), M(NS)+LPS, M(IFN-γ) + LPS and M(IL4) + LPS, each point represents a single donor of n=28 COVID-19 convalescent donors without long- COVID symptoms (n(CL-)) and 11 donors with long- COVID symptoms(n(CL+)) at T1, n(CL-) = 24 and n(CL+) = 10 with symptoms at T2, n(CL-) = 16 and n(CL+) = 5 at T3. In the graphs of M(IFNγ), n(CL-)=23 and n(CL+) = 8 at T1, n(CL-)= 17 and n(CL+) = 8 at T2, n(CL-)= 12 and n(CL+) = 3 at T3. In M(IL4), n(CL-)= 22 and n(CL+) = 9 at T1, n(CL-)= 17 and (CL+) = 7 at T2, n(CL-)= 12 and n(CL+) = 4 at T3. The diagram provides *P* values that indicate statistical significance.

Since the higher expression of YKL-40 in COVID-19 convalescent who reported the presence of long- COVID symptoms, we further analysis its role in each long-COVID symptoms at T1 (Table 17). The differential expression of YKL-40 of monocyte-differential macrophage was analysis by un-paired t-test for equal variance and welch correction t- test for unequal variance. There is no enough sample for differential expression analysis in MDM of donors who reported persistent or absence of chest tightness, cardiac arrhythmias or depression. There is no statistical difference can be found in the MDM of donors who reported persistent body pain or not. Donors with the presence of smell or (and) taste disturbance, cough or breath difficulties, and fatigue or reduced performance showed higher YKL-40 expression in all 6 types of macrophages respectively, but no statistical significance. Donors who reported persistent headache or persistent difficulty concentrating showed a lower expression of YKL-40 in all 6 types of monocytes- differentiated macrophages. The statistically significant lower expression of YKL-40 can be found in M(NS)+ LPS of donors with the presence of persistent difficult concentrating (*P*=0.034).

Table 17 The expression of YKL-40 in MDM of COVID-19 convalescent plasma donors and clinical parameter (Long- COVID symptoms)

Clinical parameter	Types of MDM	-		+[1]		P value
		n	Mean value	n	Mean value	
Persist smell or (and) taste disturbance	M(NS)	5	0.008	6	0.012	0.202
	M(NS)+LPS	5	0.010	6	0.013	0.353
	M(IFNγ)	4	0.013	5	0.015	0.659
	M(IFNγ) +LPS	5	0.009	6	0.015	0.204
	M(IL4)	4	0.010	5	0.008	0.478
	M(IL4) +LPS	5	0.011	6	0.016	0.190
Persist cough or (and) breath difficulties	M(NS)	7	0.009	4	0.012	0.436
	M(NS)+LPS	7	0.010	4	0.014	0.220
	M(IFNγ)	5	0.016	4	0.011	0.273
	M(IFNγ) +LPS	7	0.012	4	0.013	0.883
	M(IL4)	5	0.008	4	0.009	0.743
	M(IL4) +LPS	7	0.013	4	0.014	0.865
Persistent fatigue or reduced performance	M(NS)	6	0.010	5	0.011	0.640
	M(NS)+LPS	6	0.011	5	0.012	0.898
	M(IFNγ)	4	0.014	5	0.014	0.961
	M(IFNγ) +LPS	6	0.012	5	0.013	0.791
	M(IL4)	4	0.007	5	0.010	0.322
	M(IL4) +LPS	6	0.012	5	0.015	0.514
Persistent headaches	M(NS)	7	0.011	4	0.010	0.873
	M(NS)+LPS	7	0.011	4	0.012	0.816
	M(IFNγ)	6	0.014	3	0.013	0.824
	M(IFNγ) +LPS	7	0.013	4	0.011	0.581
	M(IL4)	6	0.009	3	0.008	0.660
	M(IL4) +LPS	7	0.015	4	0.011	0.294
Persistent body pain	M(NS)	8	0.011	3	0.009	0.663
	M(NS)+LPS	8	0.013	3	0.008	0.301
	M(IFNγ)	6	0.013	3	0.016	0.675
	M(IFNγ) +LPS	8	0.012	3	0.012	0.963
	M(IL4)	6	0.008	3	0.011	0.214
	M(IL4) +LPS	8	0.013	3	0.015	0.726
Persistent difficulty concentrating	M(NS)	4	0.013	7	0.009	0.212
	M(NS)+LPS	4	0.016	7	0.009	0.034(*)
	M(IFNγ)	3	0.015	6	0.013	0.724
	M(IFNγ) +LPS	4	0.015	7	0.016	0.292
	M(IL4)	3	0.009	6	0.009	0.943
	M(IL4) +LPS	4	0.017	7	0.012	0.236

[1] – Without event. + with event

4 DISCUSSION

4.1 Monocyte holds an immunological imprint after COVID-19 infection.

COVID-19, a highly infectious disease that causes devastating global disease, continues to be a worldwide public health problem due to inflammatory pathology caused by acute disease and persistent immune dysfunction during convalescence (Scott et al., 2023). There is now widespread recognition that SARS-CoV-2 infections cause long-term chronic symptoms in many patients, which may be related to a deranged immune response (Syed et al., 2023).

As the host defender, the role of the innate immune system in determining disease severity and outcome is supported by recent studies (Schultze & Aschenbrenner, 2021). In the event of a respiratory infection, monocytes from the bone marrow are rapidly released into the circulation and recruited to the lungs by heightened systemic levels of MCP-1 and enhanced Ki67 on monocytes in COVID-19 patients (Mann et al., 2020; Scott et al., 2023). Several studies suggested that an increase in circulating monocytes is correlated to COVID-19 severity (Giamarellos-Bourboulis et al., 2020; Z. Zhou et al., 2020). Early studies during the first wave of COVID-19 showed enhanced monocyte infiltrates in the lung, kidney, heart, spleen and muscle from deceased COVID-19 patients (Carsana et al., 2020; Deshmukh et al., 2021), supporting a role for the abnormal monocytes in severity of COIVD-19 infection (Scott et al., 2023). In vitro models suggested that monocyte-derived macrophages might be a major source of cytokine storm, which is associated with the severity of the disease, especially in severe disease courses (Yang et al., 2020) (Mulchandani et al., 2021). COVID-19 patients with different severity courses showed different circulating monocyte types through single-cell sequencing. Mild to moderate COVID-19 patients were marked by elevated circulating inflammatory monocytes with an IFN-stimulated gene signature (Schulte-Schrepping et al., 2020).

Immunological imprinting in monocytes determines the function fate of macrophages and susceptibility to secondary infection (Saeed et al., 2014). Whether COVID-19 can also trigger this process remains unclear. A number of studies have demonstrated that COVID-19 convalescent individuals have more CD14+ monocytes in both population and function than healthy individuals. Training immunity requires the reprogramming of epigenetic and transcriptional changes in innate cells, as opposed to adaptive immunity, which reverses antigen receptor rearrangements. (Mitroulis et al., 2018). In the post-infection period, persistent epigenetic reprogramming of monocytes in COVID-19 convalescent donors is up to 1 year (Cheong et al., 2023).

4.1.1 Monocyte holds immunity memory after SARS-Cov2 infection

Secreted by activation monocyte-derived macrophages, evaluated circulating levels of IL-1β, IL-1ra IL-10 and YKL-40 were reported during the SARS-Cov2 infection acute phase and correlated with the severity of disease (Hawerkamp et al., 2023). In this study, these 4 macrophages activation biomarkers in monocyte-derived macrophages from COVID-19 convalescent and healthy donors were first analyzed. As the result showed, compared to healthy donors, monocyte-derived macrophages from COVID-19 convalescent donors showed higher expression of YKL-40 in types of M(NS), M(NS)+LPS, and M(IL4) macrophages. IL-10 is also expressed higher in both M(NS)+LPS and M(IFNγ) macrophages. In terms of expression, there is no significant difference between IL-1β and IL-1ra. Monocyte- derived macrophages are able to

secret both pro (IL-1β, etc.) and anti- inflammatory (IL-1ra, IL-10, etc.) depending on their activation inducers (Kzhyshkowska et al., 2015). YKL-40 is described as a marker for macrophage differentiation (Kzhyshkowska et al., 2007). According to our results, after at least 40 days of recovery from COVID-19 infection, monocytes retain immunological memory and are still susceptible to induced macrophage differentiation. When challenged with LPS, macrophages are more likely to polarize into M2.

Several studies have shown, in the post-infection period, compared to healthy individuals, post-COVID-19 individuals showed a greater abundance of CD14+ monocytes in both populations and functions(Zhang et al., 2022). Compared the moderate patients, monocyte display less diversity of in patient with severity disease. Immune system dysregulation are believed to be associated with the severity of COVID-19 progress (Wen et al., 2020). A longitudinal analysis suggested the major differences in both immune type and cytokines between moderate and severe disease were apparent after day 10 of the infection, while CRS related cytokines declined steady in 10 days infection in moderate patients, patients with severe COVID maintained elevated levels (Lucas et al., 2020). Single cell sequencing suggested that compared to the moderate one, patients with severe disease showed a more prominent immune imbalance which declines of some classic immune-negative signals (Zhou et al., 2021). From patients with mild disease to severe disease, the immune imbalance developed to CRS (Zhang et al., 2022). Studies have demonstrated that, compared to a severe infection, the monocytes in patients with moderate COVID-19 infection may exhibit a pro-inflammatory response in the early acute phase, and an anti-inflammatory response in the late acute phase.

Although the immune balance was restored in the convalescent period (Zhang et al., 2022), studies showed this dysregulated monocyte can persist even to 9 months (Scott et al., 2023). After 42 days of recovery from infection, peripheral monocytes from moderate COVID-19 convalescent donors showed an IFN response signature compared to healthy donors (Li et al., 2022). After 3 months from recovery, peripheral CXCR3+ monocyte was significant increased compared to the healthy donors (Ryan et al., 2022). Recent research showed that, unlike it from acute phase, monocyte from convalescent donors was distinct and characterized by increased chromatin accessibility at AP1 and MAF loci (Brauns et al., 2022), which are the key regulator involved in the functional programming of macrophages (Hannemann et al., 2019; Lavin et al., 2014). Single- cell epigenomic landscape suggested that monocytes could maintain chromatin reprogramming in the COVID-19 convalescent period, enabling a rapid inflammatory response against subsequent infection (Maojun You et al., 2021). In this study, we showed that monocytes from COVID-19 convalescent donors showed a rapid response to stimulation compared to healthy donors. COVID-19 convalescent donors exhibited a tendency toward tissue-repaired macrophages derived from monocytes.

4.1.2 YKL-40 could be a biomarker for long- term memory of monocyte- macrophages system after COVID-19 moderate infection

It has been demonstrated in our lab and others that YKL-40 regulates inflammation, innate immunity, fibroproliferative repair, and angiogenesis (Kamle et al., 2021; Kzhyshkowska et al., 2007; J. Kzhyshkowska et al., 2006). As a result of these studies, YKL-40 appears to be dysregulated in a variety of diseases that are characterized by injury, inflammation, or tissue remodeling (Dela Cruz et al., 2012; Kamle et al., 2021; Lee, 2009). In COVID-19, YKL-40 was reported to be the potent stimulator of ACE2

(Kamle et al., 2021), which is a cell binding receptor for SARS- Co- 2 (Hoffmann et al., 2020; P. Zhou et al., 2020). Serum YKl-40 level was reported to have a strong correlation with disease severity of COVID-19 (Parlak & Laloğlu, 2022; Schoneveld et al., 2021). This higher serum YKL-40 level could persist after one month of recovery (De Lorenzo et al., 2022).

As it is already known that macrophages are the main source of serum YKL-40 in inflammation (Guo et al., 2016; Kumagai et al., 2016; Yoshio & Kanto, 2021), we focused on the long-term changes in YKL-40 levels from monocyte-differentiated macrophages from COVID-19 convalescent plasma donors. For all 3 donations up to 495 days after infection, higher YKL-40 levels were found in all 6 types of monocytes-differentiated macrophages in COVID-19 convalescent plasma donors compared to the healthy ones. This suggested that the dysregulation of monocytes could be longer than one year. YKL-40 was reported to be a marker for late macrophage differentiation (Julia Kzhyshkowska et al., 2006). Recent studies hold the view that the cells are considered to have undergone trained immunity if restimulation of these innate immune cells at a later time point results in enhanced responsiveness compared with the initial stimulation (Ochando et al., 2023). In this study, YKL-40 expression is significantly higher in the M (IL4) +LPS macrophages at T3. This might suggest that after the dysregulation of immunity returned to normal, monocytes from COVID-19 convalescent plasma donors might still hold the memory, and YKL-40 could be a biomarker for the long-term memory for the innate immunity system after COVID-19 infection.

4.2 Functional changes monocyte plays an important role in long-term infection status of COVID-19

Long COVID is an often-debilitating illness that occurs in at least 10% of SARS-CoV-2 infections. A global estimate of 65 million people suffers from long COVID, and the number continues to rise. In spite the face that long COVID occurs of all ages and acute phase disease severities, the most long COVID cases are non- hospitalized patients with mild acute illness (Davis et al., 2023). Although the cause of long COVID remains unclear, hypotheses for its pathogenesis include immune dysregulation (Phetsouphanh et al., 2022; Proal & VanElzakker, 2021), impacts of SARS-CoV-2 on the microbiota (Proal & VanElzakker, 2021; Yeoh et al., 2021), autoimmunity microvascular blood clotting with endothelial dysfunction(Wallukat et al., 2021) and dysfunctional signaling in the brainstem and/or vagus nerve(Haffke et al., 2022; Proal & VanElzakker, 2021).

A comprehensive study showed that donors with long COVID showed an increase population of non- classical monocyte compared the donors without long COVID (Klein et al., 2023). This functional reprograming of monocytes in patients with convalescing COVID-19 could have both beneficial and detrimental consequences (Brauns et al., 2022). In this study, we found that higher expression of YKL-40 in M(NS) at T3 was found in COVID-19 plasma donors with the presence of long COVID. As the marker for late macrophage differentiation, higher expression of YKL-40 suggested monocyte could improve the response to the infection after COVID-19 infection. On the also hand, YKL-40 is also the marker for the neuroinflammation (Baldacci et al., 2017), autoinflammation(Aomatsu et al., 2011), endothelial dysfunction(Jafari & Mohsenin, 2016) and fibrogenesis in liver(Kumagai et al., 2016) and bladder (Richter et al., 2010). Function epigenetic changes of YKL-40 in monocyte could plays an important role in long COVID symptoms.

4.3 The homeostatic regulation of YKL-40 plays in the biology of healing, repair, and disease.

YKL-40 was reported could be a prognostic marker of severity in COVID-19 infection (Parlak & Laloğlu, 2022; Schoneveld et al., 2021). In this study, negative correlations were found between the total amount of symptoms in acute phase and YKL-40 level from M(NS) at T1 and T2. This negative correlation can also be found in the amount of respiratory symptoms and YKL-40 expression in M(NS), M(IFNγ) at T1. With the LPS stimulation, the negative correlation in respiratory system can be seen in M(IL4)+LPS, at T2 and T3. As this study is focused on convalescent donors from mild to moderate COVID-19 infection, the amount of symptoms was seen as the symbol of severity during acute infection phase. According to this, the memory of YKL-40 from monocyte could negatively be related to the severity of respiratory symptoms in moderate acute infection.

YKL-40 plays a crucial role in inflammation, angiogenesis and abnormal ECM remodeling (Murphy et al., 2023). However, YKL-40 can have protective and pathologic effects (Kamle et al., 2021). In respiratory diseases, the level of circulating YKL-40 has been considered a promising biomarker for assessing disease severity and forecasting patient prognosis (Hozumi et al., 2017; Jiang et al., 2019). YKL-40 inhibits hyperoxia-induced acute lung injury (Sohn et al., 2010; Syed & Bhandari, 2013), is a powerful inhibitor of cellular apoptosis (Lee et al., 2009), and controls bacterial infection via the regulation of inflammasome activation(Dela Cruz et al., 2012). Recent research emphasizes the role of YKL-40 as an activator of ACE2 and SPP, showing that these induction events contribute to increased tissue inflammation and damage in COVID-19 (Kamle et al., 2021). These discoveries emphasize the vital importance of maintaining regulatory control over YKL-40 during healing, repair, and disease management (Kamle et al., 2021). Recent study showed that controlled the expression of YKL-40 can decrease the augmented AEC2 expression and diminish the consequences of SC2 infection(Kamle et al., 2021).As former studies suggested, unlike severe infection develop to the CRS, cytokines declined steady in 10 days moderate infection (Lucas et al., 2020). After moderate infection, monocyte holds the immunological memory for quick differential for macrophages, they may also hold the imprint of the homeostatic regulation of YKL-40.

4.4 Macrophage- derived YKL-40 plays an important role in mitigate neuroinflammation after COVID-19 infection, related to long-term difficulty in concentration symptoms.

YKl-40 is a well-described human biomarker of neuroinflammation (Craig-Schapiro et al., 2010). In this study, negative correlations were found between the total amount of symptoms in acute phase and YKL-40 level from M(IFNγ) +LPS at all 3 donations. Among donors with reported long-COVID symptoms, significantly higher expression of YKL-40 can be found in donors who reported difficulty in concentration symptoms.

SARS-CoV-2 is primarily recognized as a respiratory virus, but neurological symptoms may develop several days before respiratory symptoms (Harapan & Yoo, 2021; Spudich & Nath, 2022). Further, long-term effects of COVID, including headaches, sensory abnormalities, depression, and even psychosis, can persist for months after infection (Spudich & Nath, 2022). Neurological complications extend beyond the central nervous system (CNS) to include the peripheral nervous system (PNS) (Mao et al., 2020). Nevertheless, our understanding of peripheral neurological

manifestations remains incomplete (Ellul et al., 2020; Harapan & Yoo; Sampaio Rocha-Filho et al.).

Available evidence does not indicate that SARS-CoV-2 or related coronaviruses possess significant neurovirulence (Ellul et al., 2020). A histopathological analysis of deceased acute COVID-19 patients found only sparse evidence of the virus' nucleic acids or proteins (Matschke et al.; Spudich & Nath, 2022). Damage to the nervous system may be primarily caused by the body's own immune response (Ellul et al., 2020). Activation of microglia and release of proinflammatory cytokines are symptoms of a secondary neuroinflammatory response caused by cytokines from the peripheral immune system. Several upregulated plasma neuroinflammation biomarkers can be detected in convalescent COVID-19 individuals(de Paula et al., 2023). YKl-40 is a well-described human biomarker of neuroinflammation(Craig-Schapiro et al., 2010), which is elevated in neurological diseases, including Alzheimer's Disease(Craig-Schapiro et al., 2010), ALS(Alcolea et al., 2017), and frontotemporal dementia(Craig-Schapiro et al., 2010). During neuroinflammation, YKL-40 may suppress glial phagocytic activation in Alzheimer's disease(Lananna et al., 2020). Following traumatic brain injury, YKL-40 expression appears to moderate aspects of neuroinflammation related to astrocytes and microglia/macrophages (Wiley et al., 2015). In the context of stroke, the deletion of YKL-40 has been shown to hasten stroke development by significantly reducing M2 macrophage polarization and thereby exacerbating neuroinflammation (Im et al., 2020). During the acute phase of COVID-19, YKL-40 has been identified as a potential severity biomarker (Parlak & Laloğlu, 2022; Pilotto et al.), yet no studies have yet demonstrated a direct correlation between YKL-40 levels and neurological symptoms in both the acute and post-infection phases of COVID-19.

5 SUMMARY

In the complex landscape of COVID-19 research, a particular area of interest is the study of individuals who have recovered from the virus, known as COVID-19 convalescents. Changes in trained innate immunity, especially within the monocyte-macrophage system, play a crucial role in the convalescent period following virus infection. As a differentiation and activation marker of macrophages, YKL-40 can be involved in innate immune reactions during virus infection. YKL-40 has been identified as a biomarker for the severity of COVID-19 in the acute infection period. In this study, we explore the potential biomarker role of YKL-40 in monocyte-differentiated macrophages from COVID-19 convalescent individuals. 39 COVID-19 convalescent plasma donors and 36 healthy plasma donors were included in the study as part of the CORE project. The donation at time point 1 (T1) occurred 44-447 days after COVID-19 diagnosis, donation at time point 2 (T2) was conducted 21-60 days after T1, followed by a third donation (T3) 25-62 days after T2. Donors' baseline characteristics, including COVID-19 symptoms, were documented through a questionnaire. For the study CD14+ selection of monocytes has been performed, and monocytes were differentiated towards homeostatic M(NS), inflammatory M(IFN-γ), and healing M(IL4) macrophages for 2 days. Challenge with LPS has been applied after 24 h of cultivation for next 24 hours. Expression of IL-1β, IL-1ra, IL-10 and YKL-40 was analyzed by RT-PCR. Compared to healthy individuals, a statistically significantly higher expression of YKL-40 was found in M(NS) (T1), M(NS)+LPS (T1), M(IL4) (T1). YKL-40 expression was further analyzed in monocyte-derived macrophages obtained at T2 and T3. The statistically significant higher expression of YKL-40 in COVID-19 convalescent donors was found in M(NS) (T2) and M(IL4)+LPS (T3). Correlation of YKL-40 expression level with clinical parameters of acute and post/long-COVID was further analyzed. A statistically significant negative correlation was found between the expression of YKL-40 and the total amount of acute symptoms in M(NS) (T1, T2). To explore the correlation between YKL-40 expression and specific symptoms, all symptoms were classified into respiratory, systemic and neurology symptoms. Donors who reported the presence of respiratory symptoms showed statistically lower expression of YKL-40 in M(NS) (T2, T3) and in M(IFNγ) (T3). The negative correlation with YKL-40 expression was also evident with the amount of respiratory symptoms in M(NS) (T1), M(NS)+LPS (T1), M(IFNγ) (T1), M(IFNγ)+LPS (T3), and M(IL4)+LPS (T2). Donors who reported the presence of the systemic symptoms showed statistically lower expression of YKL-40 in M(NS) (T2) and M(IL4) (T3), although the YKL-40 expression had no statistical significant correlation with the amount of systemic symptoms. For neurological symptoms, no statistically significant differences in the YKL-40 expression was found between donors who reported the presence or absence of the symptoms. The statistically significant negative correlation was found with neurological symptoms in M(NS) (T1, T3), M(IFNγ) (T3), and M(IFNγ)+LPS (T1, T2, T3). As post/long COVID is consider as a public health challenge, correlation with YKL-40 with post/long COVID was analyzed. Donors who reported the post/long COVID symptoms showed a statistically significantly higher expression of YKL-40 in M(NS) (T3). Among all donors with post/long COVID symptoms, people reporting the persistent difficulty in concentration showed statistically significant lower expression of YKL-40 in M(NS)+LPS (T1). In summary, our study reveals a long-term change in YKL-40 expression in monocyte-derived macrophages in COVID-19 convalescent individuals, where the strongest correlation is detected for neurological symptoms. Given the importance of monocytes/macrophages system, YKL-40 may become a potential marker for the long-term dysregulation of the innate immune system.

6 BIBLIOGRAPHY

Akboga, M. K. & Sahinarslan, A. (2017). YKL-40 and other indicators of inflammation in coronary collateral development and SYNTAX score. *Int J Cardiol, 229*, 66. https://doi.org/10.1016/j.ijcard.2016.11.229

Akboga, M. K., Yalcin, R., Sahinarslan, A., Yilmaz Demirtas, C. & Abaci, A. (2016). Effect of serum YKL-40 on coronary collateral development and SYNTAX score in stable coronary artery disease. *International Journal of Cardiology, 224*, 323-327. https://doi.org/https://doi.org/10.1016/j.ijcard.2016.09.042

Alcolea, D., Vilaplana, E., Suárez-Calvet, M., Illán-Gala, I., Blesa, R., Clarimón, J., Lladó, A., Sánchez-Valle, R., Molinuevo, J. L., García-Ribas, G., Compta, Y., Martí, M. J., Piñol-Ripoll, G., Amer-Ferrer, G., Noguera, A., García-Martín, A., Fortea, J. & Lleó, A. (2017). CSF sAPPβ, YKL-40, and neurofilament light in frontotemporal lobar degeneration. *Neurology, 89*(2), 178-188. https://doi.org/10.1212/wnl.0000000000004088

Aomatsu, T., Imaeda, H., Matsumoto, K., Kimura, E., Yoden, A., Tamai, H., Fujiyama, Y., Mizoguchi, E. & Andoh, A. (2011). Faecal chitinase 3-like-1: a novel biomarker of disease activity in paediatric inflammatory bowel disease. *Aliment Pharmacol Ther, 34*(8), 941-948. https://doi.org/10.1111/j.1365-2036.2011.04805.x

Arts, R. J., Novakovic, B., Ter Horst, R., Carvalho, A., Bekkering, S., Lachmandas, E., Rodrigues, F., Silvestre, R., Cheng, S. C., Wang, S. Y., Habibi, E., Gonçalves, L. G., Mesquita, I., Cunha, C., van Laarhoven, A., van de Veerdonk, F. L., Williams, D. L., van der Meer, J. W., Logie, C., O'Neill, L. A., Dinarello, C. A., Riksen, N. P., van Crevel, R., Clish, C., Notebaart, R. A., Joosten, L. A., Stunnenberg, H. G., Xavier, R. J. & Netea, M. G. (2016). Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. *Cell Metab, 24*(6), 807-819. https://doi.org/10.1016/j.cmet.2016.10.008

Attiq, A., Yao, L. J., Afzal, S. & Khan, M. A. (2021). The triumvirate of NF-κB, inflammation and cytokine storm in COVID-19. *Int Immunopharmacol, 101*(Pt B), 108255. https://doi.org/10.1016/j.intimp.2021.108255

Auffray, C., Sieweke, M. H. & Geissmann, F. (2009). Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol, 27*, 669-692. https://doi.org/10.1146/annurev.immunol.021908.132557

Baldacci, F., Lista, S., Cavedo, E., Bonuccelli, U. & Hampel, H. (2017). Diagnostic function of the neuroinflammatory biomarker YKL-40 in Alzheimer's disease and other neurodegenerative diseases. *Expert Rev Proteomics, 14*(4), 285-299. https://doi.org/10.1080/14789450.2017.1304217

Bao, J., Ouyang, Y., Qiao, L., He, J., Liu, F., Wang, Y., Miao, L., Fu, A., Lou, Z., Zang, Q., Huang, W., Huang, J. & Li, Z. (2022). Serum CHI3L1 as a Biomarker for Non-invasive Diagnosis of Liver Fibrosis. *Discov Med, 33*(168), 41-49.

Bekkering, S., Arts, R. J. W., Novakovic, B., Kourtzelis, I., van der Heijden, C., Li, Y., Popa, C. D., Ter Horst, R., van Tuijl, J., Netea-Maier, R. T., van de Veerdonk, F. L., Chavakis, T., Joosten, L. A. B., van der Meer, J. W. M., Stunnenberg, H., Riksen, N. P. & Netea, M. G. (2018). Metabolic Induction of Trained Immunity through the Mevalonate Pathway. *Cell, 172*(1-2), 135-146.e139. https://doi.org/10.1016/j.cell.2017.11.025

Bergmann, O. J., Johansen, J. S., Klausen, T. W., Mylin, A. K., Kristensen, J. S., Kjeldsen, E. & Johnsen, H. E. (2005). High serum concentration of YKL-40 is associated with short survival in patients with acute myeloid leukemia. *Clin Cancer Res, 11*(24 Pt 1), 8644-8652. https://doi.org/10.1158/1078-0432.Ccr-05-1317

Bernard, I., Ransy, D. G., Brophy, J., Kakkar, F., Bitnun, A., Samson, L., Read, S., Soudeyns, H., Hawkes, M. T. & Epic Study, G. (2022). Chitinase-3-like Protein 1 Is Associated with Poor Virologic Control and Immune Activation in Children Living with HIV. *Viruses, 14*(12). https://doi.org/10.3390/v14122602

Berres, M. L., Papen, S., Pauels, K., Schmitz, P., Zaldivar, M. M., Hellerbrand, C., Mueller, T., Berg, T., Weiskirchen, R., Trautwein, C. & Wasmuth, H. E. (2009). A functional variation in CHI3L1 is associated with severity of liver fibrosis and YKL-40 serum levels in chronic hepatitis C infection. *J Hepatol, 50*(2), 370-376. https://doi.org/10.1016/j.jhep.2008.09.016

Bissel, S. J., Kofler, J., Nyaundi, J., Murphey-Corb, M., Wisniewski, S. R. & Wiley, C. A. (2016). Cerebrospinal Fluid Biomarkers of Simian Immunodeficiency Virus Encephalitis : CSF Biomarkers of SIV Encephalitis. *J Neuroimmune Pharmacol, 11*(2), 332-347. https://doi.org/10.1007/s11481-016-9666-9

Bjørn, M. E., Andersen, C. L., Jensen, M. K. & Hasselbalch, H. C. (2014). Circulating YKL-40 in myelofibrosis a potential novel biomarker of disease activity and the inflammatory state. *Eur J Haematol, 93*(3), 224-228. https://doi.org/10.1111/ejh.12332

Bohnacker, S., Hartung, F., Henkel, F., Quaranta, A., Kolmert, J., Priller, A., Ud-Dean, M., Giglberger, J., Kugler, L. M., Pechtold, L., Yazici, S., Lechner, A., Erber, J., Protzer, U., Lingor, P., Knolle, P., Chaker, A. M., Schmidt-Weber, C. B., Wheelock, C. E. & Esser-von Bieren, J. (2022a). Mild COVID-19 imprints a long-term inflammatory eicosanoid- and chemokine memory in monocyte-derived macrophages. *Mucosal Immunol, 15*(3), 515-524. https://doi.org/10.1038/s41385-021-00482-8

Bohnacker, S., Hartung, F., Henkel, F., Quaranta, A., Kolmert, J., Priller, A., Ud-Dean, M., Giglberger, J., Kugler, L. M., Pechtold, L., Yazici, S., Lechner, A., Erber, J., Protzer, U., Lingor, P., Knolle, P., Chaker, A. M., Schmidt-Weber, C. B., Wheelock, C. E. & Esser-von Bieren, J. (2022b). Mild COVID-19 imprints a long-term inflammatory eicosanoid- and chemokine memory in monocyte-derived macrophages. *Mucosal Immunol*. https://doi.org/10.1038/s41385-021-00482-8

Bonneh-Barkay, D., Bissel, S. J., Wang, G., Fish, K. N., Nicholl, G. C., Darko, S. W., Medina-Flores, R., Murphey-Corb, M., Rajakumar, P. A., Nyaundi, J., Mellors,

J. W., Bowser, R. & Wiley, C. A. (2008). YKL-40, a marker of simian immunodeficiency virus encephalitis, modulates the biological activity of basic fibroblast growth factor. *Am J Pathol, 173*(1), 130-143. https://doi.org/10.2353/ajpath.2008.080045

Brauns, E., Azouz, A., Grimaldi, D., Xiao, H., Thomas, S., Nguyen, M., Olislagers, V., Vu Duc, I., Orte Cano, C., Del Marmol, V., Pannus, P., Libert, F., Saussez, S., Dauby, N., Das, J., Marchant, A. & Goriely, S. (2022). Functional reprogramming of monocytes in patients with acute and convalescent severe COVID-19. *JCI Insight, 7*(9). https://doi.org/10.1172/jci.insight.154183

Brightling, C. E. & Evans, R. A. (2022). Long COVID: which symptoms can be attributed to SARS-CoV-2 infection? *Lancet, 400*(10350), 411-413. https://doi.org/10.1016/s0140-6736(22)01385-x

Brueggeman, J. M., Zhao, J., Schank, M., Yao, Z. Q. & Moorman, J. P. (2022). Trained Immunity: An Overview and the Impact on COVID-19. *Front Immunol, 13*, 837524. https://doi.org/10.3389/fimmu.2022.837524

Carlos, W. G., Dela Cruz, C. S., Cao, B., Pasnick, S. & Jamil, S. (2020). Novel Wuhan (2019-nCoV) Coronavirus. *Am J Respir Crit Care Med, 201*(4), P7-p8. https://doi.org/10.1164/rccm.2014P7

Carsana, L., Sonzogni, A., Nasr, A., Rossi, R. S., Pellegrinelli, A., Zerbi, P., Rech, R., Colombo, R., Antinori, S., Corbellino, M., Galli, M., Catena, E., Tosoni, A., Gianatti, A. & Nebuloni, M. (2020). Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. *Lancet Infect Dis, 20*(10), 1135-1140. https://doi.org/10.1016/s1473-3099(20)30434-5

Charfeddine, S., Ibn Hadj Amor, H., Jdidi, J., Torjmen, S., Kraiem, S., Hammami, R., Bahloul, A., Kallel, N., Moussa, N., Touil, I., Ghrab, A., Elghoul, J., Meddeb, Z., Thabet, Y., Kammoun, S., Bouslama, K., Milouchi, S., Abdessalem, S. & Abid, L. (2021). Long COVID 19 Syndrome: Is It Related to Microcirculation and Endothelial Dysfunction? Insights From TUN-EndCOV Study. *Front Cardiovasc Med, 8*, 745758. https://doi.org/10.3389/fcvm.2021.745758

Chen, G., Yang, T., Gu, Q., Ni, X. H., Zhao, Z. H., Ye, J., Meng, X. M., Liu, Z. H., He, J. G. & Xiong, C. M. (2014). Elevated plasma YKL-40 as a prognostic indicator in patients with idiopathic pulmonary arterial hypertension. *Respirology, 19*(4), 608-615. https://doi.org/10.1111/resp.12283

Chen, R., Lan, Z., Ye, J., Pang, L., Liu, Y., Wu, W., Qin, X., Guo, Y. & Zhang, P. (2021). Cytokine Storm: The Primary Determinant for the Pathophysiological Evolution of COVID-19 Deterioration. *Front Immunol, 12*, 589095. https://doi.org/10.3389/fimmu.2021.589095

Cheong, J.-G., Ravishankar, A., Sharma, S., Parkhurst, C. N., Grassmann, S. A., Wingert, C. K., Laurent, P., Ma, S., Paddock, L., Miranda, I. C., Karakaslar, E. O., Nehar-Belaid, D., Thibodeau, A., Bale, M. J., Kartha, V. K., Yee, J. K., Mays, M. Y., Jiang, C., Daman, A. W., Martinez de Paz, A., Ahimovic, D., Ramos, V., Lercher, A., Nielsen, E., Alvarez-Mulett, S., Zheng, L., Earl, A., Yallowitz, A.,

Robbins, L., LaFond, E., Weidman, K. L., Racine-Brzostek, S., Yang, H. S., Price, D. R., Leyre, L., Rendeiro, A. F., Ravichandran, H., Kim, J., Borczuk, A. C., Rice, C. M., Jones, R. B., Schenck, E. J., Kaner, R. J., Chadburn, A., Zhao, Z., Pascual, V., Elemento, O., Schwartz, R. E., Buenrostro, J. D., Niec, R. E., Barrat, F. J., Lief, L., Sun, J. C., Ucar, D. & Josefowicz, S. Z. (2023). Epigenetic memory of coronavirus infection in innate immune cells and their progenitors. *Cell, 186*(18), 3882-3902.e3824. https://doi.org/https://doi.org/10.1016/j.cell.2023.07.019

Coriati, A., Bouvet, G. F., Massé, C., Ducruet, T. & Berthiaume, Y. (2021). YKL-40 as a clinical biomarker in adult patients with CF: Implications of a CHI3L1 single nucleotide polymorphism in disease severity. *J Cyst Fibros, 20*(6), e93-e99. https://doi.org/10.1016/j.jcf.2020.11.011

Craig-Schapiro, R., Perrin, R. J., Roe, C. M., Xiong, C., Carter, D., Cairns, N. J., Mintun, M. A., Peskind, E. R., Li, G., Galasko, D. R., Clark, C. M., Quinn, J. F., D'Angelo, G., Malone, J. P., Townsend, R. R., Morris, J. C., Fagan, A. M. & Holtzman, D. M. (2010). YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry, 68*(10), 903-912. https://doi.org/10.1016/j.biopsych.2010.08.025

Czupryna, P., Kulczyńka-Przybik, A., Mroczko, B., Wondim, M. A., Grygorczuk, S., Borawski, K., Pancewicz, S. & Moniuszko-Malinowska, A. (2022). Assessment of the YKL-40 concentration in patients with tick-borne encephalitis. *Ticks Tick Borne Dis, 13*(2), 101895. https://doi.org/10.1016/j.ttbdis.2022.101895

Das, A., Kamrul-Hasan, A., Kabir, M. R., Das, S., Zaki, K. & Al Mahtab, M. (2021). Evaluation of Chitinase 3-like 1 (CHI3L1) as a noninvasive biomarker of hepatic fibrosis in patients with Hepatitis B virus-related compensated chronic liver disease. *J Family Med Prim Care, 10*(4), 1694-1698. https://doi.org/10.4103/jfmpc.jfmpc_1922_20

Davis, H. E., McCorkell, L., Vogel, J. M. & Topol, E. J. (2023). Long COVID: major findings, mechanisms and recommendations. *Nature Reviews Microbiology, 21*(3), 133-146. https://doi.org/10.1038/s41579-022-00846-2

De Lorenzo, R., Sciorati, C., Lorè, N. I., Capobianco, A., Tresoldi, C., Cirillo, D. M., Ciceri, F., Rovere-Querini, P. & Manfredi, A. A. (2022). Chitinase-3-like protein-1 at hospital admission predicts COVID-19 outcome: a prospective cohort study. *Sci Rep, 12*(1), 7606. https://doi.org/10.1038/s41598-022-11532-x

de Paula, J. J., Paiva, R., Souza-Silva, N. G., Rosa, D. V., Duran, F. L. S., Coimbra, R. S., Costa, D. S., Dutenhefner, P. R., Oliveira, H. S. D., Camargos, S. T., Vasconcelos, H. M. M., de Oliveira Carvalho, N., da Silva, J. B., Silveira, M. B., Malamut, C., Oliveira, D. M., Molinari, L. C., de Oliveira, D. B., Januário, J. N., Silva, L. C., De Marco, L. A., Queiroz, D. M. M., Meira, W., Busatto, G., Miranda, D. M. & Romano-Silva, M. A. (2023). Selective visuoconstructional impairment following mild COVID-19 with inflammatory and neuroimaging correlation findings. *Mol Psychiatry, 28*(2), 553-563. https://doi.org/10.1038/s41380-022-01632-5

Dela Cruz, C. S., Liu, W., He, C. H., Jacoby, A., Gornitzky, A., Ma, B., Flavell, R., Lee, C. G. & Elias, J. A. (2012). Chitinase 3-like-1 promotes Streptococcus pneumoniae killing and augments host tolerance to lung antibacterial responses. *Cell Host Microbe, 12*(1), 34-46. https://doi.org/10.1016/j.chom.2012.05.017

Deshmukh, V., Motwani, R., Kumar, A., Kumari, C. & Raza, K. (2021). Histopathological observations in COVID-19: a systematic review. *J Clin Pathol, 74*(2), 76-83. https://doi.org/10.1136/jclinpath-2020-206995

Ebihara, T., Matsubara, T., Togami, Y., Matsumoto, H., Tachino, J., Matsuura, H., Kojima, T., Sugihara, F., Seno, S., Okuzaki, D., Hirata, H. & Ogura, H. (2023). Combination of WFDC2, CHI3L1, and KRT19 in Plasma Defines a Clinically Useful Molecular Phenotype Associated with Prognosis in Critically Ill COVID-19 Patients. *J Clin Immunol, 43*(2), 286-298. https://doi.org/10.1007/s10875-022-01386-3

Ellul, M. A., Benjamin, L., Singh, B., Lant, S., Michael, B. D., Easton, A., Kneen, R., Defres, S., Sejvar, J. & Solomon, T. (2020). Neurological associations of COVID-19. *Lancet Neurol, 19*(9), 767-783. https://doi.org/10.1016/S1474-4422(20)30221-0

Esmat, G., Metwally, M., Zalata, K. R., Gadalla, S., Abdel-Hamid, M., Abouzied, A., Shaheen, A. A., El-Raziky, M., Khatab, H., El-Kafrawy, S., Mikhail, N., Magder, L. S., Afdhal, N. H. & Strickland, G. T. (2007). Evaluation of serum biomarkers of fibrosis and injury in Egyptian patients with chronic hepatitis C. *J Hepatol, 46*(4), 620-627. https://doi.org/10.1016/j.jhep.2006.12.010

Eurich, D., Neumann, U. P., Boas-Knoop, S., Neuhaus, R., Kiessling, A., Yahyazadeh, A., Trautwein, C., Wasmuth, H., Puhl, G., Neuhaus, P. & Bahra, M. (2013). YKL-40-gene polymorphism affects acute cellular rejection and fibrosis progression after transplantation for hepatitis C virus-induced liver disease. *J Gastroenterol Hepatol, 28*(1), 153-160. https://doi.org/10.1111/j.1440-1746.2012.07270.x

Evans, R. A., McAuley, H., Harrison, E. M., Shikotra, A., Singapuri, A., Sereno, M., Elneima, O., Docherty, A. B., Lone, N. I., Leavy, O. C., Daines, L., Baillie, J. K., Brown, J. S., Chalder, T., De Soyza, A., Diar Bakerly, N., Easom, N., Geddes, J. R., Greening, N. J., Hart, N., Heaney, L. G., Heller, S., Howard, L., Hurst, J. R., Jacob, J., Jenkins, R. G., Jolley, C., Kerr, S., Kon, O. M., Lewis, K., Lord, J. M., McCann, G. P., Neubauer, S., Openshaw, P. J. M., Parekh, D., Pfeffer, P., Rahman, N. M., Raman, B., Richardson, M., Rowland, M., Semple, M. G., Shah, A. M., Singh, S. J., Sheikh, A., Thomas, D., Toshner, M., Chalmers, J. D., Ho, L. P., Horsley, A., Marks, M., Poinasamy, K., Wain, L. V. & Brightling, C. E. (2021). Physical, cognitive, and mental health impacts of COVID-19 after hospitalisation (PHOSP-COVID): a UK multicentre, prospective cohort study. *Lancet Respir Med, 9*(11), 1275-1287. https://doi.org/10.1016/s2213-2600(21)00383-0

Fawzy, S., Ahmed, M. M., Alsayed, B. A., Mir, R. & Amle, D. (2022). IL-2 and IL-1β Patient Immune Responses Are Critical Factors in SARS-CoV-2 Infection Outcomes. *J Pers Med, 12*(10). https://doi.org/10.3390/jpm12101729

Franke, C., Ferse, C., Kreye, J., Reincke, S. M., Sanchez-Sendin, E., Rocco, A., Steinbrenner, M., Angermair, S., Treskatsch, S., Zickler, D., Eckardt, K. U., Dersch, R., Hosp, J., Audebert, H. J., Endres, M., Ploner, J. C. & Prüß, H. (2021). High frequency of cerebrospinal fluid autoantibodies in COVID-19 patients with neurological symptoms. *Brain Behav Immun, 93*, 415-419. https://doi.org/10.1016/j.bbi.2020.12.022

Fusetti, F., Pijning, T., Kalk, K. H., Bos, E. & Dijkstra, B. W. (2003). Crystal structure and carbohydrate-binding properties of the human cartilage glycoprotein-39. *J Biol Chem, 278*(39), 37753-37760. https://doi.org/10.1074/jbc.M303137200

Ge, L., Wang, Y., Liu, Z., Du, H. & Zhao, D. (2023). Chitinase 3-like 1 plays a pivotal role in airway response of RSV infection via regulating DC functional transition. *Int Immunopharmacol, 124*(Pt A), 110819. https://doi.org/10.1016/j.intimp.2023.110819

Giamarellos-Bourboulis, E. J., Netea, M. G., Rovina, N., Akinosoglou, K., Antoniadou, A., Antonakos, N., Damoraki, G., Gkavogianni, T., Adami, M. E., Katsaounou, P., Ntaganou, M., Kyriakopoulou, M., Dimopoulos, G., Koutsodimitropoulos, I., Velissaris, D., Koufargyris, P., Karageorgos, A., Katrini, K., Lekakis, V., Lupse, M., Kotsaki, A., Renieris, G., Theodoulou, D., Panou, V., Koukaki, E., Koulouris, N., Gogos, C. & Koutsoukou, A. (2020). Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. *Cell Host Microbe, 27*(6), 992-1000.e1003. https://doi.org/10.1016/j.chom.2020.04.009

Gibaldi, D., Vilar-Pereira, G., Pereira, I. R., Silva, A. A., Barrios, L. C., Ramos, I. P., Mata Dos Santos, H. A., Gazzinelli, R. & Lannes-Vieira, J. (2020). CCL3/Macrophage Inflammatory Protein-1α Is Dually Involved in Parasite Persistence and Induction of a TNF- and IFNγ-Enriched Inflammatory Milieu in Trypanosoma cruzi-Induced Chronic Cardiomyopathy. *Front Immunol, 11*, 306. https://doi.org/10.3389/fimmu.2020.00306

Groff, D., Sun, A., Ssentongo, A. E., Ba, D. M., Parsons, N., Poudel, G. R., Lekoubou, A., Oh, J. S., Ericson, J. E., Ssentongo, P. & Chinchilli, V. M. (2021). Short-term and Long-term Rates of Postacute Sequelae of SARS-CoV-2 Infection: A Systematic Review. *JAMA Netw Open, 4*(10), e2128568. https://doi.org/10.1001/jamanetworkopen.2021.28568

Gu, Y., Zuo, X., Zhang, S., Ouyang, Z., Jiang, S., Wang, F. & Wang, G. (2021). The Mechanism behind Influenza Virus Cytokine Storm. *Viruses, 13*(7). https://doi.org/10.3390/v13071362

Gumus, A., Kayhan, S., Cinarka, H., Kirbas, A., Bulmus, N., Yavuz, A., Sahin, U. & Ozkaya, S. (2013). High serum YKL-40 level in patients with COPD is related to hypoxemia and disease severity. *Tohoku J Exp Med, 229*(2), 163-170. https://doi.org/10.1620/tjem.229.163

Guo, W., Cao, S., Yan, B., Zhang, G., Li, J., Zhao, Y. & Zhang, S. (2016). Myocardial protective effects of a c-Jun N-terminal kinase inhibitor in rats with brain death. *J Cell Mol Med, 20*(7), 1214-1218. https://doi.org/10.1111/jcmm.12676

Haffke, M., Freitag, H., Rudolf, G., Seifert, M., Doehner, W., Scherbakov, N., Hanitsch, L., Wittke, K., Bauer, S., Konietschke, F., Paul, F., Bellmann-Strobl, J., Kedor, C., Scheibenbogen, C. & Sotzny, F. (2022). Endothelial dysfunction and altered endothelial biomarkers in patients with post-COVID-19 syndrome and chronic fatigue syndrome (ME/CFS). *J Transl Med, 20*(1), 138. https://doi.org/10.1186/s12967-022-03346-2

Hakala, B. E., White, C. & Recklies, A. D. (1993). Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem, 268*(34), 25803-25810.

Han, H., Ma, Q., Li, C., Liu, R., Zhao, L., Wang, W., Zhang, P., Liu, X., Gao, G., Liu, F., Jiang, Y., Cheng, X., Zhu, C. & Xia, Y. (2020). Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerg Microbes Infect, 9*(1), 1123-1130. https://doi.org/10.1080/22221751.2020.1770129

Hannemann, N., Cao, S., Eriksson, D., Schnelzer, A., Jordan, J., Eberhardt, M., Schleicher, U., Rech, J., Ramming, A., Uebe, S., Ekici, A., Cañete, J. D., Chen, X., Bäuerle, T., Vera, J., Bogdan, C., Schett, G. & Bozec, A. (2019). Transcription factor Fra-1 targets arginase-1 to enhance macrophage-mediated inflammation in arthritis. *J Clin Invest, 129*(7), 2669-2684. https://doi.org/10.1172/jci96832

Harapan, B. A.-O. & Yoo, H. J. Neurological symptoms, manifestations, and complications associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease 19 (COVID-19). (1432-1459 (Electronic)).

Harapan, B. N. & Yoo, H. J. (2021). Neurological symptoms, manifestations, and complications associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease 19 (COVID-19). *J Neurol, 268*(9), 3059-3071. https://doi.org/10.1007/s00415-021-10406-y

Hawerkamp, H. C., Dyer, A. H., Patil, N. D., McElheron, M., O'Dowd, N., O'Doherty, L., Mhaonaigh, A. U., George, A. M., O'Halloran, A. M., Reddy, C., Kenny, R. A., Little, M. A., Martin-Loeches, I., Bergin, C., Kennelly, S. P., Donnelly, S. C., Bourke, N. M., Long, A., Sui, J., Doherty, D. G., Conlon, N., Cheallaigh, C. N. & Fallon, P. G. (2023). Characterisation of the pro-inflammatory cytokine signature in severe COVID-19. *Front Immunol, 14*, 1170012. https://doi.org/10.3389/fimmu.2023.1170012

Hirano, T. & Murakami, M. (2020). COVID-19: A New Virus, but a Familiar Receptor and Cytokine Release Syndrome. *Immunity, 52*(5), 731-733. https://doi.org/10.1016/j.immuni.2020.04.003

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C. & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell, 181*(2), 271-280.e278. https://doi.org/10.1016/j.cell.2020.02.052

Hozumi, H., Fujisawa, T., Enomoto, N., Nakashima, R., Enomoto, Y., Suzuki, Y., Kono, M., Karayama, M., Furuhashi, K., Murakami, A., Inui, N., Nakamura, Y., Mimori, T. & Suda, T. (2017). Clinical Utility of YKL-40 in Polymyositis/dermatomyositis-associated Interstitial Lung Disease. *J Rheumatol, 44*(9), 1394-1401. https://doi.org/10.3899/jrheum.170373

Hu, B., Guo, H., Zhou, P. & Shi, Z. L. (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol, 19*(3), 141-154. https://doi.org/10.1038/s41579-020-00459-7

Hu, B., Huang, S. & Yin, L. (2021). The cytokine storm and COVID-19. *J Med Virol, 93*(1), 250-256. https://doi.org/10.1002/jmv.26232

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J. & Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet, 395*(10223), 497-506. https://doi.org/10.1016/s0140-6736(20)30183-5

Huang, H., Wu, T., Mao, J., Fang, Y., Zhang, J., Wu, L., Zheng, S., Lin, B. & Pan, H. (2015). CHI3L1 Is a Liver-Enriched, Noninvasive Biomarker That Can Be Used to Stage and Diagnose Substantial Hepatic Fibrosis. *Omics, 19*(6), 339-345. https://doi.org/10.1089/omi.2015.0037

Huang, L., Li, X., Gu, X., Zhang, H., Ren, L., Guo, L., Liu, M., Wang, Y., Cui, D., Wang, Y., Zhang, X., Shang, L., Zhong, J., Wang, X., Wang, J. & Cao, B. (2022). Health outcomes in people 2 years after surviving hospitalisation with COVID-19: a longitudinal cohort study. *Lancet Respir Med, 10*(9), 863-876. https://doi.org/10.1016/s2213-2600(22)00126-6

Hussman, J. P. (2020). Cellular and Molecular Pathways of COVID-19 and Potential Points of Therapeutic Intervention. *Front Pharmacol, 11*, 1169. https://doi.org/10.3389/fphar.2020.01169

Im, J. H., Yeo, I. J., Park, P. H., Choi, D. Y., Han, S. B., Yun, J. & Hong, J. T. (2020). Deletion of Chitinase-3-like 1 accelerates stroke development through enhancement of Neuroinflammation by STAT6-dependent M2 microglial inactivation in Chitinase-3-like 1 knockout mice. *Exp Neurol, 323*, 113082. https://doi.org/10.1016/j.expneurol.2019.113082

Jafari, B. & Mohsenin, V. (2016). Chitinase-3-like protein-1 (YKL-40) as a marker of endothelial dysfunction in obstructive sleep apnea. *Sleep Med, 25*, 87-92. https://doi.org/10.1016/j.sleep.2016.08.001

James, A. J., Reinius, L. E., Verhoek, M., Gomes, A., Kupczyk, M., Hammar, U., Ono, J., Ohta, S., Izuhara, K., Bel, E., Kere, J., Söderhäll, C., Dahlén, B., Boot, R. G. & Dahlén, S. E. (2016). Increased YKL-40 and Chitotriosidase in Asthma and Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med, 193*(2), 131-142. https://doi.org/10.1164/rccm.201504-0760OC

Jiang, L., Wang, Y., Peng, Q., Shu, X., Wang, G. & Wu, X. (2019). Serum YKL-40 level is associated with severity of interstitial lung disease and poor prognosis in dermatomyositis with anti-MDA5 antibody. *Clin Rheumatol, 38*(6), 1655-1663. https://doi.org/10.1007/s10067-019-04457-w

Jin, X., Fu, B., Wu, Z. J., Zheng, X. Q., Hu, J. H., Jin, L. F. & Tang, L. L. (2020). Serum chitinase-3-like protein 1 is a biomarker of liver fibrosis in patients with chronic hepatitis B in China. *Hepatobiliary Pancreat Dis Int, 19*(4), 384-389. https://doi.org/10.1016/j.hbpd.2020.05.009

Johansen, J. S., Christoffersen, P., Møller, S., Price, P. A., Henriksen, J. H., Garbarsch, C. & Bendtsen, F. (2000). Serum YKL-40 is increased in patients with hepatic fibrosis. *Journal of Hepatology, 32*(6), 911-920. https://doi.org/https://doi.org/10.1016/S0168-8278(00)80095-1

Johansen, J. S., Olee, T., Price, P. A., Hashimoto, S., Ochs, R. L. & Lotz, M. (2001). Regulation of YKL-40 production by human articular chondrocytes. *Arthritis Rheum, 44*(4), 826-837. https://doi.org/10.1002/1529-0131(200104)44:4<826::Aid-anr139>3.0.Co;2-u

Kamle, S., Ma, B., He, C. H., Akosman, B., Zhou, Y., Lee, C. M., El-Deiry, W. S., Huntington, K., Liang, O., Machan, J. T., Kang, M. J., Shin, H. J., Mizoguchi, E., Lee, C. G. & Elias, J. A. (2021). Chitinase 3-like-1 is a therapeutic target that mediates the effects of aging in COVID-19. *JCI Insight, 6*(21). https://doi.org/10.1172/jci.insight.148749

Kamle, S., Ma, B., Lee, C. M., Schor, G., Zhou, Y., Lee, C. G. & Elias, J. A. (2022). Host chitinase 3-like-1 is a universal therapeutic target for SARS-CoV-2 viral variants in COVID-19. *Elife, 11*. https://doi.org/10.7554/eLife.78273

Kang, Q., Chen, J., Luo, H., Tan, N., Gao, H., Zhang, X., Yu, M., Liu, D., Xi, H., An, Y., Han, Y., Cheng, R. & Xu, X. (2020). Decrease in Chitinase 3-Like Protein 1 Levels Reflects Improvement in Liver Fibrosis after HCV Eradication. *Dis Markers, 2020*, 8539804. https://doi.org/10.1155/2020/8539804

Kang, Q., Xu, J., Luo, H., Tan, N., Chen, H., Cheng, R., Pan, J., Han, Y., Yang, Y., Liu, D., Xi, H., Yu, M. & Xu, X. (2021). Direct antiviral agent treatment leads to rapid and significant fibrosis regression after HCV eradication. *J Viral Hepat, 28*(9), 1284-1292. https://doi.org/10.1111/jvh.13558

Karki, R. & Kanneganti, T. D. (2021). The 'cytokine storm': molecular mechanisms and therapeutic prospects. *Trends Immunol, 42*(8), 681-705. https://doi.org/10.1016/j.it.2021.06.001

Karki, R. & Kanneganti, T. D. (2022). Innate immunity, cytokine storm, and inflammatory cell death in COVID-19. *J Transl Med, 20*(1), 542. https://doi.org/10.1186/s12967-022-03767-z

Karki, R., Lee, S., Mall, R., Pandian, N., Wang, Y., Sharma, B. R., Malireddi, R. S., Yang, D., Trifkovic, S., Steele, J. A., Connelly, J. P., Vishwanath, G., Sasikala,

M., Reddy, D. N., Vogel, P., Pruett-Miller, S. M., Webby, R., Jonsson, C. B. & Kanneganti, T. D. (2022). ZBP1-dependent inflammatory cell death, PANoptosis, and cytokine storm disrupt IFN therapeutic efficacy during coronavirus infection. *Sci Immunol, 7*(74), eabo6294. https://doi.org/10.1126/sciimmunol.abo6294

Karki, R., Sharma, B. R., Tuladhar, S., Williams, E. P., Zalduondo, L., Samir, P., Zheng, M., Sundaram, B., Banoth, B., Malireddi, R. K. S., Schreiner, P., Neale, G., Vogel, P., Webby, R., Jonsson, C. B. & Kanneganti, T. D. (2021). Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. *Cell, 184*(1), 149-168.e117. https://doi.org/10.1016/j.cell.2020.11.025

Karwelat, D., Schmeck, B., Ringel, M., Benedikter, B. J., Hübner, K., Beinborn, I., Maisner, A., Schulte, L. N. & Vollmeister, E. (2020). Influenza virus-mediated suppression of bronchial Chitinase-3-like 1 secretion promotes secondary pneumococcal infection. *Faseb j, 34*(12), 16432-16448. https://doi.org/10.1096/fj.201902988RR

Kim, M. J., Shim, D. H., Cha, H. R., Moon, K. Y., Yang, C. M., Hwang, S. J., Kim, K. W., Park, J. H., Lee, C. G., Elias, J. A., Sohn, M. H. & Lee, J. M. (2019). Chitinase 3-like 1 protein plays a critical role in respiratory syncytial virus-induced airway inflammation. *Allergy, 74*(4), 685-697. https://doi.org/10.1111/all.13661

Kjaergaard, A. D., Bojesen, S. E., Nordestgaard, B. G. & Johansen, J. S. (2014). YKL-40 and Alcoholic Liver and Pancreas Damage and Disease in 86258 Individuals from the General Population: Cohort and Mendelian Randomization Studies. *Clinical Chemistry, 60*(11), 1429-1440. https://doi.org/10.1373/clinchem.2014.229096

Kjaergaard, A. D., Helby, J., Johansen, J. S., Nordestgaard, B. G. & Bojesen, S. E. (2020). Elevated plasma YKL-40 and risk of infectious disease: a prospective study of 94665 individuals from the general population. *Clin Microbiol Infect, 26*(10), 1411.e1411-1411.e1419. https://doi.org/10.1016/j.cmi.2020.01.010

Klein, J., Wood, J., Jaycox, J. R., Dhodapkar, R. M., Lu, P., Gehlhausen, J. R., Tabachnikova, A., Greene, K., Tabacof, L., Malik, A. A., Silva Monteiro, V., Silva, J., Kamath, K., Zhang, M., Dhal, A., Ott, I. M., Valle, G., Peña-Hernández, M., Mao, T., Bhattacharjee, B., Takahashi, T., Lucas, C., Song, E., McCarthy, D., Breyman, E., Tosto-Mancuso, J., Dai, Y., Perotti, E., Akduman, K., Tzeng, T. J., Xu, L., Geraghty, A. C., Monje, M., Yildirim, I., Shon, J., Medzhitov, R., Lutchmansingh, D., Possick, J. D., Kaminski, N., Omer, S. B., Krumholz, H. M., Guan, L., Dela Cruz, C. S., van Dijk, D., Ring, A. M., Putrino, D. & Iwasaki, A. (2023). Distinguishing features of long COVID identified through immune profiling. *Nature, 623*(7985), 139-148. https://doi.org/10.1038/s41586-023-06651-y

Kleinnijenhuis, J., Quintin, J., Preijers, F., Benn, C. S., Joosten, L. A., Jacobs, C., van Loenhout, J., Xavier, R. J., Aaby, P., van der Meer, J. W., van Crevel, R. & Netea, M. G. (2014). Long-lasting effects of BCG vaccination on both

heterologous Th1/Th17 responses and innate trained immunity. *J Innate Immun, 6*(2), 152-158. https://doi.org/10.1159/000355628

Kleinnijenhuis, J., Quintin, J., Preijers, F., Joosten, L. A., Ifrim, D. C., Saeed, S., Jacobs, C., van Loenhout, J., de Jong, D., Stunnenberg, H. G., Xavier, R. J., van der Meer, J. W., van Crevel, R. & Netea, M. G. (2012). Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc Natl Acad Sci U S A, 109*(43), 17537-17542. https://doi.org/10.1073/pnas.1202870109

Kleinnijenhuis, J., Quintin, J., Preijers, F., Joosten, L. A., Jacobs, C., Xavier, R. J., van der Meer, J. W., van Crevel, R. & Netea, M. G. (2014). BCG-induced trained immunity in NK cells: Role for non-specific protection to infection. *Clin Immunol, 155*(2), 213-219. https://doi.org/10.1016/j.clim.2014.10.005

Knorr, T., Obermayr, F., Bartnik, E., Zien, A. & Aigner, T. (2003). YKL-39 (chitinase 3-like protein 2), but not YKL-40 (chitinase 3-like protein 1), is up regulated in osteoarthritic chondrocytes. *Ann Rheum Dis, 62*(10), 995-998. https://doi.org/10.1136/ard.62.10.995

Konradsen, J. R., James, A., Nordlund, B., Reinius, L. E., Söderhäll, C., Melén, E., Wheelock, A. M., Lödrup Carlsen, K. C., Lidegran, M., Verhoek, M., Boot, R. G., Dahlén, B., Dahlén, S. E. & Hedlin, G. (2013). The chitinase-like protein YKL-40: a possible biomarker of inflammation and airway remodeling in severe pediatric asthma. *J Allergy Clin Immunol, 132*(2), 328-335.e325. https://doi.org/10.1016/j.jaci.2013.03.003

Kruit, A., Grutters, J. C., Ruven, H. J., van Moorsel, C. C. & van den Bosch, J. M. (2007). A CHI3L1 gene polymorphism is associated with serum levels of YKL-40, a novel sarcoidosis marker. *Respir Med, 101*(7), 1563-1571. https://doi.org/10.1016/j.rmed.2006.12.006

Kumagai, E., Mano, Y., Yoshio, S., Shoji, H., Sugiyama, M., Korenaga, M., Ishida, T., Arai, T., Itokawa, N., Atsukawa, M., Hyogo, H., Chayama, K., Ohashi, T., Ito, K., Yoneda, M., Kawaguchi, T., Torimura, T., Nozaki, Y., Watanabe, S., Mizokami, M. & Kanto, T. (2016). Serum YKL-40 as a marker of liver fibrosis in patients with non-alcoholic fatty liver disease. *Sci Rep, 6*, 35282. https://doi.org/10.1038/srep35282

Kzhyshkowska, J., Gratchev, A. & Goerdt, S. (2007). Human chitinases and chitinase-like proteins as indicators for inflammation and cancer. *Biomark Insights, 2*, 128-146.

Kzhyshkowska, J., Gudima, A., Riabov, V., Dollinger, C., Lavalle, P. & Vrana, N. E. (2015). Macrophage responses to implants: prospects for personalized medicine. *Journal of Leukocyte Biology, 98*(6), 953-962. https://doi.org/10.1189/jlb.5VMR0415-166R

Kzhyshkowska, J., Mamidi, S., Gratchev, A., Kremmer, E., Schmuttermaier, C., Krusell, L., Haus, G., Utikal, J., Schledzewski, K., Scholtze, J. & Goerdt, S. (2006). Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in

alternatively activated macrophages and secreted via lysosomal pathway. *Blood, 107*(8), 3221-3228. https://doi.org/https://doi.org/10.1182/blood-2005-07-2843

Kzhyshkowska, J., Mamidi, S., Gratchev, A., Kremmer, E., Schmuttermaier, C., Krusell, L., Haus, G., Utikal, J., Schledzewski, K., Scholtze, J. & Goerdt, S. (2006). Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway. *Blood, 107*(8), 3221-3228. https://doi.org/10.1182/blood-2005-07-2843

Kzhyshkowska, J., Yin, S., Liu, T., Riabov, V. & Mitrofanova, I. (2016). Role of chitinase-like proteins in cancer. *Biol Chem, 397*(3), 231-247. https://doi.org/10.1515/hsz-2015-0269

Lai, C. C., Hsu, C. K., Yen, M. Y., Lee, P. I., Ko, W. C. & Hsueh, P. R. (2023). Long COVID: An inevitable sequela of SARS-CoV-2 infection. *J Microbiol Immunol Infect, 56*(1), 1-9. https://doi.org/10.1016/j.jmii.2022.10.003

Lananna, B. V., McKee, C. A., King, M. W., Del-Aguila, J. L., Dimitry, J. M., Farias, F. H. G., Nadarajah, C. J., Xiong, D. D., Guo, C., Cammack, A. J., Elias, J. A., Zhang, J., Cruchaga, C. & Musiek, E. S. (2020). Chi3l1/YKL-40 is controlled by the astrocyte circadian clock and regulates neuroinflammation and Alzheimer's disease pathogenesis. *Sci Transl Med, 12*(574). https://doi.org/10.1126/scitranslmed.aax3519

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S. & Amit, I. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell, 159*(6), 1312-1326. https://doi.org/10.1016/j.cell.2014.11.018

Lee, C. G. (2009). Chitin, chitinases and chitinase-like proteins in allergic inflammation and tissue remodeling. *Yonsei Med J, 50*(1), 22-30. https://doi.org/10.3349/ymj.2009.50.1.22

Lee, C. G., Da Silva, C. A., Dela Cruz, C. S., Ahangari, F., Ma, B., Kang, M. J., He, C. H., Takyar, S. & Elias, J. A. (2011). Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. *Annu Rev Physiol, 73*, 479-501. https://doi.org/10.1146/annurev-physiol-012110-142250

Lee, C. G., Dela Cruz, C. S., Herzog, E., Rosenberg, S. M., Ahangari, F. & Elias, J. A. (2012). YKL-40, a chitinase-like protein at the intersection of inflammation and remodeling. *Am J Respir Crit Care Med, 185*(7), 692-694. https://doi.org/10.1164/rccm.201202-0203ED

Lee, C. G. & Elias, J. A. (2010). Role of breast regression protein-39/YKL-40 in asthma and allergic responses. *Allergy Asthma Immunol Res, 2*(1), 20-27. https://doi.org/10.4168/aair.2010.2.1.20

Lee, C. G., Hartl, D., Lee, G. R., Koller, B., Matsuura, H., Da Silva, C. A., Sohn, M. H., Cohn, L., Homer, R. J., Kozhich, A. A., Humbles, A., Kearley, J., Coyle, A., Chupp, G., Reed, J., Flavell, R. A. & Elias, J. A. (2009). Role of breast

regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. *J Exp Med, 206*(5), 1149-1166. https://doi.org/10.1084/jem.20081271

Lee, J. W., Kim, M. N., Kim, E. G., Leem, J. S., Baek, S. M., Kim, M. J., Kim, K. W. & Sohn, M. H. (2022). Chitinase 3-like 1 is involved in the induction of IL-8 expression by double-stranded RNA in airway epithelial cells. *Biochem Biophys Res Commun, 592*, 106-112. https://doi.org/10.1016/j.bbrc.2022.01.008

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K. S. M., Lau, E. H. Y., Wong, J. Y., Xing, X., Xiang, N., Wu, Y., Li, C., Chen, Q., Li, D., Liu, T., Zhao, J., Liu, M., Tu, W., Chen, C., Jin, L., Yang, R., Wang, Q., Zhou, S., Wang, R., Liu, H., Luo, Y., Liu, Y., Shao, G., Li, H., Tao, Z., Yang, Y., Deng, Z., Liu, B., Ma, Z., Zhang, Y., Shi, G., Lam, T. T. Y., Wu, J. T., Gao, G. F., Cowling, B. J., Yang, B., Leung, G. M. & Feng, Z. (2020). Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N Engl J Med, 382*(13), 1199-1207. https://doi.org/10.1056/NEJMoa2001316

Li, Z., Chen, X., Dan, J., Hu, T., Hu, Y., Liu, S., Chai, Y., Shi, Y., Wu, J., Ni, H., Zhu, J., Wu, Y., Li, N., Yu, Y., Wang, Z., Zhao, J., Zhong, N., Ren, X., Shen, Z. & Cao, X. (2022). Innate immune imprints in SARS-CoV-2 Omicron variant infection convalescents. *Signal Transduct Target Ther, 7*(1), 377. https://doi.org/10.1038/s41392-022-01237-y

Liu, P. P., Blet, A., Smyth, D. & Li, H. (2020). The Science Underlying COVID-19: Implications for the Cardiovascular System. *Circulation, 142*(1), 68-78. https://doi.org/10.1161/circulationaha.120.047549

Liu, Q., Mak, J. W. Y., Su, Q., Yeoh, Y. K., Lui, G. C., Ng, S. S. S., Zhang, F., Li, A. Y. L., Lu, W., Hui, D. S., Chan, P. K., Chan, F. K. L. & Ng, S. C. (2022). Gut microbiota dynamics in a prospective cohort of patients with post-acute COVID-19 syndrome. *Gut, 71*(3), 544-552. https://doi.org/10.1136/gutjnl-2021-325989

Lowery, S. A., Sariol, A. & Perlman, S. (2021). Innate immune and inflammatory responses to SARS-CoV-2: Implications for COVID-19. *Cell Host Microbe, 29*(7), 1052-1062. https://doi.org/10.1016/j.chom.2021.05.004

Lucas, C., Wong, P., Klein, J., Castro, T. B. R., Silva, J., Sundaram, M., Ellingson, M. K., Mao, T., Oh, J. E., Israelow, B., Takahashi, T., Tokuyama, M., Lu, P., Venkataraman, A., Park, A., Mohanty, S., Wang, H., Wyllie, A. L., Vogels, C. B. F., Earnest, R., Lapidus, S., Ott, I. M., Moore, A. J., Muenker, M. C., Fournier, J. B., Campbell, M., Odio, C. D., Casanovas-Massana, A., Herbst, R., Shaw, A. C., Medzhitov, R., Schulz, W. L., Grubaugh, N. D., Dela Cruz, C., Farhadian, S., Ko, A. I., Omer, S. B. & Iwasaki, A. (2020). Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature, 584*(7821), 463-469. https://doi.org/10.1038/s41586-020-2588-y

Madden, N., Mei, Y. Z. J., Jakubiak, K., Li, J., Hargus, G., Goldman, J. E. & Al-Dalahmah, O. (2023). The link between SARS-CoV-2 related microglial reactivity and astrocyte pathology in the inferior olivary nucleus. *Front Neurosci, 17*, 1198219. https://doi.org/10.3389/fnins.2023.1198219

Mann, E. R., Menon, M., Knight, S. B., Konkel, J. E., Jagger, C., Shaw, T. N., Krishnan, S., Rattray, M., Ustianowski, A., Bakerly, N. D., Dark, P., Lord, G., Simpson, A., Felton, T., Ho, L. P., Feldmann, M., Grainger, J. R. & Hussell, T. (2020). Longitudinal immune profiling reveals key myeloid signatures associated with COVID-19. *Sci Immunol, 5*(51). https://doi.org/10.1126/sciimmunol.abd6197

Mao, L., Jin, H., Wang, M., Hu, Y., Chen, S., He, Q., Chang, J., Hong, C., Zhou, Y., Wang, D., Miao, X., Li, Y. & Hu, B. (2020). Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol, 77*(6), 683-690. https://doi.org/10.1001/jamaneurol.2020.1127

Marshall, M. (2021). COVID's toll on smell and taste: what scientists do and don't know. *Nature, 589*(7842), 342-343. https://doi.org/10.1038/d41586-021-00055-6

Matschke, J., Lütgehetmann, M., Hagel, C., Sperhake, J. P., Schröder, A. S., Edler, C., Mushumba, H., Fitzek, A., Allweiss, L., Dandri, M., Dottermusch, M., Heinemann, A., Pfefferle, S., Schwabenland, M., Sumner Magruder, D., Bonn, S., Prinz, M., Gerloff, C., Püschel, K., Krasemann, S., Aepfelbacher, M. & Glatzel, M. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. (1474-4465 (Electronic)).

Matsuura, H., Hartl, D., Kang, M. J., Dela Cruz, C. S., Koller, B., Chupp, G. L., Homer, R. J., Zhou, Y., Cho, W. K., Elias, J. A. & Lee, C. G. (2011). Role of breast regression protein-39 in the pathogenesis of cigarette smoke-induced inflammation and emphysema. *Am J Respir Cell Mol Biol, 44*(6), 777-786. https://doi.org/10.1165/rcmb.2010-0081OC

Mazur, M., Zielińska, A., Grzybowski, M. M., Olczak, J. & Fichna, J. (2021). Chitinases and Chitinase-Like Proteins as Therapeutic Targets in Inflammatory Diseases, with a Special Focus on Inflammatory Bowel Diseases. *Int J Mol Sci, 22*(13). https://doi.org/10.3390/ijms22136966

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S. & Manson, J. J. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet, 395*(10229), 1033-1034. https://doi.org/10.1016/s0140-6736(20)30628-0

Merad, M. & Martin, J. C. (2020). Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol, 20*(6), 355-362. https://doi.org/10.1038/s41577-020-0331-4

Merkler, A. E., Parikh, N. S., Mir, S., Gupta, A., Kamel, H., Lin, E., Lantos, J., Schenck, E. J., Goyal, P., Bruce, S. S., Kahan, J., Lansdale, K. N., LeMoss, N. M., Murthy, S. B., Stieg, P. E., Fink, M. E., Iadecola, C., Segal, A. Z., Cusick, M., Campion, T. R., Jr., Diaz, I., Zhang, C. & Navi, B. B. (2020). Risk of Ischemic Stroke in Patients With Coronavirus Disease 2019 (COVID-19) vs Patients With Influenza. *JAMA Neurol, 77*(11), 1-7. https://doi.org/10.1001/jamaneurol.2020.2730

Millis, A. J., Hoyle, M., Reich, E. & Mann, D. M. (1985). Isolation and characterization of a Mr = 38,000 protein from differentiating smooth muscle cells. *J Biol Chem, 260*(6), 3754-3761.

Mitroulis, I., Ruppova, K., Wang, B., Chen, L.-S., Grzybek, M., Grinenko, T., Eugster, A., Troullinaki, M., Palladini, A., Kourtzelis, I., Chatzigeorgiou, A., Schlitzer, A., Beyer, M., Joosten, L. A. B., Isermann, B., Lesche, M., Petzold, A., Simons, K., Henry, I., Dahl, A., Schultze, J. L., Wielockx, B., Zamboni, N., Mirtschink, P., Coskun, Ü., Hajishengallis, G., Netea, M. G. & Chavakis, T. (2018). Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity. *Cell, 172*(1), 147-161.e112. https://doi.org/https://doi.org/10.1016/j.cell.2017.11.034

Mulchandani, R., Lyngdoh, T. & Kakkar, A. K. (2021). Deciphering the COVID ‐ 19 cytokine storm: systematic review and meta ‐ analysis. *European journal of clinical investigation, 51*(1), e13429.

Müller, R., Rink, G., Uzun, G., Bakchoul, T., Wuchter, P., Klüter, H. & Bugert, P. (2022). Increased plasma level of soluble P-selectin in non-hospitalized COVID-19 convalescent donors. *Thromb Res, 216*, 120-124. https://doi.org/10.1016/j.thromres.2022.06.014

Murakami, M., Kamimura, D. & Hirano, T. (2019). Pleiotropy and Specificity: Insights from the Interleukin 6 Family of Cytokines. *Immunity, 50*(4), 812-831. https://doi.org/10.1016/j.immuni.2019.03.027

Murphy, S. L., Halvorsen, B., Holter, J. C., Huse, C., Tveita, A., Trøseid, M., Hoel, H., Kildal, A. B., Holten, A. R., Lerum, T. V., Skjønsberg, O. H., Michelsen, A. E., Aaløkken, T. M., Tonby, K., Lind, A., Dudman, S., Granerud, B. K., Heggelund, L., Bøe, S., Dyrholt-Riise, A. M., Aukrust, P., Barratt-Due, A., Ueland, T. & Dahl, T. B. (2023). Circulating markers of extracellular matrix remodelling in severe COVID-19 patients. *J Intern Med, 294*(6), 784-797. https://doi.org/10.1111/joim.13725

Naka, K. K., Papathanassiou, K., Bechlioulis, A., Pappas, K., Tigas, S., Makriyiannis, D., Antoniou, S., Kazakos, N., Margeli, A., Papassotiriou, I., Tsatsoulis, A. & Michalis, L. K. (2018). Association of vascular indices with novel circulating biomarkers as prognostic factors for cardiovascular complications in patients with type 2 diabetes mellitus. *Clinical Biochemistry, 53*, 31-37. https://doi.org/https://doi.org/10.1016/j.clinbiochem.2017.12.010

Netea, M. G., Domínguez-Andrés, J., Barreiro, L. B., Chavakis, T., Divangahi, M., Fuchs, E., Joosten, L. A. B., van der Meer, J. W. M., Mhlanga, M. M., Mulder, W. J. M., Riksen, N. P., Schlitzer, A., Schultze, J. L., Stabell Benn, C., Sun, J. C., Xavier, R. J. & Latz, E. (2020). Defining trained immunity and its role in health and disease. *Nat Rev Immunol, 20*(6), 375-388. https://doi.org/10.1038/s41577-020-0285-6

Netea, M. G., Joosten, L. A., Latz, E., Mills, K. H., Natoli, G., Stunnenberg, H. G., O'Neill, L. A. & Xavier, R. J. (2016). Trained immunity: A program of innate immune memory in health and disease. *Science, 352*(6284), aaf1098. https://doi.org/10.1126/science.aaf1098

Netea, M. G., Quintin, J. & van der Meer, J. W. (2011). Trained immunity: a memory for innate host defense. *Cell Host Microbe, 9*(5), 355-361. https://doi.org/10.1016/j.chom.2011.04.006

Nishimura, N., De Battista, D., McGivern, D. R., Engle, R. E., Tice, A., Fares-Gusmao, R., Kabat, J., Pomerenke, A., Nguyen, H., Sato, S., Bock, K. W., Moore, I. N., Kleiner, D. E., Zamboni, F., Alter, H. J., Govindarajan, S. & Farci, P. (2021). Chitinase 3-like 1 is a profibrogenic factor overexpressed in the aging liver and in patients with liver cirrhosis. *Proc Natl Acad Sci U S A, 118*(17). https://doi.org/10.1073/pnas.2019633118

Nøjgaard, C., Høst, N. B., Christensen, I. J., Poulsen, S. H., Egstrup, K., Price, P. A. & Johansen, J. S. (2008). Serum levels of YKL-40 increases in patients with acute myocardial infarction. *Coron Artery Dis, 19*(4), 257-263. https://doi.org/10.1097/MCA.0b013e3282f40dd5

Ochando, J., Mulder, W. J. M., Madsen, J. C., Netea, M. G. & Duivenvoorden, R. (2023). Trained immunity — basic concepts and contributions to immunopathology. *Nature Reviews Nephrology, 19*(1), 23-37. https://doi.org/10.1038/s41581-022-00633-5

Ombrello, M. J. & Schulert, G. S. (2021). COVID-19 and cytokine storm syndrome: are there lessons from macrophage activation syndrome? *Transl Res, 232*, 1-12. https://doi.org/10.1016/j.trsl.2021.03.002

Outinen, T. K., Mantula, P., Jaatinen, P., Hämäläinen, M., Moilanen, E., Vaheri, A., Huhtala, H., Mäkelä, S. & Mustonen, J. (2019). Glycoprotein YKL-40 Is Elevated and Predicts Disease Severity in Puumala Hantavirus Infection. *Viruses, 11*(9). https://doi.org/10.3390/v11090767

Pandolfi, L., Fossali, T., Frangipane, V., Bozzini, S., Morosini, M., D'Amato, M., Lettieri, S., Urtis, M., Di Toro, A., Saracino, L., Percivalle, E., Tomaselli, S., Cavagna, L., Cova, E., Mojoli, F., Bergomi, P., Ottolina, D., Lilleri, D., Corsico, A. G., Arbustini, E., Colombo, R. & Meloni, F. (2020). Broncho-alveolar inflammation in COVID-19 patients: a correlation with clinical outcome. *BMC Pulm Med, 20*(1), 301. https://doi.org/10.1186/s12890-020-01343-z

Park, A. & Iwasaki, A. (2020). Type I and Type III Interferons - Induction, Signaling, Evasion, and Application to Combat COVID-19. *Cell Host Microbe, 27*(6), 870-878. https://doi.org/10.1016/j.chom.2020.05.008

Park, J., Dean, L. S., Jiyarom, B., Gangcuangco, L. M., Shah, P., Awamura, T., Ching, L. L., Nerurkar, V. R., Chow, D. C., Igno, F., Shikuma, C. M. & Devendra, G. (2023). Elevated circulating monocytes and monocyte activation in COVID-19 convalescent individuals. *Front Immunol, 14*, 1151780. https://doi.org/10.3389/fimmu.2023.1151780

Parlak, E. & Laloğlu, E. (2022). Analysis of Chitinase-3-Like Protein 1, IL-1-Alpha, and IL-6 as Novel Inflammatory Biomarkers for COVID-19. *J Interferon Cytokine Res, 42*(10), 536-541. https://doi.org/10.1089/jir.2022.0065

Pelloski, C. E., Mahajan, A., Maor, M., Chang, E. L., Woo, S., Gilbert, M., Colman, H., Yang, H., Ledoux, A., Blair, H., Passe, S., Jenkins, R. B. & Aldape, K. D. (2005). YKL-40 expression is associated with poorer response to radiation and shorter overall survival in glioblastoma. *Clin Cancer Res, 11*(9), 3326-3334. https://doi.org/10.1158/1078-0432.Ccr-04-1765

Peluso, M. J. & Deeks, S. G. (2022). Early clues regarding the pathogenesis of long-COVID. *Trends Immunol, 43*(4), 268-270. https://doi.org/10.1016/j.it.2022.02.008

Phetsouphanh, C., Darley, D. R., Wilson, D. B., Howe, A., Munier, C. M. L., Patel, S. K., Juno, J. A., Burrell, L. M., Kent, S. J., Dore, G. J., Kelleher, A. D. & Matthews, G. V. (2022). Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. *Nat Immunol, 23*(2), 210-216. https://doi.org/10.1038/s41590-021-01113-x

Pilotto, A., Masciocchi, S., Volonghi, I., De Giuli, V., Caprioli, F., Mariotto, S., Ferrari, S., Bozzetti, S., Imarisio, A., Risi, B., Premi, E., Benussi, A., Focà, E., Castelli, F., Zanusso, G., Monaco, S., Stefanelli, P., Gasparotti, R., Zekeridou, A., McKeon, A., Ashton, N. J., Blenno, W. K., Zetterberg, H. & Padovani, A. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Encephalitis Is a Cytokine Release Syndrome: Evidences From Cerebrospinal Fluid Analyses. (1537-6591 (Electronic)).

Pretorius, E., Venter, C., Laubscher, G. J., Kotze, M. J., Oladejo, S. O., Watson, L. R., Rajaratnam, K., Watson, B. W. & Kell, D. B. (2022). Prevalence of symptoms, comorbidities, fibrin amyloid microclots and platelet pathology in individuals with Long COVID/Post-Acute Sequelae of COVID-19 (PASC). *Cardiovasc Diabetol, 21*(1), 148. https://doi.org/10.1186/s12933-022-01579-5

Proal, A. D. & VanElzakker, M. B. (2021). Long COVID or Post-acute Sequelae of COVID-19 (PASC): An Overview of Biological Factors That May Contribute to Persistent Symptoms. *Front Microbiol, 12*, 698169. https://doi.org/10.3389/fmicb.2021.698169

Qudus, M. S., Tian, M., Sirajuddin, S., Liu, S., Afaq, U., Wali, M., Liu, J., Pan, P., Luo, Z., Zhang, Q., Yang, G., Wan, P., Li, Y. & Wu, J. (2023). The roles of critical pro-inflammatory cytokines in the drive of cytokine storm during SARS-CoV-2 infection. *J Med Virol, 95*(4), e28751. https://doi.org/10.1002/jmv.28751

Rajamanickam, A., Kumar, N. P., Pandiarajan, A. N., Selvaraj, N., Munisankar, S., Renji, R. M., Venkatramani, V., Murhekar, M., Thangaraj, J. W. V., Kumar, M. S., Kumar, C. P. G., Bhatnagar, T., Ponnaiah, M., Sabarinathan, R., Saravanakumar, V. & Babu, S. (2021). Dynamic alterations in monocyte numbers, subset frequencies and activation markers in acute and convalescent COVID-19 individuals. *Sci Rep, 11*(1), 20254. https://doi.org/10.1038/s41598-021-99705-y

Ramasamy, S. & Subbian, S. (2021). Critical Determinants of Cytokine Storm and Type I Interferon Response in COVID-19 Pathogenesis. *Clin Microbiol Rev, 34*(3). https://doi.org/10.1128/cmr.00299-20

Reese, T. A., Liang, H. E., Tager, A. M., Luster, A. D., Van Rooijen, N., Voehringer, D. & Locksley, R. M. (2007). Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature, 447*(7140), 92-96. https://doi.org/10.1038/nature05746

Rehli, M., Krause, S. W. & Andreesen, R. (1997). Molecular characterization of the gene for human cartilage gp-39 (CHI3L1), a member of the chitinase protein family and marker for late stages of macrophage differentiation. *Genomics, 43*(2), 221-225. https://doi.org/10.1006/geno.1997.4778

Rehli, M., Niller, H. H., Ammon, C., Langmann, S., Schwarzfischer, L., Andreesen, R. & Krause, S. W. (2003). Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. *J Biol Chem, 278*(45), 44058-44067. https://doi.org/10.1074/jbc.M306792200

Remuzzi, A. & Remuzzi, G. (2020). COVID-19 and Italy: what next? *Lancet, 395*(10231), 1225-1228. https://doi.org/10.1016/s0140-6736(20)30627-9

Richter, B., Roslind, A., Hesse, U., Nordling, J., Johansen, J. S., Horn, T. & Hansen, A. B. (2010). YKL-40 and mast cells are associated with detrusor fibrosis in patients diagnosed with bladder pain syndrome/interstitial cystitis according to the 2008 criteria of the European Society for the Study of Interstitial Cystitis. *Histopathology, 57*(3), 371-383. https://doi.org/10.1111/j.1365-2559.2010.03640.x

Ryan, F. J., Hope, C. M., Masavuli, M. G., Lynn, M. A., Mekonnen, Z. A., Yeow, A. E. L., Garcia-Valtanen, P., Al-Delfi, Z., Gummow, J., Ferguson, C., O'Connor, S., Reddi, B. A. J., Hissaria, P., Shaw, D., Kok-Lim, C., Gleadle, J. M., Beard, M. R., Barry, S. C., Grubor-Bauk, B. & Lynn, D. J. (2022). Long-term perturbation of the peripheral immune system months after SARS-CoV-2 infection. *BMC Med, 20*(1), 26. https://doi.org/10.1186/s12916-021-02228-6

Saeed, S., Quintin, J., Kerstens, H. H., Rao, N. A., Aghajanirefah, A., Matarese, F., Cheng, S. C., Ratter, J., Berentsen, K., van der Ent, M. A., Sharifi, N., Janssen-Megens, E. M., Ter Huurne, M., Mandoli, A., van Schaik, T., Ng, A., Burden, F., Downes, K., Frontini, M., Kumar, V., Giamarellos-Bourboulis, E. J., Ouwehand, W. H., van der Meer, J. W., Joosten, L. A., Wijmenga, C., Martens, J. H., Xavier, R. J., Logie, C., Netea, M. G. & Stunnenberg, H. G. (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science, 345*(6204), 1251086. https://doi.org/10.1126/science.1251086

Sampaio Rocha-Filho, P. A., Albuquerque, P. M., Carvalho, L., Dandara Pereira Gama, M. & Magalhães, J. E. Headache, anosmia, ageusia and other neurological symptoms in COVID-19: a cross-sectional study. (1129-2377 (Electronic)).

Sarma, N. J., Tiriveedhi, V., Subramanian, V., Shenoy, S., Crippin, J. S., Chapman, W. C. & Mohanakumar, T. (2012). Hepatitis C virus mediated changes in miRNA-449a modulates inflammatory biomarker YKL40 through components of the NOTCH signaling pathway. *PLoS One,* *7*(11), e50826. https://doi.org/10.1371/journal.pone.0050826

Sawatzky, J., Soo, J., Conroy, A. L., Bhargava, R., Namasopo, S., Opoka, R. O. & Hawkes, M. T. (2019). Biomarkers of Systemic Inflammation in Ugandan Infants and Children Hospitalized With Respiratory Syncytial Virus Infection. *Pediatr Infect Dis J, 38*(8), 854-859. https://doi.org/10.1097/inf.0000000000002343

Schoneveld, L., Ladang, A., Henket, M., Frix, A. N., Cavalier, E. & Guiot, J. (2021). YKL-40 as a new promising prognostic marker of severity in COVID infection. *Crit Care, 25*(1), 66. https://doi.org/10.1186/s13054-020-03383-7

Schulte-Schrepping, J., Reusch, N., Paclik, D., Baßler, K., Schlickeiser, S., Zhang, B., Krämer, B., Krammer, T., Brumhard, S., Bonaguro, L., De Domenico, E., Wendisch, D., Grasshoff, M., Kapellos, T. S., Beckstette, M., Pecht, T., Saglam, A., Dietrich, O., Mei, H. E., Schulz, A. R., Conrad, C., Kunkel, D., Vafadarnejad, E., Xu, C. J., Horne, A., Herbert, M., Drews, A., Thibeault, C., Pfeiffer, M., Hippenstiel, S., Hocke, A., Müller-Redetzky, H., Heim, K. M., Machleidt, F., Uhrig, A., Bosquillon de Jarcy, L., Jürgens, L., Stegemann, M., Glösenkamp, C. R., Volk, H. D., Goffinet, C., Landthaler, M., Wyler, E., Georg, P., Schneider, M., Dang-Heine, C., Neuwinger, N., Kappert, K., Tauber, R., Corman, V., Raabe, J., Kaiser, K. M., Vinh, M. T., Rieke, G., Meisel, C., Ulas, T., Becker, M., Geffers, R., Witzenrath, M., Drosten, C., Suttorp, N., von Kalle, C., Kurth, F., Händler, K., Schultze, J. L., Aschenbrenner, A. C., Li, Y., Nattermann, J., Sawitzki, B., Saliba, A. E. & Sander, L. E. (2020). Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. *Cell,* *182*(6), 1419-1440.e1423. https://doi.org/10.1016/j.cell.2020.08.001

Schultze, J. L. & Aschenbrenner, A. C. (2021). COVID-19 and the human innate immune system. *Cell,* *184*(7), 1671-1692. https://doi.org/10.1016/j.cell.2021.02.029

Scott, N. A., Pearmain, L., Knight, S. B., Brand, O., Morgan, D. J., Jagger, C., Harbach, S., Khan, S., Shuwa, H. A., Franklin, M., Kästele, V., Williams, T., Prise, I., McClure, F. A., Hackney, P., Smith, L., Menon, M., Konkel, J. E., Lawless, C., Wilson, J., Mathioudakis, A. G., Stanel, S. C., Ustianowski, A., Lindergard, G., Brij, S., Diar Bakerly, N., Dark, P., Brightling, C., Rivera-Ortega, P., Lord, G. M., Horsley, A., Piper Hanley, K., Felton, T., Simpson, A., Grainger, J. R., Hussell, T. & Mann, E. R. (2023). Monocyte migration profiles define disease severity in acute COVID-19 and unique features of long COVID. *Eur Respir J, 61*(5). https://doi.org/10.1183/13993003.02226-2022

Sohn, M. H., Kang, M. J., Matsuura, H., Bhandari, V., Chen, N. Y., Lee, C. G. & Elias, J. A. (2010). The chitinase-like proteins breast regression protein-39 and YKL-40 regulate hyperoxia-induced acute lung injury. *Am J Respir Crit Care Med, 182*(7), 918-928. https://doi.org/10.1164/rccm.200912-1793OC

Soy, M., Keser, G., Atagündüz, P., Tabak, F., Atagündüz, I. & Kayhan, S. (2020). Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. *Clin Rheumatol, 39*(7), 2085-2094. https://doi.org/10.1007/s10067-020-05190-5

Spudich, S. & Nath, A. (2022). Nervous system consequences of COVID-19. *Science, 375*(6578), 267-269. https://doi.org/10.1126/science.abm2052

Su, Y., Yuan, D., Chen, D. G., Ng, R. H., Wang, K., Choi, J., Li, S., Hong, S., Zhang, R., Xie, J., Kornilov, S. A., Scherler, K., Pavlovitch-Bedzyk, A. J., Dong, S., Lausted, C., Lee, I., Fallen, S., Dai, C. L., Baloni, P., Smith, B., Duvvuri, V. R., Anderson, K. G., Li, J., Yang, F., Duncombe, C. J., McCulloch, D. J., Rostomily, C., Troisch, P., Zhou, J., Mackay, S., DeGottardi, Q., May, D. H., Taniguchi, R., Gittelman, R. M., Klinger, M., Snyder, T. M., Roper, R., Wojciechowska, G., Murray, K., Edmark, R., Evans, S., Jones, L., Zhou, Y., Rowen, L., Liu, R., Chour, W., Algren, H. A., Berrington, W. R., Wallick, J. A., Cochran, R. A., Micikas, M. E., Wrin, T., Petropoulos, C. J., Cole, H. R., Fischer, T. D., Wei, W., Hoon, D. S. B., Price, N. D., Subramanian, N., Hill, J. A., Hadlock, J., Magis, A. T., Ribas, A., Lanier, L. L., Boyd, S. D., Bluestone, J. A., Chu, H., Hood, L., Gottardo, R., Greenberg, P. D., Davis, M. M., Goldman, J. D. & Heath, J. R. (2022). Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell, 185*(5), 881-895.e820. https://doi.org/10.1016/j.cell.2022.01.014

Sutherland, T. E. (2018). Chitinase-like proteins as regulators of innate immunity and tissue repair: helpful lessons for asthma? *Biochemical Society Transactions, 46*(1), 141-151. https://doi.org/10.1042/BST20170108

Swank, Z., Senussi, Y., Manickas-Hill, Z., Yu, X. G., Li, J. Z., Alter, G. & Walt, D. R. (2023). Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike Is Associated With Post-acute Coronavirus Disease 2019 Sequelae. *Clin Infect Dis, 76*(3), e487-e490. https://doi.org/10.1093/cid/ciac722

Syed, M. A. & Bhandari, V. (2013). Hyperoxia exacerbates postnatal inflammation-induced lung injury in neonatal BRP-39 null mutant mice promoting the M1 macrophage phenotype. *Mediators Inflamm, 2013*, 457189. https://doi.org/10.1155/2013/457189

Syed, U., Subramanian, A., Wraith, D. C., Lord, J. M., McGee, K., Ghokale, K., Nirantharakumar, K. & Haroon, S. (2023). Incidence of immune-mediated inflammatory diseases following COVID-19: a matched cohort study in UK primary care. *BMC Med, 21*(1), 363. https://doi.org/10.1186/s12916-023-03049-5

Tay, M. Z., Poh, C. M., Rénia, L., Macary, P. A. & Ng, L. F. P. (2020). The trinity of COVID-19: immunity, inflammation and intervention. *Nature Reviews Immunology, 20*(6), 363-374. https://doi.org/10.1038/s41577-020-0311-8

Tercan, H., Riksen, N. P., Joosten, L. A. B., Netea, M. G. & Bekkering, S. (2021). Trained Immunity: Long-Term Adaptation in Innate Immune Responses. *Arterioscler Thromb Vasc Biol, 41*(1), 55-61. https://doi.org/10.1161/atvbaha.120.314212

Tso, W. W., Kwan, M. Y., Kwok, J. S., Tsang, J. O., Yip, C. C., Leung, L. K., Li, C., Wang, Y., Chow, M. S., Tsang, A. M., Chim, S., Chow, C. Y., Ho, A. C., Chan, S. H., Tai, S. M., Lee, W. C., Chan, V. C., Yau, E. K., Sun, J. K., Chow, H. M., Lau, Y. L., Ip, P. & Chan, J. F. (2023). Clinical characteristics of unvaccinated or incompletely vaccinated children with neurological manifestations due to SARS-CoV-2 Omicron infection. *J Med Virol, 95*(7), e28895. https://doi.org/10.1002/jmv.28895

Ueland, T., Laugsand, L. E., Vatten, L. J., Janszky, I., Platou, C., Michelsen, A. E., Damås, J. K., Aukrust, P. & Åsvold, B. O. (2020). Extracellular matrix markers and risk of myocardial infarction: The HUNT Study in Norway. *European Journal of Preventive Cardiology, 24*(11), 1161-1167. https://doi.org/10.1177/2047487317703826

Ural, U. M., Tekin, Y. B., Cüre, M. & Şahin, F. K. (2015). Serum YKL-40 levels as a novel marker of inflammation in patients with endometriosis. *Clin Exp Obstet Gynecol, 42*(4), 495-497.

Väänänen, T., Kallio, J., Vuolteenaho, K., Ojala, A., Luukkaala, T., Hämäläinen, M., Tammela, T., Kellokumpu-Lehtinen, P. L. & Moilanen, E. (2017). High YKL-40 is associated with poor survival in patients with renal cell carcinoma: a novel independent prognostic marker. *Scand J Urol, 51*(5), 367-372. https://doi.org/10.1080/21681805.2017.1327885

Vabret, N., Britton, G. J., Gruber, C., Hegde, S., Kim, J., Kuksin, M., Levantovsky, R., Malle, L., Moreira, A., Park, M. D., Pia, L., Risson, E., Saffern, M., Salomé, B., Esai Selvan, M., Spindler, M. P., Tan, J., van der Heide, V., Gregory, J. K., Alexandropoulos, K., Bhardwaj, N., Brown, B. D., Greenbaum, B., Gümüş, Z. H., Homann, D., Horowitz, A., Kamphorst, A. O., Curotto de Lafaille, M. A., Mehandru, S., Merad, M. & Samstein, R. M. (2020). Immunology of COVID-19: Current State of the Science. *Immunity, 52*(6), 910-941. https://doi.org/10.1016/j.immuni.2020.05.002

Vanderbeke, L., Van Mol, P., Van Herck, Y., De Smet, F., Humblet-Baron, S., Martinod, K., Antoranz, A., Arijs, I., Boeckx, B., Bosisio, F. M., Casaer, M., Dauwe, D., De Wever, W., Dooms, C., Dreesen, E., Emmaneel, A., Filtjens, J., Gouwy, M., Gunst, J., Hermans, G., Jansen, S., Lagrou, K., Liston, A., Lorent, N., Meersseman, P., Mercier, T., Neyts, J., Odent, J., Panovska, D., Penttila, P. A., Pollet, E., Proost, P., Qian, J., Quintelier, K., Raes, J., Rex, S., Saeys, Y., Sprooten, J., Tejpar, S., Testelmans, D., Thevissen, K., Van Buyten, T., Vandenhaute, J., Van Gassen, S., Velásquez Pereira, L. C., Vos, R., Weynand, B., Wilmer, A., Yserbyt, J., Garg, A. D., Matthys, P., Wouters, C., Lambrechts, D., Wauters, E. & Wauters, J. (2021). Monocyte-driven atypical cytokine storm and aberrant neutrophil activation as key mediators of COVID-19 disease severity. *Nat Commun, 12*(1), 4117. https://doi.org/10.1038/s41467-021-24360-w

Vetter, P., Vu, D. L., L'Huillier, A. G., Schibler, M., Kaiser, L. & Jacquerioz, F. (2020). Clinical features of covid-19. *BMJ, 369*.

Vom Dorp, F., Tschirdewahn, S., Niedworok, C., Reis, H., Krause, H., Kempkensteffen, C., Busch, J., Kramer, G., Shariat, S. F., Nyirady, P., Rübben, H. & Szarvas, T. (2016). Circulating and Tissue Expression Levels of YKL-40 in Renal Cell Cancer. *J Urol, 195*(4 Pt 1), 1120-1125. https://doi.org/10.1016/j.juro.2015.09.084

Wallukat, G., Hohberger, B., Wenzel, K., Fürst, J., Schulze-Rothe, S., Wallukat, A., Hönicke, A. S. & Müller, J. (2021). Functional autoantibodies against G-protein coupled receptors in patients with persistent Long-COVID-19 symptoms. *J Transl Autoimmun, 4*, 100100. https://doi.org/10.1016/j.jtauto.2021.100100

Wang, B., Li, R., Lu, Z. & Huang, Y. (2020). Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. *Aging (Albany NY), 12*(7), 6049-6057. https://doi.org/10.18632/aging.103000

Wang, J., Jiang, M., Chen, X. & Montaner, L. J. (2020). Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: Review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. *J Leukoc Biol, 108*(1), 17-41. https://doi.org/10.1002/jlb.3covr0520-272r

Wen, W., Su, W., Tang, H., Le, W., Zhang, X., Zheng, Y., Liu, X., Xie, L., Li, J., Ye, J., Dong, L., Cui, X., Miao, Y., Wang, D., Dong, J., Xiao, C., Chen, W. & Wang, H. (2020). Immune cell profiling of COVID-19 patients in the recovery stageby single-cell sequencing. *Cell Discovery, 6*(1), 31. https://doi.org/10.1038/s41421-020-0168-9

Whitaker, M., Elliott, J., Chadeau-Hyam, M., Riley, S., Darzi, A., Cooke, G., Ward, H. & Elliott, P. (2022). Persistent COVID-19 symptoms in a community study of 606,434 people in England. *Nat Commun, 13*(1), 1957. https://doi.org/10.1038/s41467-022-29521-z

Wiersinga, W. J., Rhodes, A., Cheng, A. C., Peacock, S. J. & Prescott, H. C. (2020). Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *Jama, 324*(8), 782-793. https://doi.org/10.1001/jama.2020.12839

Wiley, C. A., Bonneh-Barkay, D., Dixon, C. E., Lesniak, A., Wang, G., Bissel, S. J. & Kochanek, P. M. (2015). Role for mammalian chitinase 3-like protein 1 in traumatic brain injury. *Neuropathology, 35*(2), 95-106. https://doi.org/10.1111/neup.12158

Winsz-Szczotka, K., Kuźnik-Trocha, K., Gruenpeter, A., Wojdas, M., Dąbkowska, K. & Olczyk, K. (2020). Association of Circulating COMP and YKL-40 as Markers of Metabolic Changes of Cartilage with Adipocytokines in Juvenile Idiopathic Arthritis. *Metabolites, 10*(2). https://doi.org/10.3390/metabo10020061

Wu, C., Chen, X., Cai, Y., Xia, J., Zhou, X., Xu, S., Huang, H., Zhang, L., Zhou, X., Du, C., Zhang, Y., Song, J., Wang, S., Chao, Y., Yang, Z., Xu, J., Zhou, X., Chen, D., Xiong, W., Xu, L., Zhou, F., Jiang, J., Bai, C., Zheng, J. & Song, Y. (2020). Risk Factors Associated With Acute Respiratory Distress Syndrome and Death

in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med, 180*(7), 934-943. https://doi.org/10.1001/jamainternmed.2020.0994

Wu, Y., Kang, L., Guo, Z., Liu, J., Liu, M. & Liang, W. (2022). Incubation Period of COVID-19 Caused by Unique SARS-CoV-2 Strains: A Systematic Review and Meta-analysis. *JAMA Netw Open, 5*(8), e2228008. https://doi.org/10.1001/jamanetworkopen.2022.28008

Wu, Z. & McGoogan, J. M. (2020). Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *Jama, 323*(13), 1239-1242. https://doi.org/10.1001/jama.2020.2648

Xu, X., Ma, H., Xu, J., Huang, H., Wu, X., Xiong, Y., Zhan, H. & Huang, F. (2014). Elevation in circulating YKL-40 concentration in patients with cerebrovascular disease. *Bosn J Basic Med Sci, 14*(3), 120-124. https://doi.org/10.17305/bjbms.2014.3.42

Yang, D., Chu, H., Hou, Y., Chai, Y., Shuai, H., Lee, A. C.-Y., Zhang, X., Wang, Y., Hu, B. & Huang, X. (2020). Attenuated interferon and proinflammatory response in SARS-CoV-2–infected human dendritic cells is associated with viral antagonism of STAT1 phosphorylation. *The Journal of infectious diseases, 222*(5), 734-745.

Yeoh, Y. K., Zuo, T., Lui, G. C., Zhang, F., Liu, Q., Li, A. Y., Chung, A. C., Cheung, C. P., Tso, E. Y., Fung, K. S., Chan, V., Ling, L., Joynt, G., Hui, D. S., Chow, K. M., Ng, S. S. S., Li, T. C., Ng, R. W., Yip, T. C., Wong, G. L., Chan, F. K., Wong, C. K., Chan, P. K. & Ng, S. C. (2021). Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. *Gut, 70*(4), 698-706. https://doi.org/10.1136/gutjnl-2020-323020

Yoshio, S. & Kanto, T. (2021). Macrophages as a source of fibrosis biomarkers for non-alcoholic fatty liver disease. *Immunol Med, 44*(3), 175-186. https://doi.org/10.1080/25785826.2020.1868664

You, M., Chen, L., Zhang, D., Zhao, P., Chen, Z., Qin, E.-Q., Gao, Y., Davis, M. M. & Yang, P. (2021). Single-cell epigenomic landscape of peripheral immune cells reveals establishment of trained immunity in individuals convalescing from COVID-19. *Nature Cell Biology, 23*(6), 620-630. https://doi.org/10.1038/s41556-021-00690-1

You, M., Chen, L., Zhang, D., Zhao, P., Chen, Z., Qin, E. Q., Gao, Y., Davis, M. M. & Yang, P. (2021). Single-cell epigenomic landscape of peripheral immune cells reveals establishment of trained immunity in individuals convalescing from COVID-19. *Nat Cell Biol, 23*(6), 620-630. https://doi.org/10.1038/s41556-021-00690-1

Zhang, F., Mears, J. R., Shakib, L., Beynor, J. I., Shanaj, S., Korsunsky, I., Nathan, A., Donlin, L. T. & Raychaudhuri, S. (2021). IFN-γ and TNF-α drive a CXCL10+ CCL2+ macrophage phenotype expanded in severe COVID-19 lungs and

inflammatory diseases with tissue inflammation. *Genome Med, 13*(1), 64. https://doi.org/10.1186/s13073-021-00881-3

Zhang, Y., Wang, S., Xia, H., Guo, J., He, K., Huang, C., Luo, R., Chen, Y., Xu, K., Gao, H., Sheng, J. & Li, L. (2022). Identification of Monocytes Associated with Severe COVID-19 in the PBMCs of Severely Infected Patients Through Single-Cell Transcriptome Sequencing. *Engineering (Beijing), 17*, 161-169. https://doi.org/10.1016/j.eng.2021.05.009

Zhao, T., Su, Z., Li, Y., Zhang, X. & You, Q. (2020). Chitinase-3 like-protein-1 function and its role in diseases. *Signal Transduct Target Ther, 5*(1), 201. https://doi.org/10.1038/s41392-020-00303-7

Zhao, T., Su, Z., Li, Y., Zhang, X. & You, Q. (2020). Chitinase-3 like-protein-1 function and its role in diseases. *Signal Transduction and Targeted Therapy, 5*(1), 201. https://doi.org/10.1038/s41392-020-00303-7

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., Si, H. R., Zhu, Y., Li, B., Huang, C. L., Chen, H. D., Chen, J., Luo, Y., Guo, H., Jiang, R. D., Liu, M. Q., Chen, Y., Shen, X. R., Wang, X., Zheng, X. S., Zhao, K., Chen, Q. J., Deng, F., Liu, L. L., Yan, B., Zhan, F. X., Wang, Y. Y., Xiao, G. F. & Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature, 579*(7798), 270-273. https://doi.org/10.1038/s41586-020-2012-7

Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Qi, Y., Sun, R., Tian, Z., Xu, X. & Wei, H. (2020). Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. *Natl Sci Rev, 7*(6), 998-1002. https://doi.org/10.1093/nsr/nwaa041

Zhou, Y., Peng, H., Sun, H., Peng, X., Tang, C., Gan, Y., Chen, X., Mathur, A., Hu, B., Slade, M. D., Montgomery, R. R., Shaw, A. C., Homer, R. J., White, E. S., Lee, C. M., Moore, M. W., Gulati, M., Lee, C. G., Elias, J. A. & Herzog, E. L. (2014). Chitinase 3-like 1 suppresses injury and promotes fibroproliferative responses in Mammalian lung fibrosis. *Sci Transl Med, 6*(240), 240ra276. https://doi.org/10.1126/scitranslmed.3007096

Zhou, Y., Zhang, J., Wang, D., Wang, D., Guan, W., Qin, J., Xu, X., Fang, J., Fu, B., Zheng, X., Wang, D., Zhao, H., Chen, X., Tian, Z., Xu, X., Wang, G. & Wei, H. (2021). Profiling of the immune repertoire in COVID-19 patients with mild, severe, convalescent, or retesting-positive status. *Journal of Autoimmunity, 118*, 102596. https://doi.org/https://doi.org/10.1016/j.jaut.2021.102596

Zhou, Z., Ren, L., Zhang, L., Zhong, J., Xiao, Y., Jia, Z., Guo, L., Yang, J., Wang, C., Jiang, S., Yang, D., Zhang, G., Li, H., Chen, F., Xu, Y., Chen, M., Gao, Z., Yang, J., Dong, J., Liu, B., Zhang, X., Wang, W., He, K., Jin, Q., Li, M. & Wang, J. (2020). Heightened Innate Immune Responses in the Respiratory Tract of COVID-19 Patients. *Cell Host Microbe, 27*(6), 883-890.e882. https://doi.org/10.1016/j.chom.2020.04.017

Zhu, C. B., Chen, L. L., Tian, J. J., Su, L., Wang, C., Gai, Z. T., Du, W. J. & Ma, G. L. (2012). Elevated serum YKL-40 level predicts poor prognosis in hepatocellular

carcinoma after surgery. *Ann Surg Oncol, 19*(3), 817-825. https://doi.org/10.1245/s10434-011-2026-3

Zhu, Q. & Kanneganti, T. D. (2017). Cutting Edge: Distinct Regulatory Mechanisms Control Proinflammatory Cytokines IL-18 and IL-1β. *J Immunol, 198*(11), 4210-4215. https://doi.org/10.4049/jimmunol.1700352

Zhu, Z., Zheng, T., Homer, R. J., Kim, Y. K., Chen, N. Y., Cohn, L., Hamid, Q. & Elias, J. A. (2004). Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science, 304*(5677), 1678-1682. https://doi.org/10.1126/science.1095336

Zubchenko, S., Kril, I., Nadizhko, O., Matsyura, O. & Chopyak, V. (2022). Herpesvirus infections and post-COVID-19 manifestations: a pilot observational study. *Rheumatol Int, 42*(9), 1523-1530. https://doi.org/10.1007/s00296-022-05146-9

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2. Peng W, Sheng Y, Xiao H, et al. Lung Adenocarcinoma Cells Promote Self-Migration and Self-Invasion by Activating Neutrophils to Upregulate Notch3 Expression of Cancer Cells. Front Mol Biosci. 2022; 8:762729. Published 2022 Jan 18. doi:10.3389/fmolb.2021.762729
3. Kwantwi LB, Wang S, Sheng Y, Wu Q. Multifaceted roles of CCL20 (C-C motif chemokine ligand 20): mechanisms and communication networks in breast cancer progression. Bioengineered. 2021;12(1):6923-6934. doi:10.1080/21655979.2021.1974765
4. Kwantwi, L. B., Wang, S., Zhang, W., Peng, W., Cai, Z., Sheng, Y., Xiao, H., Wang, X., & Wu, Q. (2021). Tumor-associated neutrophils activated by tumor-derived CCL20 (C-C motif chemokine ligand 20) promote T cell immunosuppression via programmed death-ligand 1 (PD-L1) in breast cancer. Bioengineered, 12(1), 6996–7006.
5. Cheng, Z., Wei, W., Wu, Z., Wang, J., Ding, X., Sheng, Y., Han, Y., & Wu, Q. (2019). ARPC2 promotes breast cancer proliferation and metastasis. Oncology reports, 41(6), 3189–3200.
6. Ding, K., Wu, Z., Li, X., Sheng, Y., Wang, X., & Tan, S. (2018). LMO4 mediates trastuzumab resistance in HER2 positive breast cancer cells. American journal of cancer research, 8(4), 594–609.

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