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The impact of COVID-19 on children and clinical paediatric care in Munich: an observational study during the first wave of the 2020 pandemic

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Abstract

While significant research has been conducted to examine the impact COVID-19 had on adults during the early phases of the ongoing pandemic, the extent to which children were affected remains largely unexplored. This thesis presents data on how the clinical care of the children's hospital of the Ludwig-Maximilians-University (LMU) in Munich was affected during the first wave of the pandemic (March to May 2020) and provides a qualitative description of how COVID-19 manifested in children. Additionally, preliminary clinical parameters as potential predictors for the development of severe COVD-19 in children are presented. To do so, data from the paediatric hospital were collected, and a cohort of 12 hospitalised children with COVID-19 was recruited and observed.

During the first wave of the SARS-CoV-2 pandemic in 2020, the total number of children presented at the paediatric care in Munich (LMU) decreased by approximately 40% in comparison to the same time period in 2019 (P=.017), and the number of children admitted due to common infectious diseases (such as respiratory or gastrointestinal infections other than COVID-19) decreased by 74% (P=.013). In context of nationally overrun and exhausted health care systems, these findings indicate that children were less impacted by the first wave of the pandemic in comparison to older age groups. Of the children that were admitted to Munich's paediatric hospitals due to a SARS-CoV-2 infection during the first wave, the majority (58%) presented with mild symptoms such as fever, cough and rhinitis. Two children within the cohort developed life-threatening severe hyperinflammatory syndromes such as PIMS and sHLH with ARDS, while three children remained asymptomatic during their entire period of infection.

On the basis of 12 hospitalised patients, the following factors can be described to be potentially associated with having a higher risk of developing a severe course of COVID-19 in children: young age (mean=2 years), pre-existing conditions including congenital heart diseases (PFO, ASD II and AVSD) or Down Syndrome. Additionally, the following clinical parameters were observed in children with a severe course of disease: highly elevated levels of IL-6, IL-2, CRP and Procalcitonin, occurrence of emesis, longer duration of fever, development of hyperinflammatory syndromes (sHLH, PIMS), prolonged duration of hospitalisation and treatment with IVIG and corticosteroids.

The small cohort size (n=12) associated to the low hospitalisation rates of children in the first wave, was insufficient to identify statistically significant predictors for developing a severe course of COVID-19 and only provide the description of the observations we made in this period of time. This remains subject of ongoing research within the hospital following an immunological and genetic approach.

To my parents and my brother

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Terms and abbreviations

ACE	Angiotensin-converting enzyme
ACE2	Angiotensin-converting enzyme 2
Ang I	Angiotensin I
Ang II	Angiotensin II
ARDS	Acute respiratory distress syndrome
AST	Serum aspartate aminotransferase
AT I	Angiotensin I
AVSD	Atrioventricular septal defect
AWMF	Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften
A&E	Accident and emergency
BMI	Body mass index
CHD	Congenital heart disease
CMV	Continuous mandatory ventilation
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computed tomography
Ct	Cycle threshold
DGPI	Deutsche Gesellschaft für pädiatrische Infektiologie
DIC	Disseminated Intravascular Coagulation
DS	Down Syndrome
EB	Epidermolysis bullosa
EBV	Epstein-Barr-Virus
EMA	European medicines agency
ESR	Erythrocyte sedimentation rate
GOT	Glutamate-Oxaloacetate-Transaminase
GPT	Glutamat-Pyruvat-Transaminase
HLA	Human leukocyte antigen
HPV	Human papillomavirus
HRV	Human rhinovirus
HSV	Herpes simplex virus
ICD-10	International statistical classification of diseases and related health problems
IEIs	Inborn errors of immunity
IFN	Interferon

IL-6	Interleukin-6
IVIG	Intravenous immune globulin
KD	Kawasaki disease
LDH	Lactatedehydrogenase
LGL	Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit
MAS	Macrophage activation syndrome
MERS-CoV	Middle east respiratory syndrome coronavirus
MHC	Major Histocompatibility Complex
MIS-C	Multi inflammatory syndrome in children
NT-proBNP	N-terminal pro hormone B-type natriuretic peptide
pCAP	Paediatric community-acquired pneumonia
PEC	Pre-existing condition
PICU	Paediatric intensive care unit
PIMS	Paediatric inflammatory multisystem syndrome
pTT	Partial-thromboplastin-time
PIMS	Paediatric inflammatory multi-system syndrome
RAS	Renin-angiotensin-system
RBD	Receptor binding domain
RSV	Respiratory syncytial virus
SAP	Systemanalyse Programmentwicklung
SOP	Solid organ transplant
SARS-CoV	Severe acute respiratory syndrome coronavirus
sHLH	Secondary hemophagocytic lymphohistiocytosis
s.p.	Status post
STIKO	Ständige Impfkommission
TMPRSS2	Type 2 transmembrane serin protease
TNF-alpha	Tumor necrosis factor alpha
TLR	Toll-like receptor

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

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Affidavit

Kaltenhauser, Carola

Surname, first name

I hereby declare, that the submitted thesis entitled

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the dissertation presented here has not been submitted in the same or similar form to any other institution for the purpose of obtaining an academic degree.

München, den 31.07.2023	Carola Kaltenhauser
Place, Date	Signature doctoral candidate

This thesis contains material which has previously been published in [1]:

[1] S. Kim-Hellmuth *et al.*, "SARS-CoV-2 Triggering Severe Acute Respiratory Distress Syndrome and Secondary Hemophagocytic Lymphohistiocytosis in a 3-Year-Old Child With Down Syndrome," *J. Pediatric Infect. Dis. Soc.*, vol. 0, no. 0, pp. 1–14, Nov. 2020, doi: 10.1093/jpids/piaa148.

1. Introduction

1.1. COVID-19 – a global pandemic

Outbreak – In December 2019 several pneumonia cases with unknown cause were noticed in Wuhan, China. Since then, a global pandemic challenges the world. A new human corona virus, formally named 2019-nCoV has been isolated from epithelial lung cells in January 2020. On January 30th the WHO called out a global state of emergency. Referring to its viral family tree, the virus became known as SARS-CoV 2 (Severe Acute Respiratory Syndrome Corona-Virus 2) and the associated disease as COVID-19 (Corona-Virus-Disease 2019) [2]. Infected individuals appeared with flu-like symptoms, pneumonia or respiratory failure, with potentially fatal outcome [3].

Virus Origin – Early cases of the outbreak have been linked to the Wuhan Seafood Market in China, frequently identified as the origin of the pandemic. Previously hosted in bats and other mammals, the corona virus transmitted into humans, potentially causing severe respiratory illness. In a short period of time the virus spread over the globe, reaching 1 million confirmed cases on the 1st of April 2020. Within 12 months of the first outbreak outside of China, in the middle of January 2020, the virus had reached nearly every country in the world, counting 94 million confirmed cases and 2 million deaths (January 19th 2021) [4]. In comparison, AIDS as one of the most challenging global virus diseases has led to 76 million HIV infections and 33 million deaths since its first diagnosis in the 1980s [5].

First wave – Figure 1.1 illustrates key events of the pandemic spreading globally, as context for the first 6 months of the pandemic. This thesis looks at observations collected during the 1st wave of the pandemic in Germany (marked red in Figure 1.1), starting with first cases at the beginning of March (calendar week 10) and after a peak, to the drop of confirmed cases per day in the middle of May (calendar week 20).

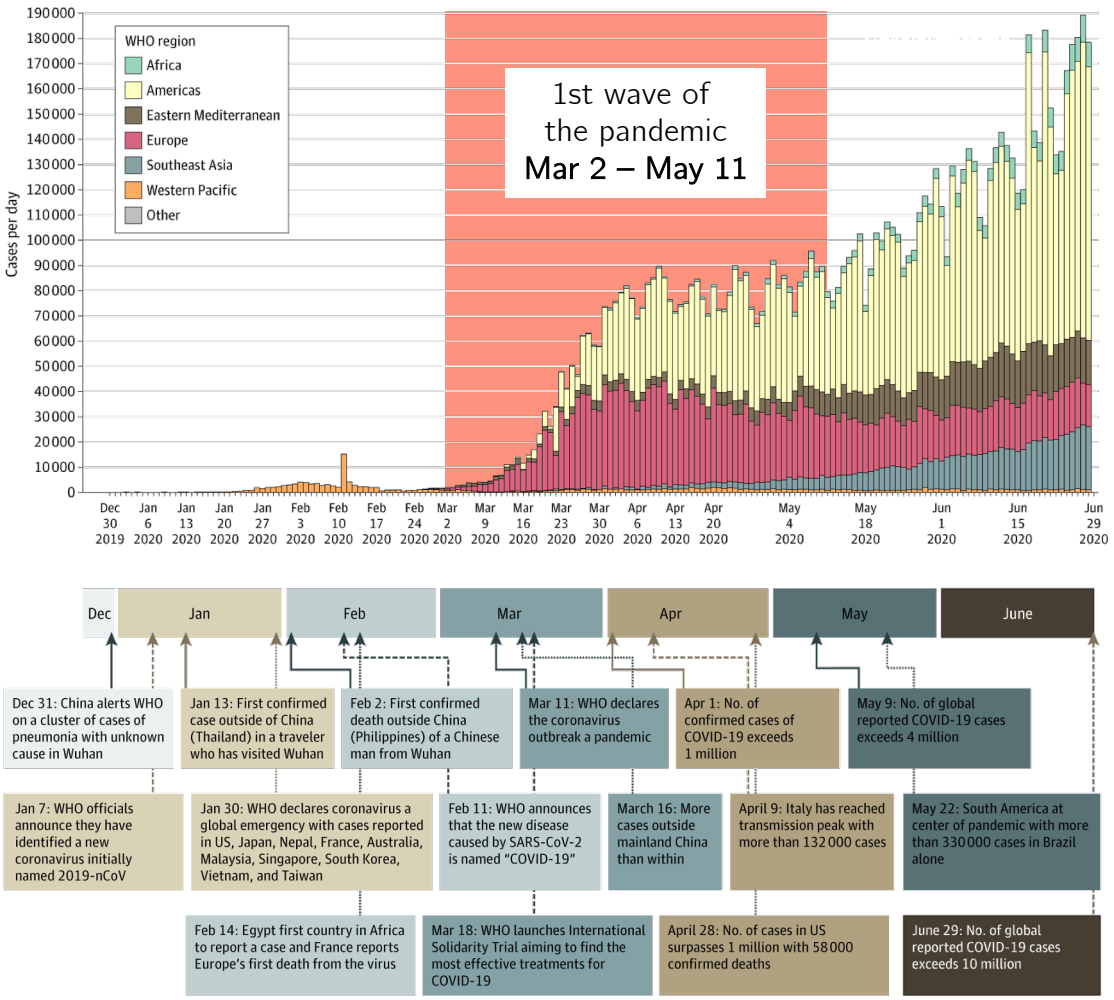

Figure 1.1: Key Events of the COVID-19 pandemic spreading globally, extracted from [6]. The red area marks the so called "first wave" in Germany: calendar week 10 to calendar week 20 [7].

Distribution – The number of cases and deaths related to COVID-19 were not evenly distributed across the globe. In addition to some identified risk factors, such as pre-existing diseases (diabetes, cardiovascular, neurological and pulmonary diseases) or clinical parameters (SOFA score, inflammation values), in particular older age was associated with fatal outcomes [7] [8]. Health care reports indicate that children appeared with less severe symptoms and were less likely to be infected than adults [9]. During the first wave in Germany, children aged 0-4 years accounted for only 1.0% and those aged 5-20 for only 5.2% of all infections, although these age groups account for

approximately 12% of the population. The mortality rate (0.0-0.1%) associated to COVID-19 for the age group under 20 years of age was also significantly lower when compared to the COVID-19 mortality rate of the entire population (5.6%) [7].

Transmission – The virus owes its rapid spread from person to person to its transport in respiratory droplets and aerosols. These are produced by speaking (600 droplets/min), singing, coughing (3,000 droplets), sneezing (40,000 droplets), as well as by medical procedures like intubation or dental procedures. Larger droplets (>5 ym) can reach 1-2 meters before sinking to the ground. Aerosols (<5 ym) are lighter and can stay in the air for some hours [10]. Therefore, social distancing, wearing masks and air ventilation were recommended as prevention measurements.

1.1.1. The coronavirus

The questions from where this novel virus took its origin and how the primary transmission from its previous host to humans took place, are most relevant issues for future pandemic preventions, reflecting human interactions with wild living animals.

Virus structure

Characteristics – The coronavirus owes its name to multiple spike glycoproteins (commonly referred to as the S-spike) surrounding a spherical body - shaped like a crown.

Figure 1.2 schematically illustrates the coronavirus with its spike (S) proteins, membrane (M) proteins and envelope (E) proteins on the surface. The M-proteins function is to link with other M- or E-proteins enabling viral connections. E-proteins regulate the M-M interactions and trigger the Golgi-system to produce larger vesicles. As linked to the RNA the nucleocapsid-protein (N), is associated with viral replication and localization of the replication-transcription-complexes [11].

S-protein – Special attention should be given to the S-protein as it is responsible for both: receptor mediation, as well as membrane fusion to invade a cell. Therefore, it plays a major role in infection and pathogenesis. What makes the coronavirus remarkable, is its way of different cell entry mechanisms: they can either (i) fuse directly into the host cell or (ii) use endosomal transport. Depending on the coronavirus, the S-protein can not only use proteinaceous but also carbohydrate receptors [12], expanding the options to diffuse into other cells.

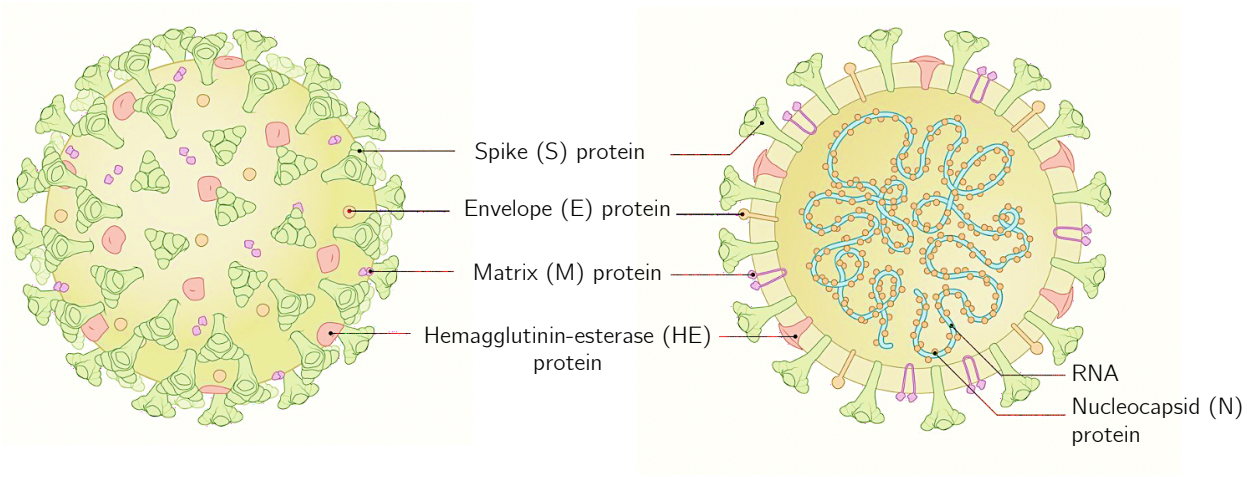

Figure 1.2: Schematic of the coronavirus showing: the Spike (S) protein (crucial for receptor mediation and membrane fusion), Matrix (M) protein, Envelope (E) protein, Hemagglutinin-esterase (HE) protein, Nucleocapsid (N) protein and single-strand RNA; extracted from [13].

Virus classification

Taxonomy – Figure 1.3 illustrates the family tree of the coronaviruses. They all belong to the group of Nidovirales, further classified into the family of Coronaviridea and subfamily of Coronavirinae. These can be clustered into categories: (a) alpha- and (b) beta-coronaviruses infect humans, (c) gamma- and (d) delta-coronaviruses only infect animals [14]. The viruses (i) hCoV-229E, hCoV-NL63, and (ii) hCoV-HKU1, hCoV-OC43 are known to cause mild respiratory illnesses like the seasonal "common cold" [15].

SARS/MERS – Apart from these (i-ii) rather harmless human coronaviruses, only the beta-coronaviruses (iii) SARS-CoV (severe acute respiratory syndrome coronavirus), MERS-CoV (middle east respiratory syndrome coronavirus) and SARS-COV-2 were identified to potentially cause severe disease in humans [14].

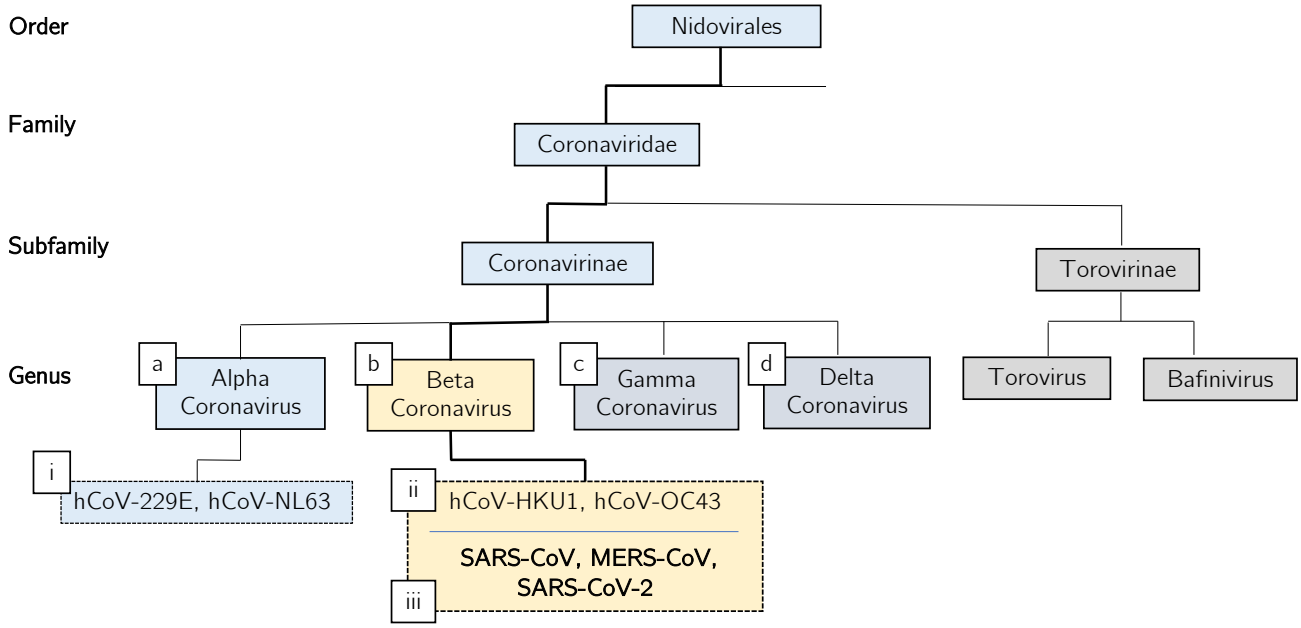

Figure 1.3 Taxonomy of the coronaviruses with beta-coronaviruses, that are able to infect human cells: hCoV-HKU1 and hCoV-OC43 (usually causing the common cold) and SARS-CoV and MERS-CoV (causing respiratory disease), based on [14]

Cellular enter & reproduction of the coronavirus

The virus itself has no cellular equipment to live on its own. As an obligate intracellular parasite it is dependent on a host cell, providing resources for metabolism and reproduction. Especially the genome and reproduction cycle of the coronavirus makes it stand out from other viruses and having an impact on its success in infection.

Cellular connection – To enter the host cell, the RBD (receptor binding domain) of the S-protein connects with the human ACE2-receptor with very high affinity. ACE2 is a human interferon activated gene, located together with TMPRSS2 (Type 2 transmembrane serin protease) in various systems: (a) type 2 pneumocytes, (b) intestinal epithelial cell, (c) nasal goblet secretory cells, (d) nasal respiratory epithelium, endothelial cells of (e) smooth muscle cells, (g) blood vessels and (f) renal tubules [16].

Cellular entry – For final endocytosis into the host cell, the TMPRSS2 is a main mediator enabling the cell-fusion by priming the S-protein. As next step the virus is uncoating and releases its RNA into the cytoplasm to be translated and replicated by the host cell [17].

Genome – Coronaviruses have the largest genome (26-32 kb) of all positive stranded RNA viruses, that is twice as big as others. With its 13 genes, however, it can create even more than 27 proteins by using a special multiprotein fusion technique [18]. Compared to other viruses, the replication/transcription complex is more similar as what is known from DNA-based structures. The replicase is composed by a multi-protein complex with specific tools such as proofreading exonuclease and other cofactors. This may be one reason for the unusually large genome of the virus [19].

1.1.2. Manifestation of COVID-19 in the human body

As an antigen in the body SARS-CoV-2 activates and challenges the immune system, that is trying to fight the virus. An infection can escalate from a primarily anti-viral-response to a state of inflammation and sometimes hyperinflammatory.

Pathogenesis of organ damage

Antiviral state – When virus-loaded droplets are inhaled, the virus first enters the ACE2 receptor-containing epithelial cells of the nasal mucosa and from there spreads into the respiratory tract. After settling in the cells, the anti-viral-state gets activated. This means that plasmacytoid dendritic cells secrete Type-I-Interferon, which protects local cells. Patients who recover early, keep the virus under control with (1) Type-I-Interferon, (2) the additionally triggered NK-cell activation and (3) possibly also by CD8+ T-cell activation. In mild cases, the antiviral response is favoured over inflammation and the virus gets rapidly controlled with minimal damage [20].

Inflammatory response – Inflammation develops when viremia is not controlled and eradicated in the early stage: Neutrophils and Macrophages, as well as inflammatory cytokines (Il-6, TNF alpha, IFN gamma, etc) migrate to the focus of infection in the lung. Infected pneumocytes get destroyed, followed by a disruption of the cell barrier, resulting in a thickened, diffuse endotheliitis [21]. Now a vicious circle can start: due to the endothelial damage, the complement system, proinflammatory cytokines, and chemokines (IL-6, IFNγ, MCP1 and IP-10) are released, neutrophile-migration and NETosis (Neutrophil extracellular traps) development are activated and coagulation increases, causing thrombotic events and again damaging endothelia. This tissue injury and thus organ damage can lead to ARDS (acute respiratory distress syndrome), but also DIC (disseminated intravascular coagulation) and multiple organ failure [20].

Symptoms – The course of the disease varies between individuals: some remain asymptomatic and are identified by routine testing, while others develop symptoms which primarily manifest in the respiratory system, but can also affect the

gastrointestinal, neurological, hematological and cardiovascular system. The CDC (Centre for Disease Control and Prevention) reported symptoms in the study population <18 years: over half presented with fever (56%), closely followed by cough (53%), followed by fatigue (no exact values), myalgia (23%), headache (28%), shortness of breath (13%), sore throat (24%), rhinorrhoea (7.2%), diarrhoea (13%), nausea/vomiting (11%), and abdominal pain (5.8%) [22].

Time frame – The average incubation period (time from virus exposure to symptom start) lasts 4-7 days. During the incubation time, the latency period of viral exposure transitions to an infectious interval after about 3 days, which lasts an average of 4 days. More than 95% of patients, that develop symptoms, had an onset within 12 days. The cessation of symptoms and the time to recovery takes about 2 weeks in mild cases, and about 6 weeks or more in severe cases [23].

Phases of infection

Figure 1.4 shows how COVID-19 disease can be clinically categorized into 3 phases. While the majority of patients recover from the infection after some days of mild to moderate symptoms, some courses of the disease progress to a severe or critical illness. Siddiqi et al. [24] describes them as follows:

Phase I – The first symptoms appear in the early infection phase, which are expressed in the form of mild and non-specific complaints such as fever, fatigue, and coughing. The frequent impairment of the respiratory tract is probably due to the distribution and role of the ACE2 receptors (described in Chapter 1.1.1). Blood counts are except for lymphopenia and neutrophilia usually without remarkable changes [24].

Phase II – The second phase is characterised by increased pulmonary inflammation with viral pneumonia, cough, fever and following hypoxia. Inflammation parameters in the blood are moderately elevated. Patients often need inpatient care at this stage. [24]. Chest images can show bilateral subpleural fluid retention and milk-glass-opacities as

signs of inflammation of the lung parenchyma [25]. In this phase it seems like the immune system is still able to control the infection.

Phase III – The third, "hyperinflammatory phase", is only present in a small proportion of patients. In this phase, the excessive immune response to the virus seems to be the reason for exacerbation of the illness. After activation of both the adaptive and innate immune systems, several chemokines and cytokines are produced. It is likely that an uncontrolled excess of this production, leads to a fulminant inflammatory reaction. This cytokine storm can manifest itself in multi-organ failure with ARDS, SIRS or cardiac shock. There is a rapid deterioration of the patient's condition with strongly increased inflammation markers such as CRP, LDH, IL-6, D-dimer, Ferritin, Troponin and NT-proBNP [24] [26]. These patients require treatment in intensive care units. Hyperinflammatory syndromes are described in Chapter 1.1.3.

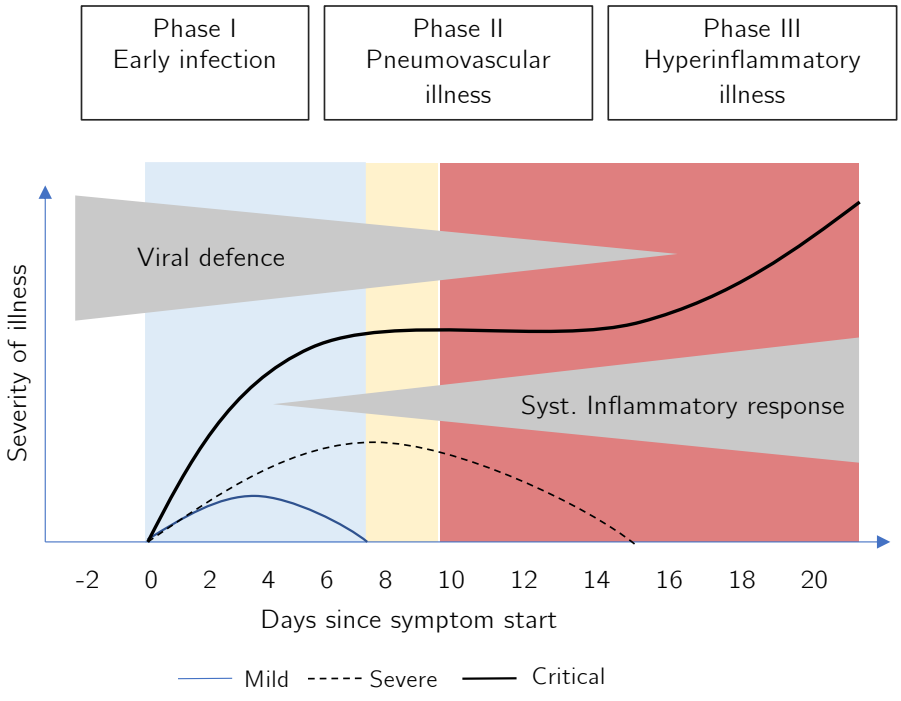

Figure 1.4 The 3 phases of infection, with progression of severity depending on the state of immune reaction, according to days since symptom start; based on [26].

1.1.3. Hyperinflammatory syndromes

Especially in later phases of COVID-19, it is possible that it is no longer the virus itself that is at the forefront, but rather the uncontrolled immune response. There are a variety of hyperinflammatory syndromes associated with COVID-19 outbreak. Especially in paediatrics, the hyperinflammatory syndromes PIMS and sHLH sometimes occur in association with COVID-19.

PIMS – Paediatric multisystem inflammatory syndrome (PMIS), multisystem inflammation syndrome in children (MIS-C), Kawasaki-like syndrome, Kawasaki-like disease, hyperinflammatory shock or hyperinflammatory syndrome with multiorgan involvement, are terms used synonymously for a newly described severe inflammatory picture occurring primarily in children since the COVID-19 outbreak [27]. This new syndrome was first described in April 2020, when the COVID-19 pandemic was already spreading worldwide. From March to June 2020 783 patients with suspected PIMS were reported. The syndrome has great clinical similarity to other severe inflammatory syndromes. It occurs in children who had a history of SARS-CoV-2 exposure in the immediate past, suggesting a post-infectious immune reaction. The Centre of disease Control (CDC) therefore suggested the following criteria to make the diagnosis, excluding other causes: (a) age $<$ 21 years, (b) the need for hospitalisation, (c) fever for at least 24 hours of over 38°C, (d) $>$2 organ system dysfunctions (i.e. cardial, renal, etc.), (e) laboratory evidence of an inflammatory response (elevated IL-6, CRP, ESR, etc.) and (f) association with a SARS-CoV-2 infection. Most common symptoms were fever, gastrointestinal symptoms like emesis, diarrhoea, abdominal pain, neurological symptoms like headache, meningism, and abnormalities of the mucous membranes, similar to Kawasaki syndrome. Respiratory symptoms did not necessarily have to occur [28]. A smaller proportion of cases had detectable active SARS-CoV-2 infections (RT-PCR), however, the majority of cases were found to have a positive serology with SARS-CoV-2 antibodies, or at least had some kind of exposure within the last 4 weeks.

All 3 phases of illness (described in Figure 1.4) may occur one after the other. In most cases, this serious condition of dysregulated and overreactive immune respond required therapy with high doses of steroids and IVIG (intravenous immunoglobulins) [29].

sHLH/MAS – Another hyperinflammatory syndrome in the context of COVID-19 is the secondary hemophagocytic lymphohistiocytosis (sHLH), or sometimes referred to as macrophage activation syndrome (MAS). MAS has been observed mainly in patients with systemic juvenile idiopathic arthritis (sJIA). Rheumatoid or autoimmune diseases can lead to an overactivation of hemophagocytic macrophages, which can cause a cytokine storm, leading to fatal consequences for the patient [30]. Familial or primary HLH often has a genetic defect as the cause for the dysregulation of the immune cells [31]. This reaction can also occur in response to an infection (usually virus-triggered), and is called secondary HLH (sHLH) [32]. The clinical picture is very similar to Sepsis and is often not diagnosed correctly. Early diagnosis and correct therapy can prevent multiple organ failure and reduce mortality. Fardet et al. have therefore developed the HScore (attached in Annex 9 with calculation of the individual values of our study patient). This enables a better risk assessment and helps to better differentiate these very similar inflammatory syndromes.

1.2. Paediatric hospitals considered for this study

The first objective of this thesis is specifically dedicated to the changes in the number of patients compared to previous years during the first wave of the COVID-19 pandemic at the LMU Children's Hospital.

Figure 1.5 The Dr. von Hauner Children's Hospital one of the main tertiary care providers for children in the city-centre of Munich [33].

The Dr. von Hauner Children's Hospital of the Ludwig-Maximilians-University (shown in Figure 1.5) is one of the main tertiary care providers for the ca. 230,000 children (0-17 years) of Munich. With numerous special outpatient clinics and highly specialised research projects, it is one of the main hubs for medicine and research on children's health in Bavaria. Diseases that require high expertise and experience, such as severe COVID-19, are treated and researched here. The Dr. von Hauner Children's Hospital, which has 285 employees and ca. 120 beds covers everything from basic care to the treatment of rare diseases. The COVID-19 pandemic was a major challenge for complex and multi-faceted institutions like this hospital [33].

For answering the research objectives concerning the analysis of COVID-19 courses in children, the Children's Hospital Schwabing (TUM) was equally involved within the CHANCE programme. Details will follow in 3.1.2.

2. Motivation and key research objectives for this thesis

This thesis provides an overview, as well as specific case studies, of COVID-19 in hospitalised children at Munich's Paediatric University during the first wave of the SARS-CoV-2 Pandemic in 2020

2.1. Motivation & novelty of work

Regional influences – Policies to mitigate effects of the pandemic aim to reduce the burden on health systems. The precautions differ not only with regards to the disciplines, but also according to regional conditions such as municipal regulations on contact restrictions or hygiene measures. To carry out proactive management ensuring optimal health care, and also drive research in infection control in children, it is important to examine what changes in the first wave of the COVID-19 pandemic occurred specifically in the paediatric hospital in Munich. These considerations give rise to → **Objective 1** (detailed on the next page, in Section 2.2).

Paediatric influences – Despite COVID-19 having attracted significant attention in the scientific community, with numerous reports of the impact COVID-19 has had on adults, the literature available for children was sparse at the time of the first wave, when this research was undertaken. The unknowns in pathogenesis of the virus demands more detailed insights for all demographics, including children, to be able to pre-emptively respond and maintain health. Specifically, the overall impact the virus had on the clinical course of children, and also a detailed analysis of symptoms and potentially predictable risk factors are of high interest and the basis for → **Objective 2, 3 (**detailed in Section 2.2).

2.2. Research objectives

(1) The impact of COVID-19 on paediatric care

To what extent was the paediatric hospital of the LMU in Munich, Germany affected by the COVID-19 outbreak during the first wave of the SARS-CoV-2 pandemic (March to May 2020)? Specifically, how did the number of patients and number of infectious diseases reported at the hospital differ compared to previous years?

(2) Manifestation of how COVID-19 manifested in children

How did SARS-CoV-2 infections manifest in children? Were only relatively mild cases reported? And if not, what did severe manifestations look like?

(3) Investigation of clinical and laboratory parameters as predictors for the development of severe COVID-19 in children

Can clinical or laboratory parameters be used as predictors for a severe course of COVID-19 in children?

The methodology for answering each objective is detailed in Chapter 3. Results are detailed in Chapter 4, and discussed in Chapter 1. Finally, an outlook is provided in Chapter 1.

3. Materials & Methods

3.1. Design of the clinical study

This doctoral project took place within the framework of a prospective non-randomised observational study. It was a mono-centre study launched by the Child Health Alliance Munich (Dr. von Hauner Children's Hospital, Children's Hospital Schwabing) in March 2020 and is still being continued and intensified with cooperation partners and an extended team at the time of publication. In order to assess the effect of SARS-CoV-2 on children, the entire patient journey was considered.

Patient Journey – From initial contact - often already in the emergency department - to the collection of follow-up data until discharge, multiple touchpoints with the patient were run through. Figure 3.1 provides an overview of the touchpoints this study had with the patients.

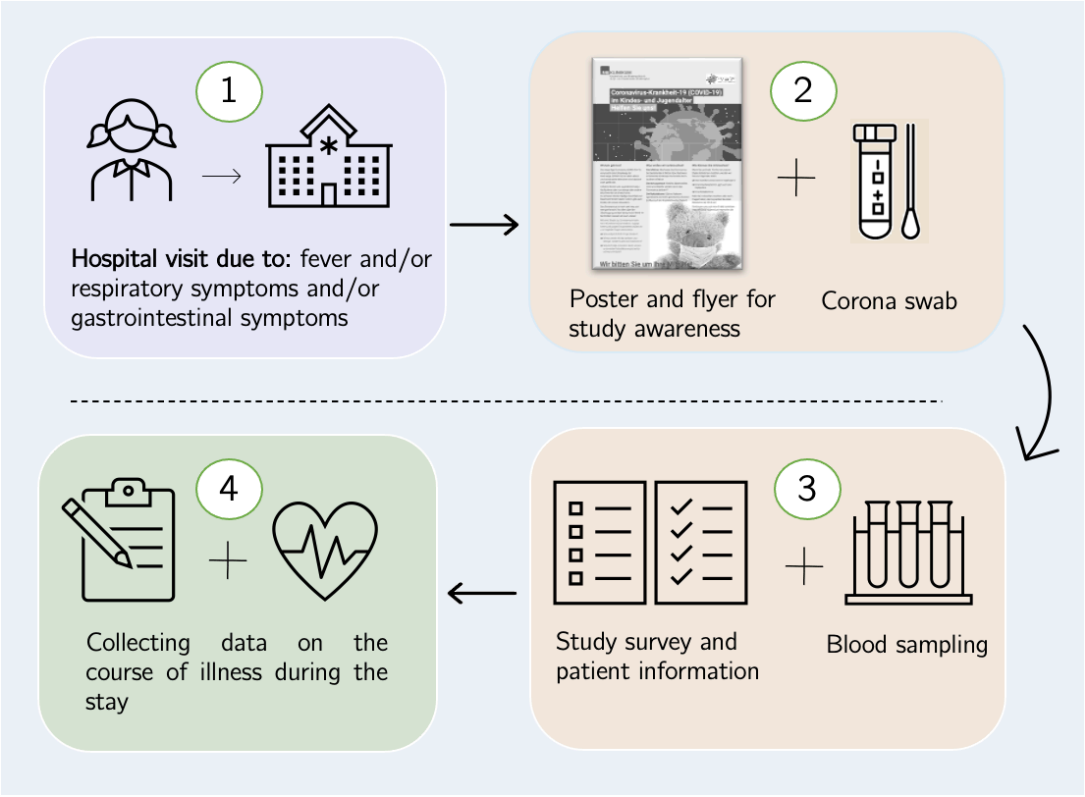

Figure 3.1 Patients journey & interaction with the study. Steps from (1) hospital arrival, (2) testing and (3) sampling, to (4) holistic data collection.

Step 1 – Each patient that visited the hospital due to (i) COVID-19 associated symptoms like fever, respiratory symptoms (cough, shortness of breath, etc.), gastrointestinal symptoms (diarrhoea, emesis, abdominal pain, etc.) or (ii) any other medical conditions that require inpatient care, like status epilepticus, dialysis etc. was principally considered for this study.

Step 2 – Each patient received a naso/oropharyngeal swab for a SARS-CoV-2 PCR-test. All patients were briefed and informed about the ongoing study by posters and flyers (see Annex 1) in the waiting areas. Every patient or their parents who were contacted, participated in the study.

Step 3 – After all questions about the study procedures were answered, the patient and/or parents were asked to give their written consent (patient information and declaration of consent can be found in Annex 4 and Annex 5). Participants filled out the clinical survey on day of admission. As stated in the study ethics approval, blood samples were only taken for the study if there was a medical reason to do so (i.e. only in cases where there was a medical indication), which usually happened on the first day and was repeated depending on the severity of the illness.

Step 4 – All relevant health data collected during the hospital stay like clinical manifestation, progression of infection, as well as therapy and outcome were carefully compiled, processed, and supplemented by secondary data (data sources listed in Chapter 0). The participants were given a pseudonym upon enrolment, which was used continuously for all further analysis.

3.1.1. Cohort selection

Information about newly admitted children who were eligible for the study or of the arriving of follow-up blood samples was communicated by a dedicated mobile phone for the study. Study participants were recruited from several institutions following the same standards and were selected for this thesis according to specific inclusion criteria.

Inclusion criteria – The study participants had to fulfil the following inclusion criteria in order to be considered a suitable cohort for answering the research questions of this thesis:

- The participants must be between 0 and 18 years old.
- They must be RT-PCR-tested positive for SARS-CoV-2 or have a positive antibody test when viral load is no longer detectable.
- They must have been hospitalised during the infection/illness in one of the participating hospitals during the first wave (March to May 2020).
- According to the requirements, the child and both legal guardians had to agree to participation.

Exclusion criteria – There were no restrictions in terms of demographic factors, such as sex, country of origin, ethnicity, or social state. There were also no limitations regarding the children's previous medical history like pre-existing conditions or regularly taken medication.

Cohort selection criteria – 63 patients were recruited between March and August 2020, of which 12 met the inclusion criteria (see above) for further analysis.

At the beginning of this project, there was an attempt to collect 3 different groups of patients in order to compare them with each other: (i) a group of COVID-19 positive children with (ii) a group of children with other infectious diseases (preferably respiratory infections) and (iii) a group of healthy children. However, due to a lack of children with respiratory or other infections and the distinct inhomogeneity of the two

'control groups' at the time of the first wave, they will not be thematised here. Only the group of COVID-19 positive children (n=12) meeting all the inclusion criteria will be descriptively presented in the results of this thesis. Further recruitment of the two other groups was continued within the research group.

Table 3.1 summarizes the cohort recruited, also including the patients that did not meet inclusion criteria for this thesis. The COVID-19 cohort consists out of 6 female and 6 male patients, aged from 0 to 18 years. Recruitment of the included children took place within the period from the 31st of March 2020 to the 22nd of May 2020. All patients were included during their stationary stay in one of the participating hospitals listed in 3.1.2 below.

		COVID-19	Control 1 (ill)	Control 2 (healthy)
Sample size (n=63)		15	25	23
Age	0 – 18	12	17	19
	18+	3	8	4

Table 3.1 showing the entire cohort recruited: (i) one COVID-19 group and two control groups containing (ii) participants with any illnesses except COVID-19 and (iii) healthy participants from the surgery department. Green highlighted: COVID-19 patients aged 0-18 years make the study cohort for this thesis, the others will not be thematised any further, but are listed here for completeness.

Additional research – Blood samples of the entire cohort (n=63) were shared with the research group for further analysis and formed the basis/starting point for research on immunophenotyping and multi-omics (genome-, transcriptome-, proteome- and metabolome-analyses). Results of this research will be published after the submission of this thesis.

3.1.2. Institutional collaborations

LMU – To achieve comprehensive coverage, several institutions agreed to participate in the study. The Dr. von Hauner Children's Hospital was the main study centre from where the study originated. To ensure an optimal distribution of medical capacity during the pandemic, the Hospital Großhadern has set up a paediatric emergency unit especially for children with severe COVID-19. There was a continuous exchange with the team of this ward about procedures and outcome of the patients.

TUM – Children with a positive SARS-CoV-2 PCR test, were informed about the study and recruited using the same procedure on site. Considering the rather small number of paediatric COVID-19 cases in Munich, this network proved to be particularly important.

Institutions	COVID-19	Control 1 (ill)	Control 2 (healthy)	all
Dr. von Hauner Children's Hospital	4	18	23	45
Children's Hospital Schwabing	8	1	-	9
Hospital Großhadern	3	-	-	3
General practice	-	6	-	6
all	15	25	23	63

Table 3.2 shows the number of all patients that were recruited in the cooperating institutions. As mentioned above only 12 children met the inclusion criteria for this thesis.

3.2. Classifications of the severity of COVID-19

International classifications – Cases of COVID-19 may be categorised based on their severity. Institutions including the Robert-Koch-Institute, the World Health Organisation (WHO) and the National Institute of Health (NIH), each have slightly different definitions, usually ranging from "light" to "critical". Table 3.3 contains the definitions and classifications we have used for categorising the cohort of this thesis.

	Clinical presentation	Definition	Source	Nr. of patients
1	Asymptomatic	Individuals who test positive for SARS-CoV-2 by virologic testing using a molecular diagnostic or antigen test, but have no symptoms	NIH COVID-19	3
2	Mild	various sign and symptoms of COVID-19 (e.g. fever, cough, sore throat, malaise, headache, muscle pain) without shortness of breath, dyspnoea, or abnormal chest imaging	NIH COVID-19	7
	Moderate	Child with clinical signs of non-severe pneumonia (cough or difficulty breathing + fast breathing and/or chest indrawing) and no signs of severe pneumonia.	WHO clinical management	
3	Severe	Child with clinical signs of pneumonia (cough or difficulty in breathing) + at least one of the following: Central cyanosis or SpO2 < 90%; severe respiratory distress (e.g. fast breathing, grunting, very severe chest indrawing); general danger sign: inability to breastfeed or drink, lethargy or unconsciousness, or convulsions	WHO clinical management	2
		Fast breathing (in breaths/min): < 2 months: ≥60; 2–11 months: ≥ 50; 1–5 years: ≥ 40	WHO clinical management	
	Critical	ARDS, Sepsis, Septic shock	WHO clinical management	

Table 3.3: Categorisation of COVID-19 severity from (1) asymptomatic to (3) critical according to criteria adapted from NIH [34] and WHO [35]. ARDS (acute respiratory distress syndrome)

3.3. Methods for data collection

Sources – Table 3.4 provides an overview of the primary and secondary data used to answer the respective research objectives.

Research Objective	Primary data	Secondary data
1	-	LMU clinic internal statistics unit
2 + 3	Clinical survey demographic information, symptoms, medical history, pre-existing conditions,	Discharge letters course of disease, medication, therapy, current state of health
	Blood samples routine laboratory, COVID-19 specific values	Nurse reports clinical parameters: e.g. body temperature, weight
	Body fluids SARS-CoV-2 RT-PCR test: naso/oropharyngeal swabs, stool, tracheal secretion	Electric health record monitored values: e.g. SpO2, HCO2, pH, RR

Table 3.4 Sources used for generating primary and secondary data.

3.3.1. Primary data sources

The clinical documentation associated to this study included: a symptom-oriented clinical survey and laboratory parameters (clinical chemistry and SARS-CoV-2 PCR diagnostics). The SARS-CoV-2 PCR diagnostics were taken from throat swab material and stool, as well as supplementary serological tests.

The Clinical Survey

The items provide general demographic information, details to the patient's medical history and a focus to specifics to the infection (survey attached in Annex 2).

Conception – The clinical survey was newly created for this study. It is a 3 page questionnaire with 9 items, consisting of 48 subscales, of which 12 are open and 36 are in a closed question format. The closed questions include dichotomous and single choice answer options with 3 to 8 categories.

Survey contents – Item 1 starts with the generated study pseudonym for each patient and the date of the hospital arrival, to which the following symptom starts are referring. Item 2 contains demographic information such as age in years, gender, weigh, height and country of origin of the parents. These are the only patient characteristics included in the questionnaire. Item 3 asks about vaccinations and the frequency of previous febrile infections in the past 6 months. Item 4 and 5 provide information whether there has already been a SARS-CoV-2 infection/test and the potential initial contact that led to the infection. Item 6 is the most comprehensive with 22 subscales and contains detailed information on the symptoms of the disease. Item 7 and 8 deal with the medical history of the patient, such as pre-existing conditions and medication. Item 9 offers as a free text field the possibility of adding any additional information.

Conduction – The survey was handed out on the first day of hospitalisation after consent was given to participate. The same survey was used for all participants. Completion of the clinical survey took approximately 5-10 minutes. In order to ensure

comparability, the clinical survey was filled out in a standardised way together with myself. The surveys were subsequently scanned, and the values digitized directly.

Laboratory Parameters

Clinical chemistry – A special COVID-19 profile was created for processing blood samples in the digital hospital working software (SAP). It contained the following values: Differential (blood count, Lymphocytes, Leukocytes, Thrombocytes), C-reactive protein, Procalcitonin, Interleukin-6, Creatinkinase, Troponin T, GOT, GPT, LDH, Creatinine, Urea, Ferritin, Cystatin C, Quick, pTT, Fibrinogen, D-Dimer, Antithrombin. These parameters were selected on the assumption that they cover a wide range of potential systems.

SARS-CoV-2 diagnostic – The hospital's infection control policy was to diagnose all patients with a SARS-CoV-2 infection directly upon presentation. All patients therefore received a naso/oropharyngeal swab for a RT-PCR-test. The samples were processed at the in-house laboratory or in the Max-von-Pettenkofer-Institut.

3.3.2. Secondary data sources

Clinical documentation – In order to better understand the course of the disease and infection during and before the inpatient stay, it was necessary to bring together patient data from several sources. For this purpose, data from discharge letters, nursing reports and intensive care protocols were collected and analysed. Some of these sources were available electronically, others had to be digitized manually.

Research for Objective 1 was compiled on the basis of data on exact patient numbers, diagnosis etc. from the hospital history provided by the LMU intern 'Stabsstelle Kaufmännisches Controlling und Entgelte' [36] and also from the DGPI [37], which is a German medical association group of infectiology in paediatrics. These provided a platform to document and collect COVID-19 cases and course of disease in paediatrics on a national level with a detailed online survey.

3.3.3. Data processing

After consultation with the Institute for Statistics of the LMU, data evaluation and visualisation was mainly performed with Excel and Matlab. The *T-test (paired two samples for means)* was used to compare the difference in the mean value of measurements at two points in time (comparing 2019/2020 and 2018/2020) [38]. To avoid the bias of multiplicity through multiple testing or also called FWER (familywise error rate) [39] - the level of significance was adjusted with the *"Bonferroni Correction"* by dividing the usual level of significance to adjust (α =0.05) through the number of tests performed (α/n). [40] It is one of the most conservative correction methods and increases the probability of generating false negative results in terms of their significance [41]. The *T-test* plus corresponding *"Bonferroni Correction"* were applied in Table 4.1.

An excerpt from the Excel sheet used to collect the data obtained is attached as Annex 3.

3.4. Ethics committee approval

The study protocol corresponded to the Helsinki Declaration from 1996 and was approved by the Ethics Council of the LMU Munich (approval attached as Annex 6). Participation was voluntary. Enrolment only took place after written consent. Blood samples were only taken in the context of a clinical indication, so there was no increased patient risk from participating in the study. Participants were initially pseudonymised and data management applies in accordance with the Basic Data Protection Regulation (DGSVO). Participants had the possibility to have their data deleted or irrevocably anonymised after the study.

4. Results

4.1. The impact of COVID-19 on paediatric care

While hospitals and intensive care units across the country struggled with overcrowding from COVID-19 patients, the paediatric hospitals were less affected. This was due to the overall low infection rates in this age group compared to the general population, and on the other hand to milder courses.

4.1.1. The role of children in epidemiological infection events

Incidence – The heatmap in Figure 4.1 (a) illustrates the level of nationwide infections per age group. White and light shades indicate a low number of infections per 100,000 inhabitants. The darker the shade, the more infected individuals there are in this age group. It shows the relatively small extent to which the age group up to 19 years was affected. Especially the age groups from 0 to 14 years show the lowest infection rates. Taking the numbers of infections per respective age group, it can be seen that in the age group under 19 year had on average 70% fewer infections per week (on average 6 infections/week) than the age groups above 19 years (avg. 21 infections/week). The peak of infections in all age groups was reached from the end of March to the end of April for the first period. At this peak the age group up to 14 years is at a maximum of 12 infections per 100,000 inhabitants. This is doubled in the group from 15 to 19 years with 27 infections per 100,000. With a value of 49 from the age group 20 upwards, this value is doubled again.

Correlation – The hospitalisation figures of infected children within this study correlate directly with the infection numbers of the general population. Figure 4.1 (b) on the right shows the reported infections in Germany per 100,000 inhabitants in red. The infected study participants are shown in blue bars. These refer to the right axis and are given in total numbers. One can observe the temporal interplay of the rise and fall of study patients with nationwide infections. The orange curve additionally

illustrates the total number of hospitalised children with COVID-19 on the normal ward, collected by the DGPI (a scientific working group for infectious paediatrics, in whose platform German doctors could register the number and course of paediatric COVID-19 patients they treated). The first cases appeared in March and rapidly increased till a peak in April with above 50 infections per 100,000, a number of 26 hospitalised children nationwide, of which 7 children were in paediatric hospitals in Munich. Following this peak, in May infection numbers decreased and the curve flattened again. After a few months we now know that subsequent to the first wave, hospitalisations remained low throughout the summer until the second wave started in autumn, when infections and hospitalisations rose sharply to a far higher level than in the first wave.

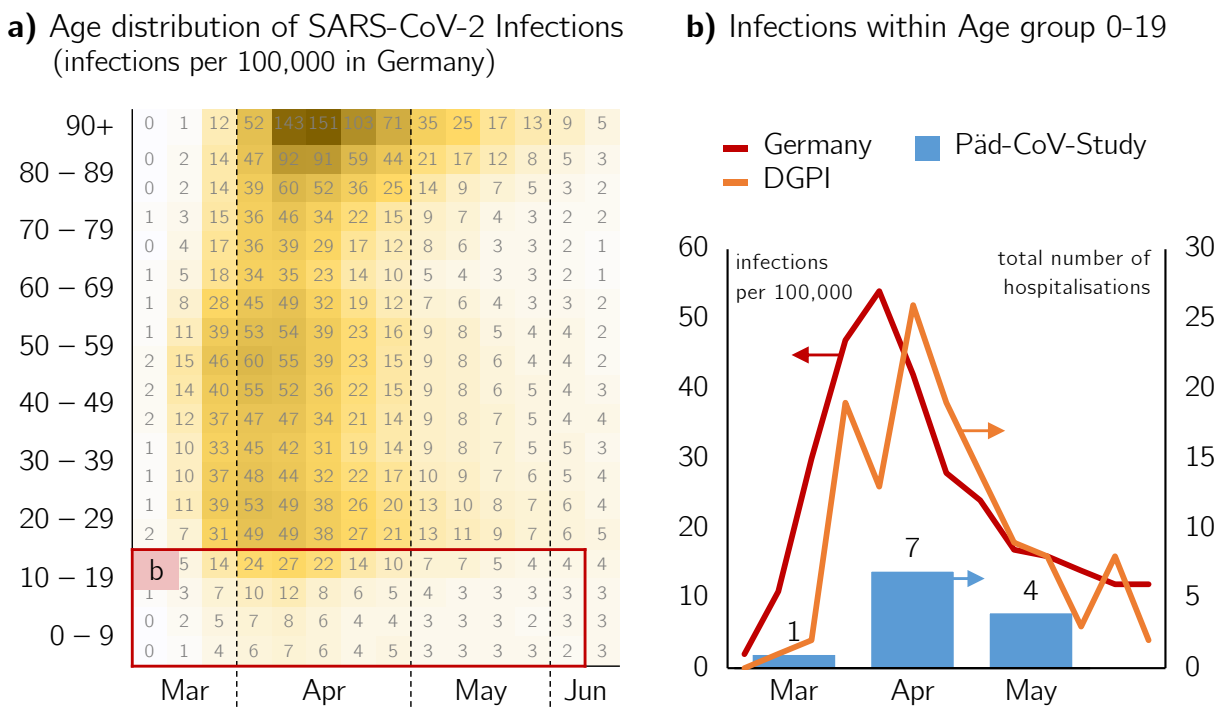

Figure 4.1 (a) A heatmap showing the average number of confirmed SARS-CoV-2 infections in Germany per 100,000 inhabitants and by age group during the first wave of the pandemic (data extracted from RKI [42]), and (b) the rise and fall of the number of nationwide infections within the age group 0-19 alongside the number of children hospitalised with COVID-19 in orange (data extracted from DGPI, only considering normal ward [43]) and recruited as part of this study in blue.

4.1.2. Influence on the number of children visiting the hospital

With the onset of the COVID-19 pandemic, health providers faced the challenges of managing new safety measures and regulations. The Dr. von Hauner Children's Hospital also changed its policy, regulated visiting permits, tightened hygiene measures, checked all staff for symptoms before entering the hospital and carried out a SARS-CoV-2 test on all new admissions. These measures naturally meant an additional burden for the hospital and the staff. As a result, e.g. elective, non-essential interventions were postponed. Another phenomenon occurred regarding the number of patients coming to the emergency room. The A&E department was less frequently visited and patients were less frequently diagnosed with all kinds of infections.

Treatment cases – Figure 4.2 (a) shows the number of patients visiting the Dr. von Hauner Children's Hospital from February to July in 2018, 2019 and 2020. It includes patients presenting via the A&E unit and the paediatric outpatient department, as well as those further admitted as inpatients in thousands. In February, the numbers were close to each other, with a 6% difference to 2019 and an 8% difference to 2018. With onset of the first wave, there was a significant drop in the number of patients by 40% in March, by 51% in April and by 38% in May compared to 2019. Compared to 2018 the number of patients decreased by 44% in March, by 53% in April and 42% in May. On average, the number of patients in the first wave 2020 decreased by 43.3% (P=.017) compared to 2019 and by 46.7% (P=.012) compared to 2018. More detailed explanations of how the P-values were calculated are given in Table 4.1.

Infections – Figure 4.2 (b) shows the number of patients diagnosed with infectious diseases according to ICD-10 codes (International Statistical Classification of Diseases and Related Health Problems) in thousands. The following codes were used for creating the figure: COVID-19, virus detected (U07.1); COVID-19, virus not detected (U07.2); Post-COVID-19 condition, unspecified (U07.4); multisystemic inflammatory syndrome

associated with COVID-19, unspecified (U07.5); fever of other and unknown cause (R50.-); acute Rhinopharyngitis (J00); acute Bronchitis (J20.-); acute Bronchiolitis (J21.-); acute lower respiratory tract infection, unspecified (J22); viral and other specified intestinal infections (A08.-); other and unspecified Gastroenteritis and Colitis of infectious and unspecified origin (A09.-); viral disease of unspecified localisation (B34.-); other and unspecified infectious diseases (B99); acute Sinusitis (J01.-); acute infections in several or unspecified localisations of the upper respiratory tract (J06.-); Influenza due to seasonal detected influenza viruses (J10.-); Influenza, viruses not detected (J11.-), Viral pneumonia, not elsewhere classified (J12.-); severe acute respiratory syndrome (U04,-). In February 2020, the month before onset of the first wave, there were 3% more cases in comparison to 2018, and 37% less cases in comparison to 2019. Regarding the first wave there were 54% less cases in March, 87% less in April and again 87% less cases in May than in 2019. Compared to 2018 there was a decrease in cases in March by 44%, in April by 78% and in May by 77%. Averaged over the months March to May, this results in a drop of 74.2% (P=.013) compared to 2019 and 62.2% (P=.005) compared to 2018.

Surgery – Figure 4.2 (c) shows the number of operations performed in the paediatric hospital and the paediatric surgery hospital in Munich. In February before the outbreak began, the number of operations were 158 in 2018, 163 in 2019 and 158 in 2020. After the outbreak, the number of operations decreased by 36% in March, 31% in April, and 34% in May compared to the previous year. The drop decreased to 20% in June and to 13% compared to 2019. Averaged over the first wave, there were 34% (P=.007) fewer operations in the paediatric hospital compared to 2019. In comparison to 2018 there was a decrease of operations by 21% in March, 30% in April, 20% in May, 31% in June and 11% in July. Thus, on average 24% (P=.025) fewer operations were performed during the first wave than in the 2 years before.

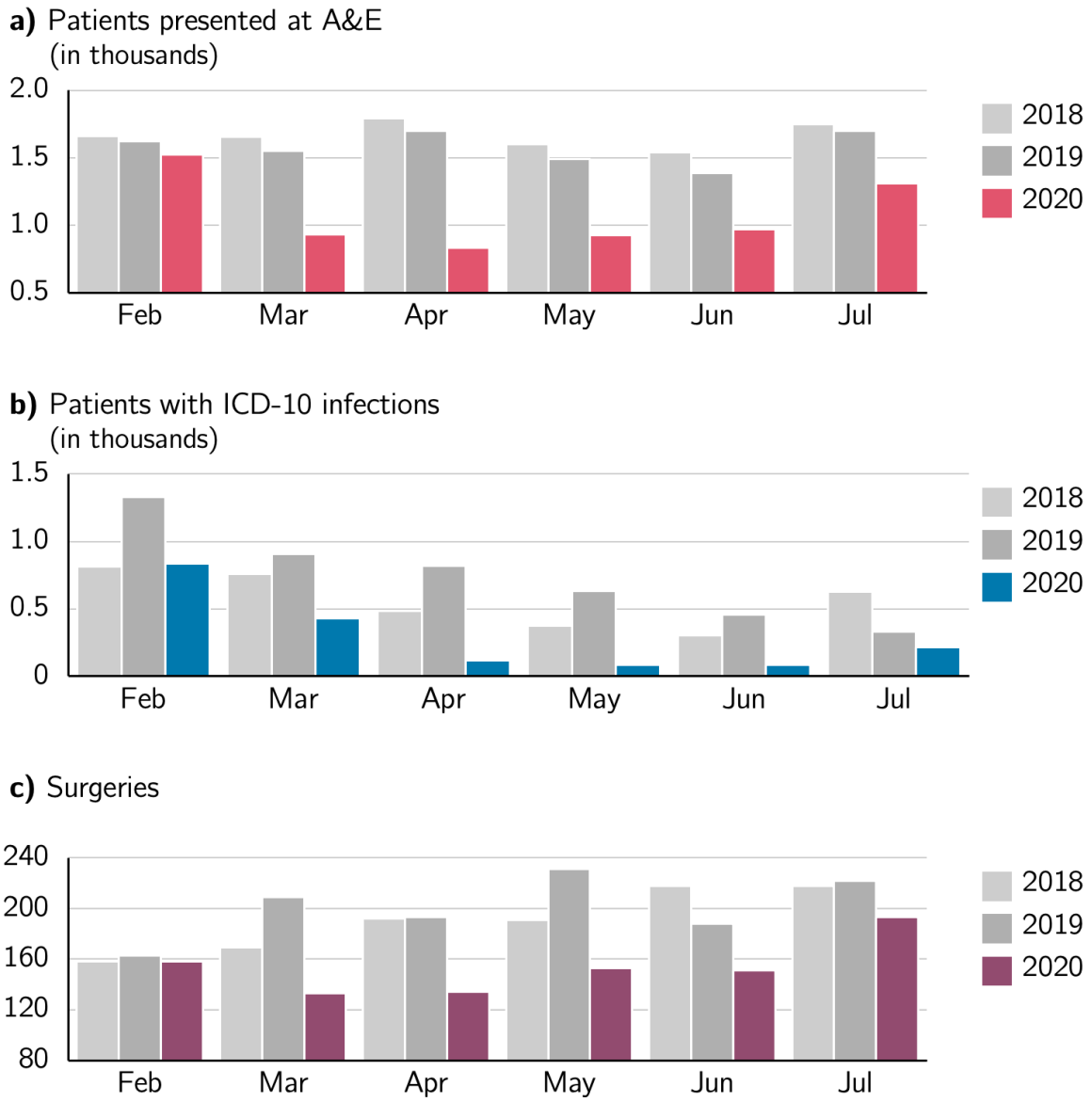

Figure 4.2 shows the numbers of 3 different events at the Dr. von Hauner Children's Hospital, comparing the last 3 years from February to July: (a) The number of patients in thousands who presented at A&E. (b) The number of patients in thousands with an infection according to ICD-10 (the included ICD-10 diagnoses are listed in the text below). (c) The number of surgeries performed.

T-test: paired two samples for means								
	2018			2019			2020	
	Mean	SD	*P*-value	Mean	SD	*P*-value	Mean	SD
Pat. presented at A&E	1682	99	0.012	1581	105	0.017	897	55
Pat. with ICD-10 infections	538	198	0.005	787	140	0.013	211	192
Surgeries performed	184	13	0.025	211	19	0.007	140	12

Table 4.1 The performed T-test (paired two samples for means) provides the mean, standard deviation (SD) of the number of (a) patients, that were admitted to the A&E, (b) patients, that presented with predefined ICD-10 infections and (c) operations performed in the hospital averaged over the months MARCH-MAY and additionally the *P*-values (*P*(T<=t) two-tail) of 2018 and 2019 comparing to 2020. To avoid the bias of multiplicity through multiple testing the level of significance is adjusted with the Bonferroni Correction to ***P'*=.0083** by dividing the usual level of significance to adjust (α=.05) through the number of tests performed (n=6) → $\frac{\alpha}{n} = \frac{0.05}{6} = 0.0083$.

4.2. Manifestation of COVID-19 in children

This Chapter presents data characterizing the study cohort, including demographic factors, clinical parameters and providing insights into the differences and similarities of the infected children and their manifestation of disease.

4.2.1. Cohort diversity

Table 4.2 shows an overview of patient characteristics and health impairment of the cohort recruited for this study.

Clinical presentation The cohort (n=12) was classified by *health status* according to recognized WHO and NIH categories (see Chapter 0): (1) Asymptomatic, (2) Mild & Moderate and (3) Severe. The categories consisted of 3, 7 and 2 children respectively, ranging in age between 0 and 18 years old.

Symptoms All children in the "Mild & Moderate" and "Severe" groups reported fever during infection. Other frequently occurring symptoms in these two groups included: cough, rhinitis and fatigue. The "Severe" group exceeded the length of symptom duration by a factor of 3 (19 days) compared to the "Mild & Moderate" group.

Pre-existing conditions – No prior pulmonary disease or Diabetes mellitus was reported for any patients in the cohort. Both children in the "Severe" group had a previous cardiovascular disease. There were patients with other pre-existing conditions in each group. These include pre-existing conditions of any organ system and are detailed in the respective groups.

Therapy – The length of hospital stay of the "Severe" group exceeds that of the "Mild & Moderate" group by a factor of 3, and that of the "Asymptomatic" group by a factor of 4. The average length of hospitalisation of the "Asymptomatic" group (4 days) was only slightly less than that of the "Mild & Moderate" group (5 days).

Origin of infection – In the "Severe" group, the origin of the infection of both children was unknown and none of the family members were tested positive. All children in the

"Asymptomatic" group and 83% in the "Mild & Moderate" group had concurrent SARS-CoV-2 infections in the family and were traceable to a single person in 50% and 71%, respectively.

Cohort n=12				
Clinical presentation		**Asymptomatic**	**Mild&Moderate**	**Severe**
Characteristics				
Sample size (n)		3	7	2
Sex (% male)		100%	29%	50%
BMI (kg/m²)		20,0	19,4	16,4
Age	0 – 2	0	1	1
	3 – 6	0	2	1
	7 – 14	2	0	0
	15 – 18	1	4	0
Symptoms				
Duration (days)		0	6	19
Fever			100%	100%
Cough			100%	50%
Shortness of breath		0%	29%	50%
Rhinitis			57%	100%
Fatigue			43%	100%
Pre-existing conditions				
Pulmonary		0%	0%	0%
Cardiovascular			14%	100%
Others		33%	71%	100%
Therapy				
Inhospital stay (days)		4	5	18
Antibiotics		33%	43%	100%
Suppl. Oxygen		0%	14%	50%
Antiviral Therapy		0%	0%	50%
Index case				
Index case known		50%	71%	0%
Index case in family		100%	83%	0%

Table 4.2 shows the cohort divided into three groups "Asymptomatic", "Mild and Moderate" and "Severe" according to their clinical presentation, giving an overview to general characteristics, symptoms, pre-existing conditions, therapy and specific information. For note: The figures refer to the available data, missing information was not included in the percentage.

4.2.2. Group 1: Asymptomatic infection

Table 4.3 shows two types of patients that can be found in the "Asymptomatic" group: (1) those without any health restrictions, and (2) those who developed recent symptoms that required treatment (independent of a SARS-CoV-2 infection).

Characteristics – All three patients are male and between 10 and 14 years old. The BMI ranges from 16.9 to 22.5 kg/m^2, which corresponds to normal weight for all of them according to the age percentile.

Hospitalisation – A SARS-CoV-2 PCR test was carried out on both children without any health restrictions, due to a hospital visit with a sick family member. Both children were admitted as inpatients for social reasons, as care at home (refugee home and single mother in hospital) could not be guaranteed. The third child was tested positive when he came for readjustment of his epilepsy medication after cerebral seizure.

Symptoms and Therapy – The two children without health impairment had no symptoms and did not receive any therapy. The third child had scabies with a bacterial superinfection in addition to his asymptomatic SARS-CoV-2 infection. This required treatment with systemic antibiotics and local ointments.

Pre-medication and conditions – Except for one child's epilepsy, treated with Lamotrigine and Levetiracetam, there were no other pre-existing conditions in this group.

Asymptomatic Infection (n=3)			
Archetyp	**Without any Health Impairment**		**With further Health Impairment**
Patient Reference	COV004	COVS008	COVS005
Characteristics			
Sex	male	male	male
Age (years)	12	10	14
BMI (kg/m²)	16,9 (normal)	20,6 (normal)	22,5 (normal)
Hospitalization			
Virus detection event	with ill mother	A&E with ill sibling	hospital routine swab
Performing hospital	Harlaching/TUM	Schwabing TUM	Schwabing TUM
Reason for hospitalisation	social indication	social indication	epilepsy medication adjustment
Days of inhospital stay	6	1	6
Current diagnosis	asympt. SARS-CoV-2 infection	asympt. SARS-CoV-2 infection	asympt. SARS-CoV-2 infection, cerebral seizure, scabies with bact. Superinfection
Symtoms and Therapy			
Covid-19 assoc. symptoms	none		none
Other symptoms	none		itching in skabies
Covid-19 specific therapy	none		none
Other therapy	none		Cephalexin, Infektoscab, Fucidin + Cortisone ointment
Laboratory values	NA	CRP!, D-Dimer!	CRP! Eosinophils!
Pre-medication and conditions			
Regulary taken medication	none		Lamotrigin, Levetriacetam
Pre-existing conditions	none		idiopatic epilepsy

Table 4.3 Characteristics and health care information from the hospital stay during the infection of the 3 asymptomatic study participants, clustered into archetypes according to the presence of any health impairment. Elevated laboratory values are marked with (!).

4.2.3. Group 2: Mild & moderate infection

Before the presentation of the group "Mild & Moderate" as a whole, Table 4.4 shows in addition three archetypes of patients in detail I chose as proxies for: (1) those with no pre-existing conditions, (2) those with mild pre-existing conditions, and (3) those with severe pre-existing conditions.

Symptoms – All children showed elevated temperature or high fever (38-40°C) and rhinitis. Other frequently occurring symptoms in these two groups included diarrhoea and fatigue. One child appeared a sudden onset of numbness and paralysis of the facial nerve, which was cause for admission. She developed a facial nerve palsy with painful trigeminal neuralgia.

Therapy and Laboratory Values –The child with severe pre-existing conditions (mentioned in Table 4.4 below) was monitored for 13 days due to his high risk profile and received Azithromycin prophylaxis. The girls facial nerve palsy improved considerably under physiotherapy and almost disappeared at discharge. Blood values of this group were predominantly normal. A decrease in haemoglobin, neutrophils and monocytes, as well as an increase in triglycerides was observed.

Mild&Moderate illness (n=7)			
Archetypes	**no pre-existing conditions**	**mild pre-existing conditions**	**severe pre-existing conditions**
Patient Reference	COV001	COVS007	COV009
Characteristics			
Sex	male	female	male
Age (years)	0	17	18
BMI (kg/m²)	15,6 (slight underweight)	23,1 (normal)	18,4 (slight underweight)
Hospitalisation (days)	3	4	13
Symptoms			
Fever (max. temp.)	40°C	38°C	38,4°C
Respiratory tract	cough, rhinitis	rhinitis	cough, rhinitis
Gastrointestinal tract	diarrhoea	diarrhoea	none
Neuronal symptoms	fatigue	facial nerve paralysis, fatigue, headache, ageusie & anosmie	none
Max duration (days)	5	12	2
Therapy and Lab. Values			
Laboratory values	Hb- Neutrophils- Triglycerides!	Monocytes -	Hb- Leucocytes-
Therapy	Paracetamol	physiotherapy	monitoring due to high risk, Paracetamol, Azithromycin
Pre-medication and conditions			
Regular medication	none		**Mycophenol Mofetil**, Hydrochlorothiazid, ...
Pre-existing conditions	none	pollen allergy	Alagille-syndrome, renal insufficiency grade III, liver transplanted, pers. left vena cava, pers. foramen ovale, ...
Performing hospital	Hauner LMU	Schwabing TUM	Großhadern LMU

Table 4.4 Characteristics and health care information from the hospital stay of study participants with mild symptoms as a proxy for 3 different degrees of pre-existing conditions. (Hb) means Haemoglobin, (-) means decrease and (!) increase of the laboratory value. Values of the rest of the cohort are addressed in the following.

Mild and moderate infection – Summary of symptoms

Symptoms were assessed at admission with the clinical survey and supplemented by discharge letters and nursing reports. Figure 4.3 depicts the symptoms before and during hospitalisation. The grey-shaded area marks the period of hospital stay. Each patient from the group "Mild & Moderate" is shown in a separate column (except for COV022 as discussed below).

Type – Fever was the most common symptom and occurred in all children, with a time span of 1 to 4 days. Second most common symptoms: cough, rhinitis, fatigue and headache. Two out of five children reported about: Shortness of breath, sore throat and diarrhoea. The symptoms 'Abdominal pain' and 'Emesis' were listed in the survey but were not reported by any child. One child (see COVS007 in Table 4.4) also developed a facial palsy. One five-year old girl (COV005) showed no symptoms during her infection except fever the day before admission. This is assumed to be due to the chemotherapy she received at that time for her acute lymphatic B-cell leukaemia (ALL-B).

Frequency – Symptom onset reached from -10 to +1 days before/after hospital admission. A seventeen-year-old, previously healthy girl (COV008 in Figure 4.3) showed with 13 days the longest duration of symptoms, while it ranged on average from 4 to 7 days. All children developed 5 to 6 symptoms, with exception of the immunosuppressed patient (COV009), who is noticeable for developing fewer symptoms for a shorter period of time compared to the others.

Outlier – The one child excluded from Figure 4.3 is a three-year old girl (COV022), which suffered from transfusion-dependent beta-thalassemia and was tested SARS-CoV-2 positive during her visit for a blood transfusion. She was then sent home and was only included in the study when she was hospitalised again 2 weeks later. At that time, she was asymptomatic and only had antibodies against SARS-CoV-2, but no virus could be detected by PCR. The date of onset and duration of the symptoms during her infection could no longer be reconstructed exactly.

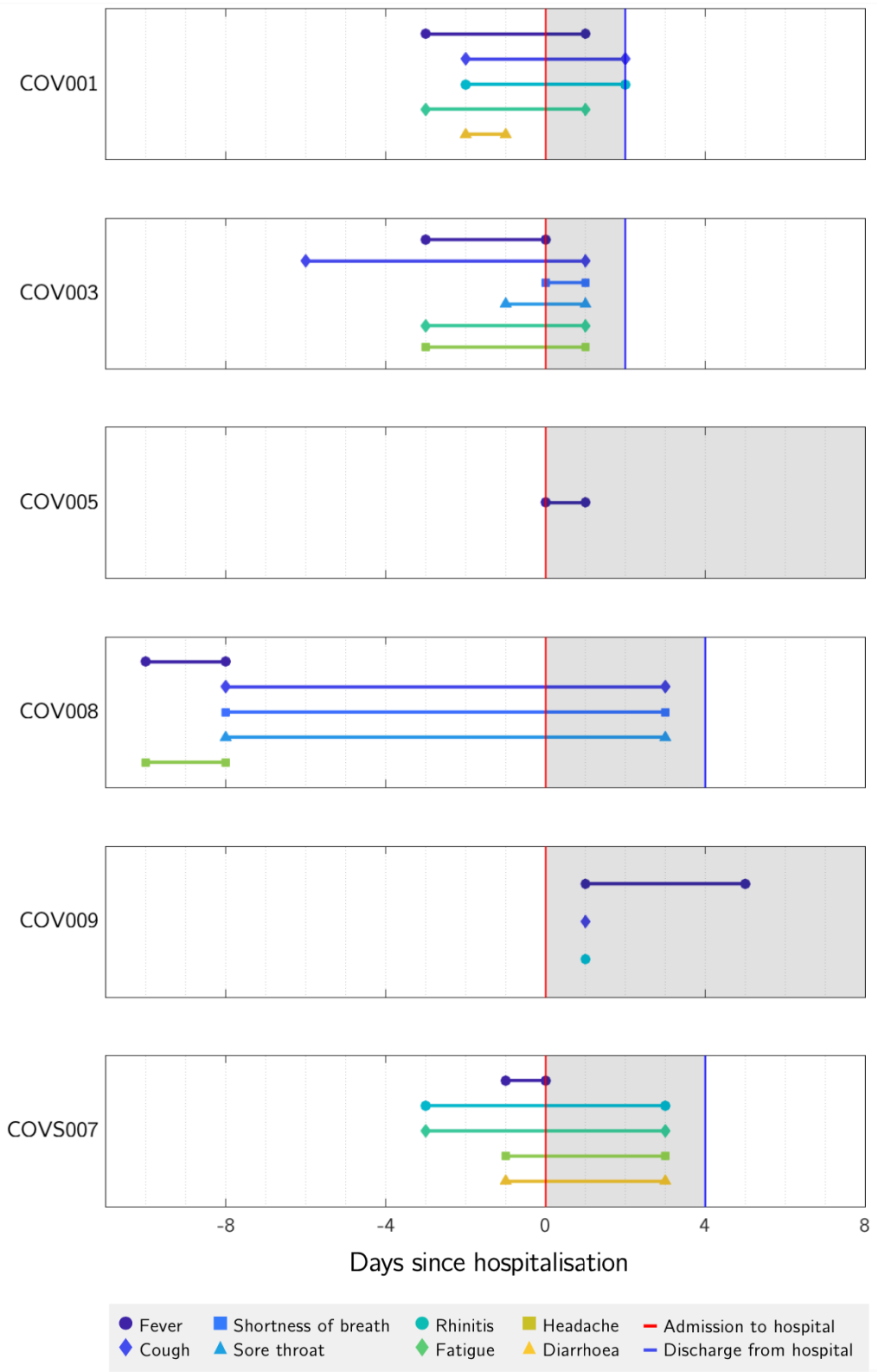

Figure 4.3 Type and duration of symptoms in relation to the time of hospitalisation in the "Mild & Moderate" cohort. Patients COV001, COVS007 and COV009 can be found in Table 4.4 above - exemplary for three different/ascending grades of health preload. *COV022 is not shown in the graph. Note: fever in COV009 appeared on day 1 and day 5, in between he was largely within normal temperatures and is counted as "2 days of fever" in further calculations.

4.2.4. Group 3: Severe infection

Table 4.5 shows the study cohort (n=2) with severe illness. Both children developed hyperinflammatory syndromes. One child underwent a severe course of COVID-19 with sHLH, whereas the other child developed SARS-CoV-2 antibodies during hospital stay but had no detectable viral load. By definition this is not COVID-19 but could be SARS-CoV-2 associated PIMS (see Hyperinflammatory in Section 1.1.3).

Characteristics – The children, one boy and one girl belonged with 1 and 3 years both to the younger of the study cohort.

Symptoms and Therapy – Figure 4.4 shows the symptom duration during hospital stay: Both children showed high fever with temperatures above 40°C. Respiratory symptoms were developed by both (rhinitis), whereas they were much more pronounced in the child with COVID-19 with additional cough and shortness of breath. The gastrointestinal system was also affected in both children: the severe COVID-19 patient had nausea and emesis in his early days of illness, the PIMS patient additionally developed an accompanying nephritis. Both cases progressed into a hyperinflammatory syndrome, which required immunosuppressive treatment. More detailed therapeutic measures of the severe COVID-19 patient will follow in Chapter 4.2.5.

Pre-medication and conditions – Both children presented with cardiovascular PECs (pre-existing condition) including: s.p. atrioventricular septal defect correction (AVSD) and pulmonary hypertension in COV006, and atrial septal defect (ASD) and persistent foramen ovale (PFO) in COVS009. Further PECs consisted of: Epidermolysis bullosa, iron deficiency anaemia (COVS009) and Down Syndrome (COV006).

Severe illness (n=2)		
	Severe COVID-19	**PIMS**
Patient Reference	COV006	COVS009
Characteristics		
Sex	male	female
Age (years)	3	1
BMI	16 (normal)	16,8 (normal)
Hospitalisation (days)	21	14
Symptoms		
Fever (max. temp.)	40,2°C	40°C
Respiratory tract	Cough, Rhinitis, Shortness of breath	Rhinitis
Gastrointestinal tract	Emesis	accompanying Nephritis, Emesis
Neuronal symptoms	Fatigue	NA
Critical symptoms	hyperinflammatory syndrome, severe ARDS, sHLH	hyperinflammatory syndrome, sepsis
Max duration (days)	24	13
Therapy		
Antibiotics	Ampicillin + Azithromycin, Piperacillin/Tazobactam	Meropenem + Vancomycin Piperacillin/Tazobactam + Flucloxacillin
Antivirals	Remdesivir	none
Immunomodulators	Prednisolon, IVIG	Prednisolon, IVIG
Ventilation support	Intubation, mechanical ventilation	none
Pre-medication and conditions		
Pre-existing conditions	Down Syndrome, s.p. AVSD correction, s.p. pulmonary hyertension	Epidermolysis bullosa, hypoferric anaemia, ASDII
Regular medication	none	Morphin retard , Esomeprazol ...
Performing Hospital	Hauner/Großhadern LMU	Schwabing TUM

Table 4.5 Characteristics and health care information about two study participants with severe illness but - by definition presumably different classifications of pathogenesis - categorised into severe COVID-19 with sHLH and PIMS with COVID-19 association. AVSD (Atrioventricular septal defect), ASD (atrio septal defect).

Severe illness – Summary of symptoms

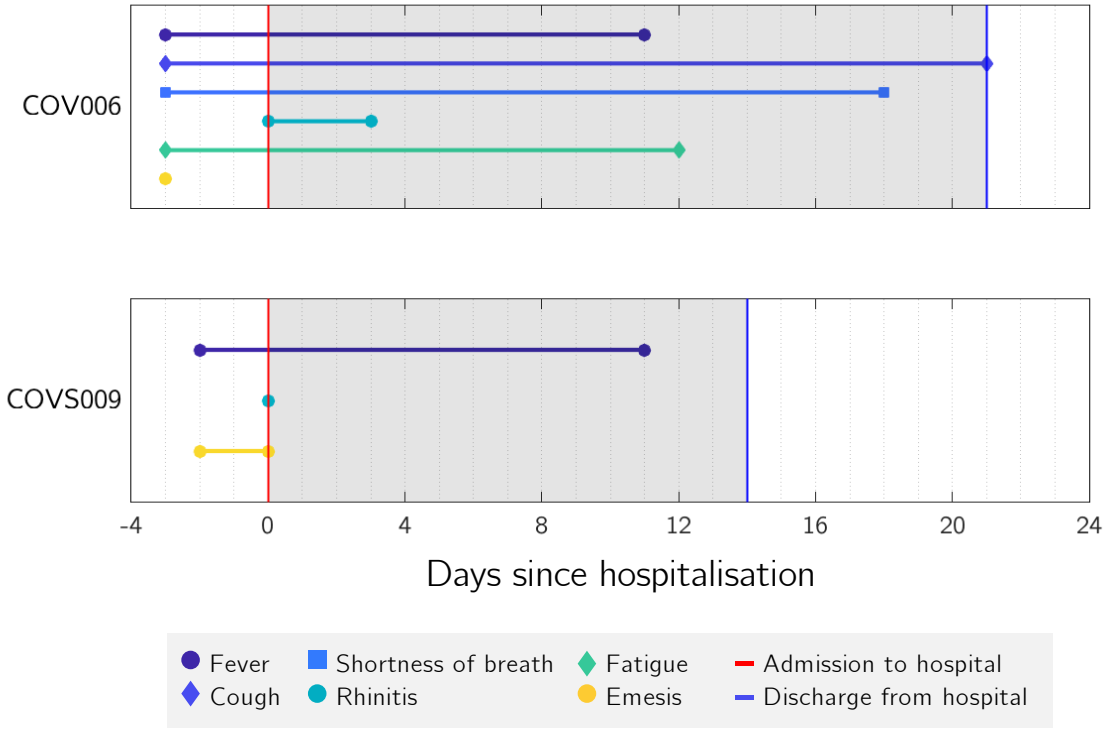

Figure 4.4 Type and duration of symptoms in relation to the time of hospitalisation in the 'Severe' cohort. (a) COV006 represents the child with severe COVID-19, he shows a long period of illness (24 days) with mainly respiratory symptoms, but also fever and fatigue. (b) COVS009 represents the child with suspected PIMS, which prominently appeared with high fever for 13 days, respiratory symptoms were of subordinate role.

PIMS

The 1-year-old girl with Epidermolysis bullosa was admitted to hospital with suspected sepsis. The child was in a reduced general condition with high fever and highly elevated inflammatory parameters (**CRP 20,6 mg/l, Il-6 102.5 ng/l**). A meningitis was excluded through a performed cerebrospinal fluid (CSF) puncture. The PCR throat swab for SARS-CoV-2 was negative and no pathogens were detected in the CSF, blood culture or stool, but the serology was positive for anti-SARS-CoV-2 IgG. Chest X-ray showed a small increase in hyperdensity of striae right perihilar, but she never required oxygen and was cardiorespiratory stable at any time. After 4 days without improvement, the medication with Piperacillin/Tazobactam plus Flucloxacillin was changed to Vancomycin plus Meropenem (for another 8 days). In addition to the persistent fever, a swelling of the lymph nodes and a systolic murmur developed during this therapy. The oral mucositis had not improved since admission and a tubulointerstitial concomitant nephritis was diagnosed. According to the CDC she met 5/6 criteria specified as diagnostic factors for PIMS (attached in Annex 7), as she: (a) was at a young age, (b) was hospitalised for 14 days, (c) had fever for 12 days, (d) had elevated inflammatory markers, and (e) had an association with a SARS-CoV-2 infection. She therefore received two IVIG (intravenous immunoglobulin) doses (2g/kg) and a therapy with cortisone (2mg/kg) was started and continued for 14 days. Due to a previous gastrointestinal bleeding and the severe pre-existing condition, ASA (Acetylsalicylic acid) was not given. With this therapy, the fever decreased, and her general condition improved. After 2 weeks of hospitalisation, she could be discharged to outpatient care, free of fever and in an improved general state of health.

4.2.5. Case study – the course of disease of a child with severe COVID-19

Our 3-year-old patient with descent from Central Africa and Down Syndrome (see Table 4.5) went through several stages of severity during his illness, from moderate symptoms at the beginning to acute life-threatening exacerbation. Of his 21-day hospital stay, he spent 9 days in the PICU (paediatric intensive care unit). Intensive care was necessary for treatment of the viral-triggered hyperinflammatory syndrome and ARDS (acute respiratory distress syndrome).

Course of illness

Figure 4.6 illustrates the course of illness with laboratory parameters, treatment and different phases of his disease. Some of the contents of the following case study have already been published in [1].

Phase I (early infection) – (DAYS: -3 to 0) At the end of March 2020 the child came to the A&E department because of fever, cough, mild shortness of breath, fatigue and emesis three days before admission, but was first sent home again. Despite Salbutamol inhalation, symptoms worsened at home and the child was finally admitted to the general ward. By this time, pulmonary involvement had already begun (phase II).

Phase II (pneumovascular infection) – (DAYS: 0 to 3) At admission the child presented in a reduced general condition with tachypnoea, jugular retraction, increased abdominal breathing, expiratory humming and coarse bubbly rales, increased temperature, lymphopenia (18%), elevated inflammatory parameters with high levels of CRP (15 mg/dl), IL-6 (494 pg/ml), Procalcitonin (22,80 ng/ml) and Ferritin (877 ng/ml). The PCR-test of an oropharyngeal swab was SARS-CoV-2 positive (Ct-values: E-gene 22.52, RdRP-gene 26.07, N-gene 26.00). An immunofluorescence test for RSV

(respiratory-syncytial-virus) and influenza A and B was negative. Origin and timepoint of infection were not known, none of the family members was tested positive.

The chest ray in Figure 4.5 (a) showed: perihilar infiltrations on both sides, centrally accentuated, small-cornered, partially confluent infiltrates, resulting in bronchopneumonia with ventilation dysfunction. Therapy was started with Ampicillin (3x650mg i.v.) and Azithromycin (1x120 p.o.), Salbutamol inhalations, NaCl 0,9% substitution, antipyresis and 1l/min oxygen supply.

During the night to the third day of treatment, his oxygen demand increased to over 3l/min and he became increasingly tachypnoeic (60-100/min). The findings of the computed tomography in Figure 4.5 (b) showed atypical features for a COVID-19 pneumonia but were most likely an atypical pneumonia with potential superinfection. Compared to the X-ray on day 0, there was a marked deterioration, especially in the right upper lobe. Due to his worsened respiration and condition, he was transferred to the PICU in the morning of day 3.

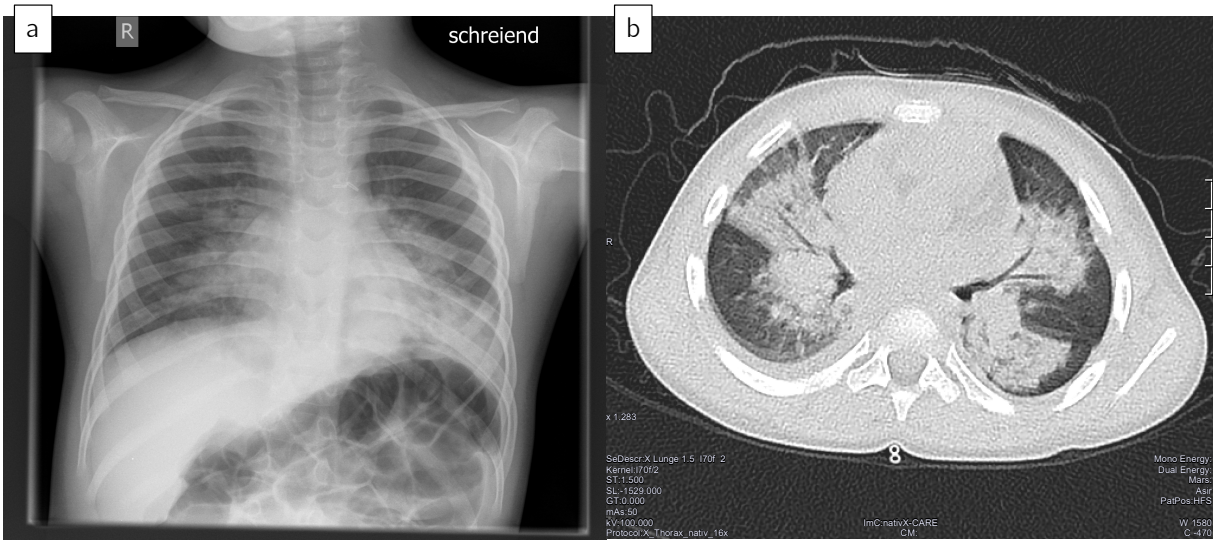

Figure 4.5 (a) chest ray posterior-anterior recorded in sitting position on day 0 of hospital admission, showing confluent infiltrates on both sides leading to bronchopneumonia with ventilation dysfunction. (b) computed tomography recorded on day 3 with features of an atypical pneumonia, with marked deterioration compared to the recording of the 0.

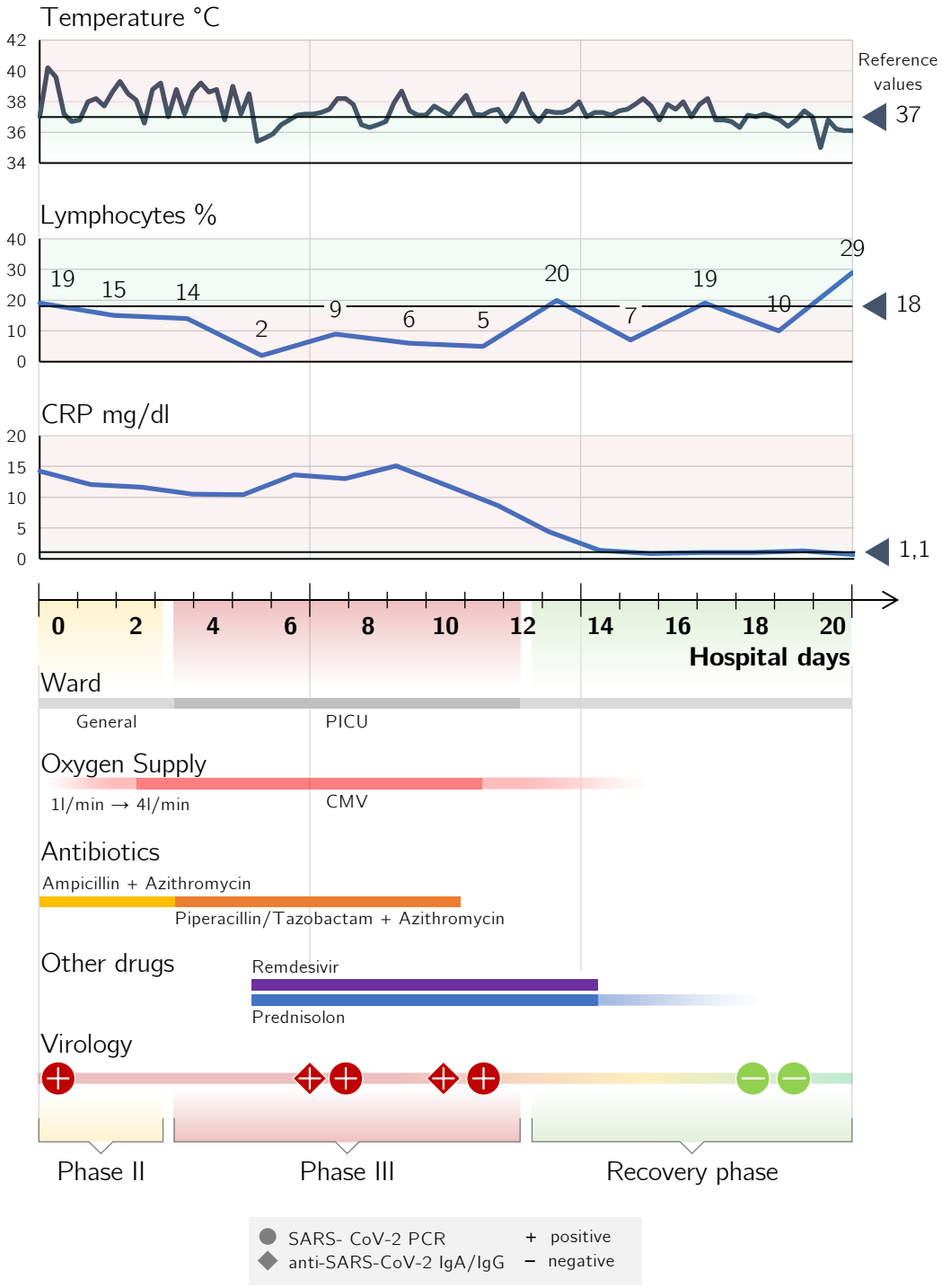

Figure 4.6 showing clinical parameters, treatment and phases of disease of a patient with severe COVID-19 according to the days of hospital stay. Phase I is not shown in the graph as it appeared before hospital admission. For note: the patient received a single IVIG dose on the same day when Remdesivir and Prednisolon were started. Values within the reference range are coloured in green, above/under the reference in red. Reference values: body temperature (<37°C), lymphocytes (18-67 %), CRP (0-1.1 mg/dl). CMV (Continuous mandatory ventilation), CRP (C-reactive protein), IVIG (intravenous immunoglobulins), PICU (paediatric intensive care unit). Parts of this figure (therapeutics, virology) have already been published in [1].

Phase III - Hyperinflammation and critical disease

(DAYS: 3 to 12) The steadily worsened respiratory state of the child developed into an ARDS (acute respiratory deficiency syndrome). Drops in saturation <90% continued and the child was intubated and put on continuous invasive mechanical ventilation under Clonidin at arrival at the PICU.

ARDS – According to the AWMF [44], our patient fulfilled 4/4 criteria of ARDS: (1) the respiratory situation worsened **acutely** during the night of the 3rd day of treatment, (2) the CT image subsequently taken on the day of PICU admission showed a marked deterioration, with pleural effusions and extensive **bilateral consolidations** in all lobes and segments, (3) shortly after intubation, the measured **oxygenation index** of 65.2 mmHg, was already far below the defined limit of <200 mmHg and (4) persisting pulmonary hypertension could be excluded by echocardiography.

Lung injury – The extent of our patient's lung impairment can be measured with the Murray scale (attached in Annex 8). The values obtained on admission to the PICU gave the following score: (a) the chest CT showed large scale consolidations in all segments and lobes (4/4 points), (b) there was severe hypoxia with an oxygenation index of 65.2 (4/4 points), and (c) ventilation was performed with a PEEP of 12 cm H2O (3/4 point). The total score (adding the points and then dividing them by 3) gave a value of 3.33 (>2,5), which corresponds to **severe lung injury** in our patient.

On day 7 the performed SARS-CoV-2 PCR test was still positive (Ct-values in stool 37), now anti-SARS-CoV-2 IgG/IgA could also be detected. As bacterial superinfection was suspected and finally diagnosed (Haemophilus Influenza, Streptococcus Pneumoniae), antibiotic therapy was changed from Ampicillin plus Azithromycin to Piperacillin/Tazobactam (3x1.3g i.v.) plus Azithromycin. Noradrenalin and volume substitution was necessary to control the haemodynamic. The child's state of health therefore, already was highly critical on his 3rd day after admission.

Sepsis/SIRS – Table 4.6 contains criteria, references and the referring values of our patient at the time of admission to the PICU according to the AWMF guideline 'Sepsis in children beyond the neonatal period'. With (1) a body temperature of 39°C, (2) a heart rate of 155 min^-1/bpm and (3) a respiratory rate of 86 /min^-1 (both above 2 standard deviations of the age norm), three of the four possible SIRS categories are fulfilled. (4) Leukocytes were still within the normal range at that time, but already at the lower limit (3.73 G/l).

Four organ systems of our patient were strongly affected by the inflammatory reaction: (i) the cardiovascular and circulation system had to be supported with Noradrenalin, (ii) respiration was insufficient (oxygenation index of 65.2), and therefore he had to be intubated and mechanically ventilated, (iii) platelet count (81/nl) was below the minimum value of 100/nl. (iv)(v) Kidney and liver did not show any dysfunction with values within the normal ranges. (vi) Making a statement about the neuronal system is difficult, as the patient was intubated and anaesthetised with Midazolam quickly at the onset of severe drops in saturation, without first documenting the neurological status using the Glasgow-Coma-Scale.

Definition of SIRS			
Criteria	**Reference**	COV006	
Temperature (T)	>38.5°C or <36.0°C	39°C	✓
Heart rate (HR)	>140 min^-1/bpm	155 min^-1/bpm	✓
Respiratory rate (RR)	>40 /min^-1	86 /min^-1	✓
Leukocytes (WBC)	3.5-14.0 G/l	3.72 G/l	-
> 2 criteria for SIRS			

Definition of SIRS with organ dysfunction			
Criteria		COV006	
Cardiovascular	Catecholamine requirement	Noradrenalin	✓
Respiration	(PaO2/FiO2 < 300)	*pO2/>FiO2= 65.2	✓
	non-selective ventilation	Intubation	✓
Nerval system	Glasgow Coma Scale (GCS) ≤ 11	-	-
Blood	Platelet count < 100/nl	81/nl	✓
Kidney	Creatinine > 2x 0.3-0.4 mg/dl	0.6 mg/dl	-
Liver	Bilirubin (ges.) ≥ 4 mg/dl	Bili – 0.3 mg/dl	-
	ALT (GPT) > 118 U/l	ALT 33 U/l	-

Table 4.6 showing SIRS criteria with references (extracted from the AWMF-register [45]) and how our patient fulfilled 3 out of 4 SIRS criteria with involvement of the cardiovascular-, respiration- and blood-system. *Horowitz ratio/Oxygenation index = paO2/FiO2

In the case of a COVID-19 diagnosis, it is necessary to also think of sHLH if there are signs of SIRS or sepsis.

sHLH – Using the HScore (attached in Annex 9) for calculating the individual risk for a secondary haemophagocytic lymphohistiocytosis (sHLH) for our patient with values from the 5th day of treatment: a maximum body temperature of 38.5°C (33 points), no spleno-or hepatomegaly (0 points), two lineages of cytopenia (Platelet count 73 G/l, WBC 4,75 G/l) (24 points) (he did not receive transfusion products to compensate his anaemia), Triglycerides 151 mg/dL (44 points), Fibrinogen (455 mg/dL) (30 points), Ferritin 7499 ng/ml (50 points), Serum Aspartate Aminotransferase 108 IU/L (19 points), no known immunosuppression (0 points). No bone marrow aspirate was performed (0 points). This total value of 200 points suggests

a high possibility for our child presenting with sHLH (HScores > 169 points have 93% sensitivity and 86% specificity).

Due to this hyperinflammatory syndrome, a therapy with Prednisolone (2 mg/kg i.v.) and a single dose IVIG (1g/kg) was started on the 5th day of treatment. In addition, a therapy trial with the antiviral Remdesivir (1x32.5 mg) via the compassionate use programme was carried out for nine days, as well as other medications used for sedation (Clonidin) and diuretic therapy (Furosemid, Kalium). The chest ray made on the 9th day (Figure 4.7 (a)) still shows bilateral increase of perihilar opacity with adjacent ventilation disturbance in known bronchopneumonia, with infiltrates typical for COVID-19.

Phase of Recovery – (DAYS: 11-21) Still SARS-COV-2 PCR positive (Ct-value 30 in tracheal secretion) and detectable anti-SARS-CoV-2 IgG/IgA on day 10 and 11, the inflammatory parameters decreased under this therapy: CRP 1 mg/dl, Ferritin 413,00 ng/ml, Procalcitonin 0,5 ng/ml, Thrombocytes 183 G/l, IL-6 18.8 pg/ml, Lymphocytes 19%. On day 10, the antibiotic therapy was stopped. The day after, the child was extubated and transferred back to the general ward in a stable condition with 1-liter O2. The therapy with Remdesivir and Prednisolon could both be stopped or faded out after nine days of treatment. On day 15 the O2 supplement was not needed anymore. The chest ray of the last day of stay (Figure 4.7 (b)) showed compared to the previous images a clearly regressive reduction of transparency in the middle and lower lobes and a completely disappeared shading in the apical middle lobe on the right. There remained constantly increased consolidations on the right and a discrete difference in transparency. With two negative SARS-CoV-2 PCR tests and instructions to continue inhaling with Salbutamol, the child could be discharged in a good state of health after 21 days of hospital stay. At follow-up after 6 weeks, the child presented in good general health with no residual symptoms of the infection.

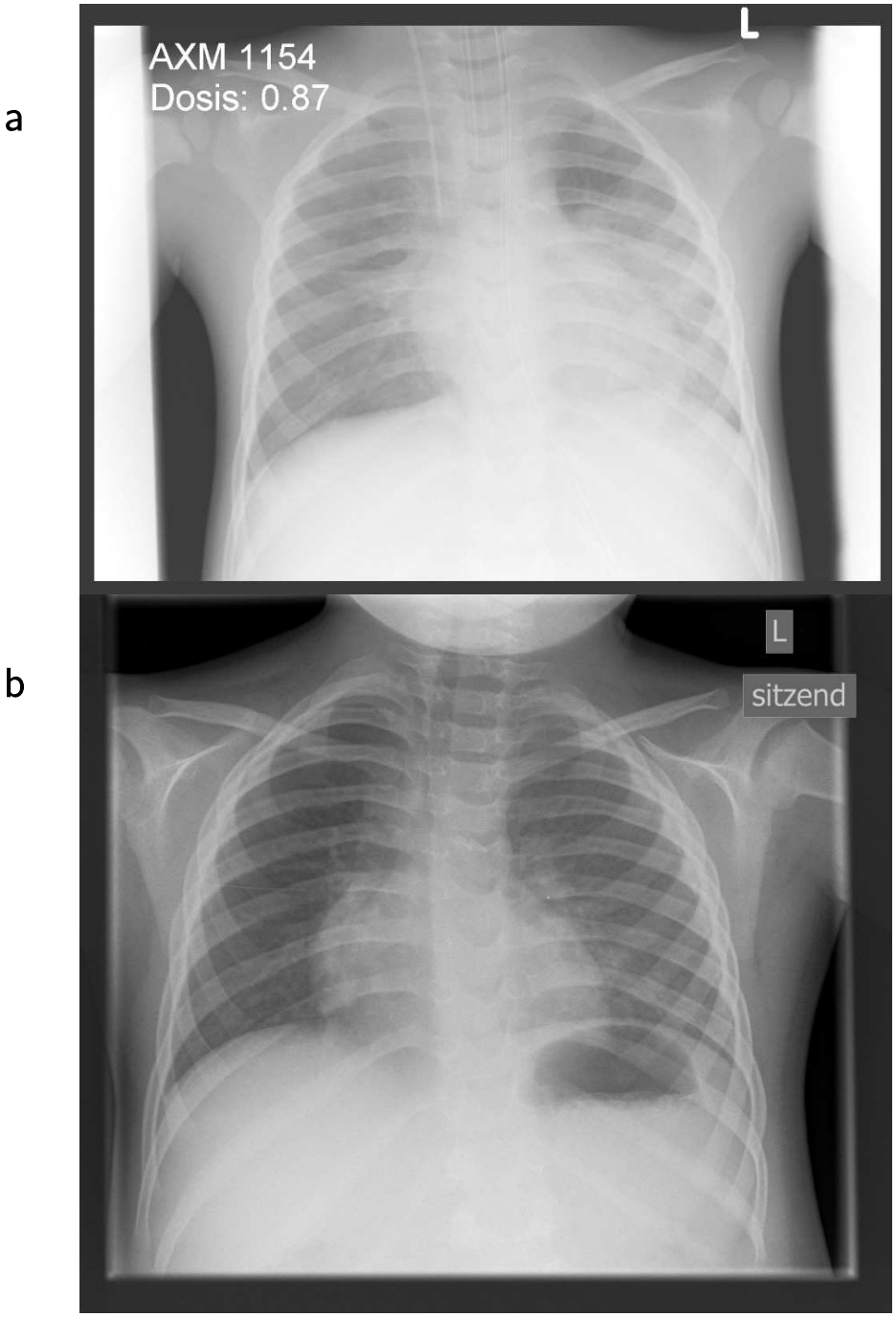

Figure 4.7 (a) chest X-ray from the 9th day of treatment with bilateral shadowing, (b) chest X-ray from the 20th day of treatment showing regressive reduction in transparency in the middle and lower lobes and complete regression of shading in the apical right middle lobe.

4.3. Clinical parameters as predictors for severe COVID-19

In the following section, tables and graphs illustrate the clinical parameters of the 3 groups in comparison. The data shown in this section must be interpreted in the context of the small sample size. Extensive statistical analysis was not performed to avoid biased results that would arise from the small cohort.

	Asymptomatic (n=3)	Mild&Moderate (n=7)	Severe (n=2)
	Mean	Mean	Mean
Specific information			
Sex (% male)	100%	29%	50%
STIKO vaccination	100%	100%	100%
Febrile infections (last month)	0%	40%	0%
Index case known	50%	71%	0%
Index case in family	100%	83%	0%
Bacterial Coinfection	33%	0%	50%
Resp. Vir. Coinfection	0%	0%	0%
Symptoms			
Fever		100%	100%
Cough		100%	50%
Shortness of breath		29%	50%
Rhinitis		57%	100%
Sore throat		29%	50%
Fatigue	0%	43%	100%
Headache		43%	50%
Emesis		0%	100%
Diarrhoea		29%	0%
Abdominal pain		14%	0%
Anosmia&Ageusia		50%	0%
Therapy			
Suppl. Oxygen	0%	14%	50%
Bronchodilatators	0%	29%	50%
Antibiotics	33%	33%	100%
Antiviral Therapy	0%	0%	50%
Syst. Corticosteroids	0%	0%	100%
Pre-existing conditions			
Pulmonary		0%	0%
Cardiovascular		14%	100%
Hematological/Oncological	0%	29%	0%
Allergies		29%	0%
Nephrological		14%	0%
Inborn Error of Immunity		14%	50%
Neurological/Neuromuscular	33%	0%	0%
Diabetes mellitus	0%	0%	0%
Other		14%	100%

Table 4.7 showing the mean (in percent) for the categories including specific information, symptoms, therapy, pre-existing conditions. Figures visualising these contents follow below.

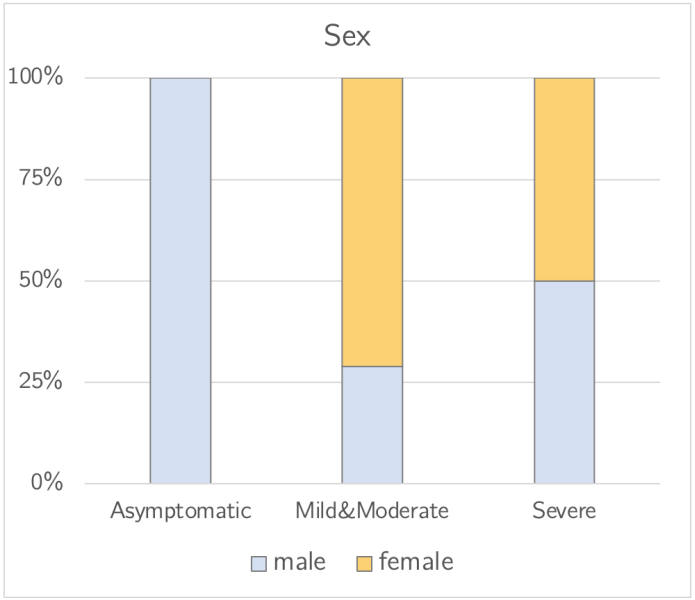

Figure 4.8 bar chart visualising the sex distribution in the three groups in percent.

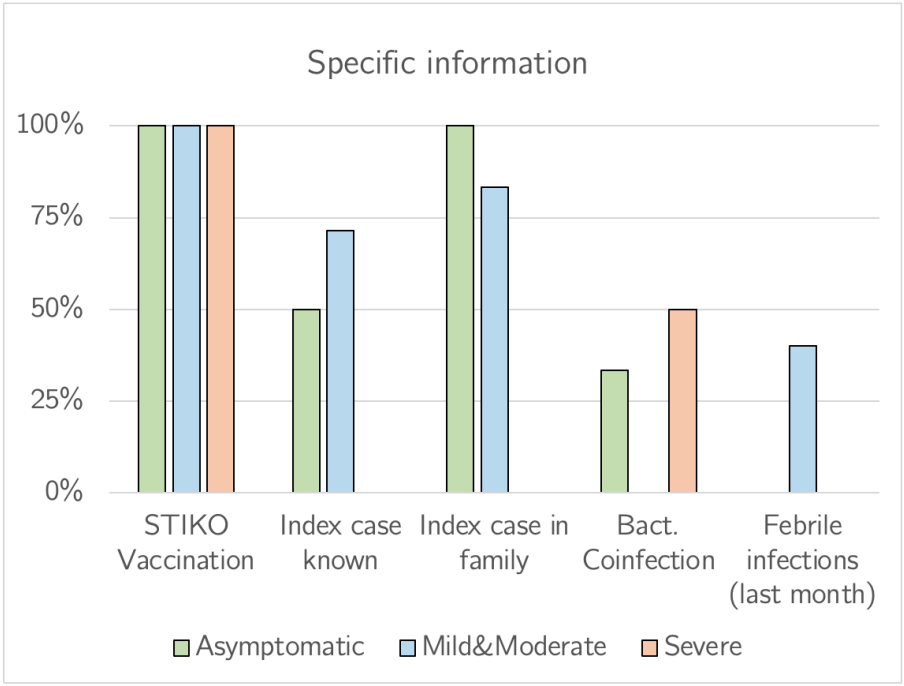

Figure 4.9 bar chart showing the percentage of the cohort with: complete STIKO vaccination status, cases where the index case is known (person that directly transmitted the infection), where bacterial coinfection during illness and previous febrile infections occurred within the last months before hospital admission. Respiratory infections were not reported and are therefore not shown.

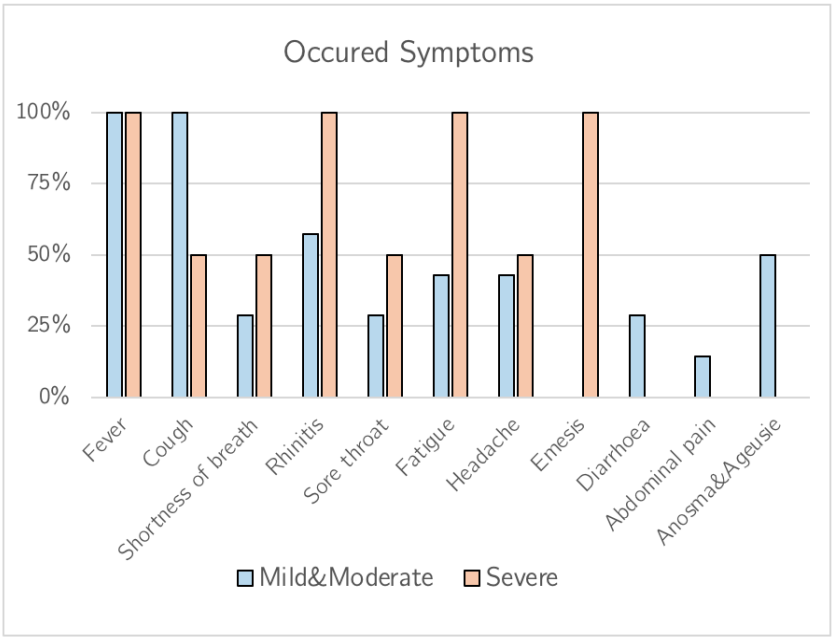

Figure 4.10 bar chart providing an overview of which symptoms occurred at any time during the illness (yes/no). By definition, the "Asymptomatic" group is not shown.

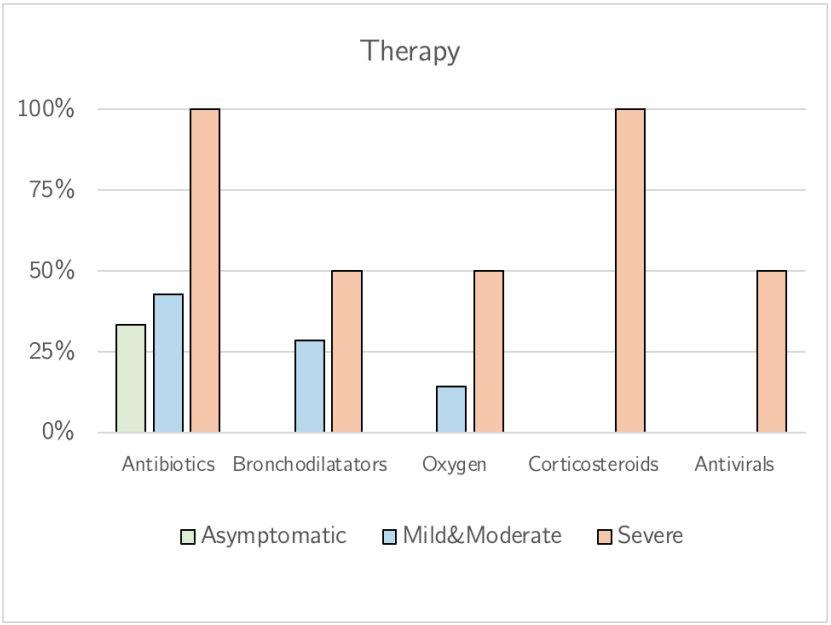

Figure 4.11 Bar chart showing the average of therapeutical measurements such as anti-infectives, systemic corticosteroids, bronchodilatators and supplemental oxygen in the three groups.

Pre-existing conditions – Figure 4.12 shows that children in the "Asymptomatic" group had no pre-existing conditions, except for one child with Epilepsy (33% in "Neurological"). Only 18% in "Mild & Moderate" comparing to 100% in "Severe" presented with cardiovascular pre-existing conditions, namely PFO (persistent foramen ovale), ASD II (atrio septal defect), and AVSD (atrioventricular septal defect). The same situation appears in the category "Other": 18% in "Mild & Moderate" (Alagille-Syndrome) and 100% in "Severe" (including: Down Syndrome, iron deficiency anaemia). The following diseases are included in the categories: "Hematological" (Acute Lymphatic Leukaemia, beta Thalassaemia), "Allergies" (pollen, grasses, house dust mite), "Nephrological" (renal insufficiency class III), Inborn Error of Immunity" (Epidermolysis bullosa).

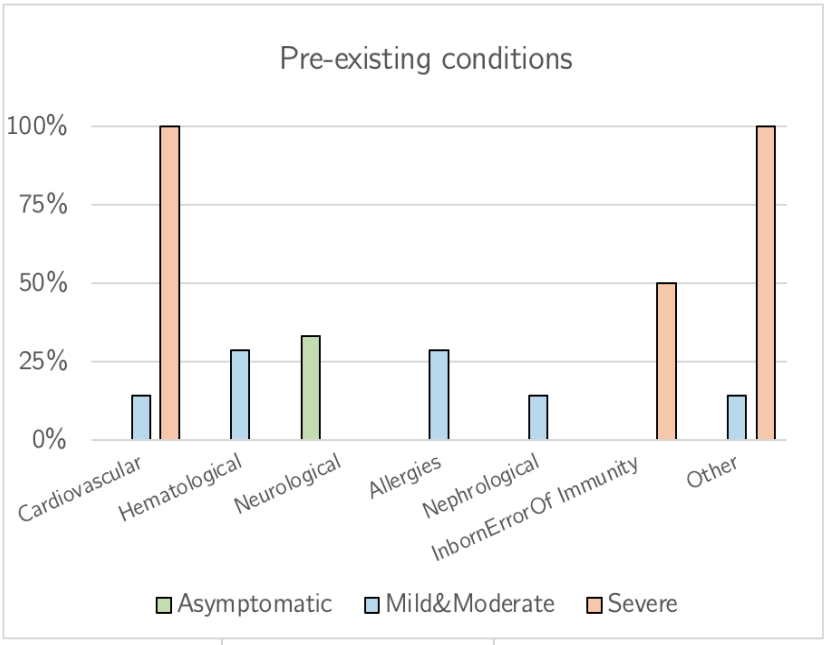

Figure 4.12 bar chart showing the average of reported pre-existing conditions. "Others" include Down Syndrome, Alagille Syndrome, iron deficiency anaemia.

	Asymptomatic (n=3)		Mild&Moderate (n=7)		Severe (n=2)	
	Mean	SD	Mean	SD	Mean	SD
Characteristics						
Age	12,0	2,0	10,7	7,7	2,0	1,4
BMI (kg/m²)	20	2,9	19	3,5	16	0,5
Duration of Symptoms (days)						
Longest Symptom			6	4,3	19	7,8
Fever			2	1,4	14	0,7
Cough			3	4,4	12	17,0
ShortnessOfBreath			2	4,2	11	14,8
SoreThroat			2	4,1	0	0,0
Rhinitis	0	0	2	2,5	1	0,7
Diarrhoea			1	1,5	1	1,4
Emesis			0	0,0	2	0,7
Headache			1	1,9	0	0,0
Anosmia/Ageusia			3	4,5	0	0,0
Fatigue			2	2,6	8	10,6
Duration of Treatment (days)						
Inhospital stay	4	2,9	5	4,5	18	4,9
Antibiotics	2	4,0	2	3,4	12	0,7
Syst. Corticosteroids	0	0,0	0	0,0	12	3,5
Suppl. Oxygen	0	0,0	1	1,5	7	9,9
Bronchodilatators	0	0,0	1	1,7	7	9,9

Table 4.8 comparing the average (mean value) and SD (standard deviation) of the three different groups of severity.

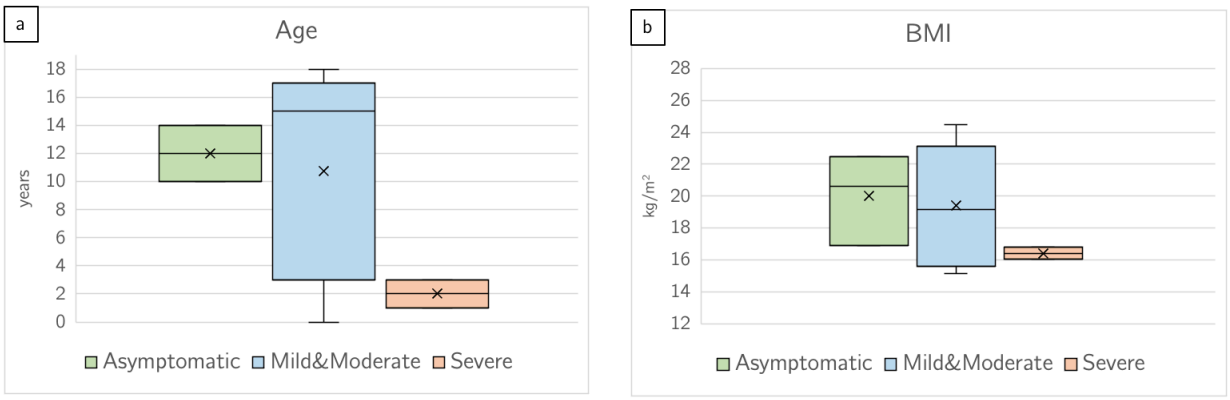

Figure 4.13 (a) boxplot (from bottom to top consisting of: minimum, lower quantile, mean (x), median, upper quantile, maximum), showing the age distribution of the three groups and (b) showing the differing Body-Mass-Index in kg/m² of the three groups.

Duration of Symptoms – Figure 4.14 shows the minimum of duration of symptoms in "Severe" (13 days) is still higher than the maximum (12 days) of "Mild & Moderate". A significant difference can be observed in duration of fever in "Severe" with a mean of 14 days compared to a mean of 2 days in "Mild & Moderate". The span of cough (min=0 days, max= 24 days) and shortness of breath (min=0 and max=21 days) in "Severe" is high, as one child developed symptoms for a long period of time, whereas the other did not at all. The duration of sore throat, headache and anosmia and ageusia could not be assessed in the "Severe" group due to the difficulty of measuring them objectively by external judgement.

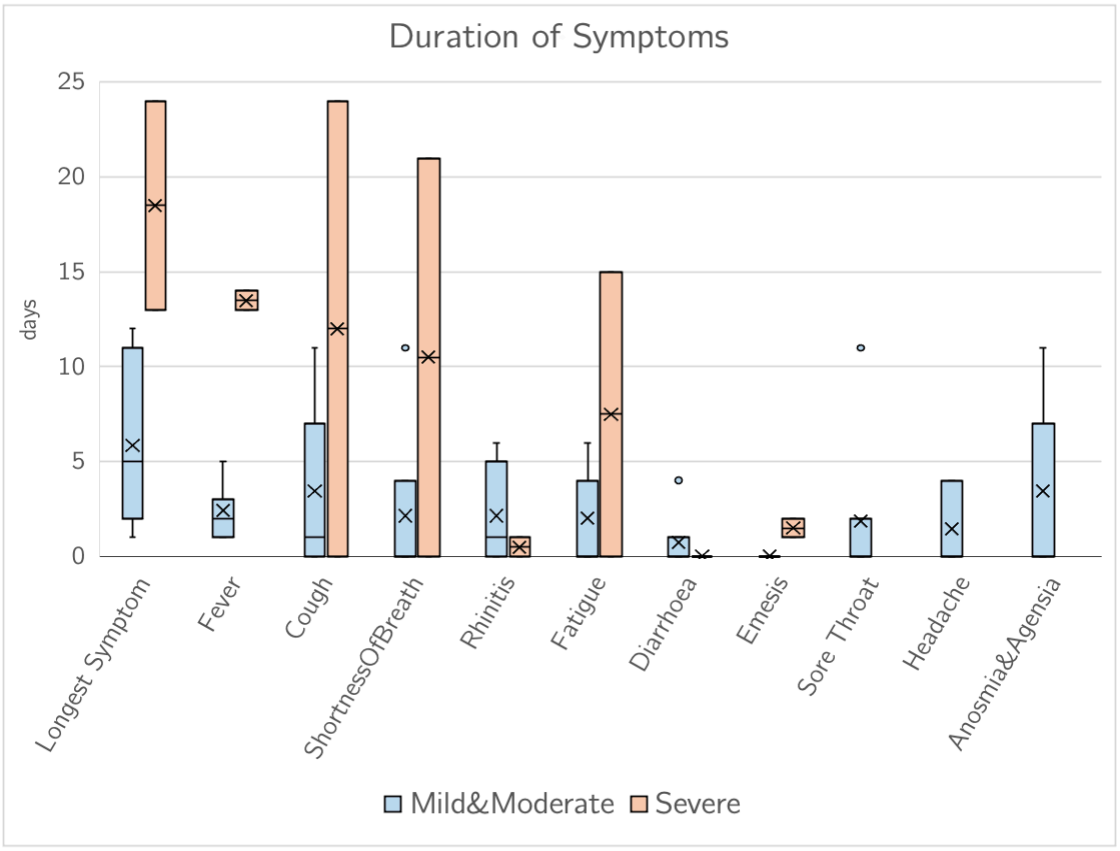

Figure 4.14 boxplots showing the duration of symptoms in days, comparing the groups "Mild & Moderate" and "Severe".

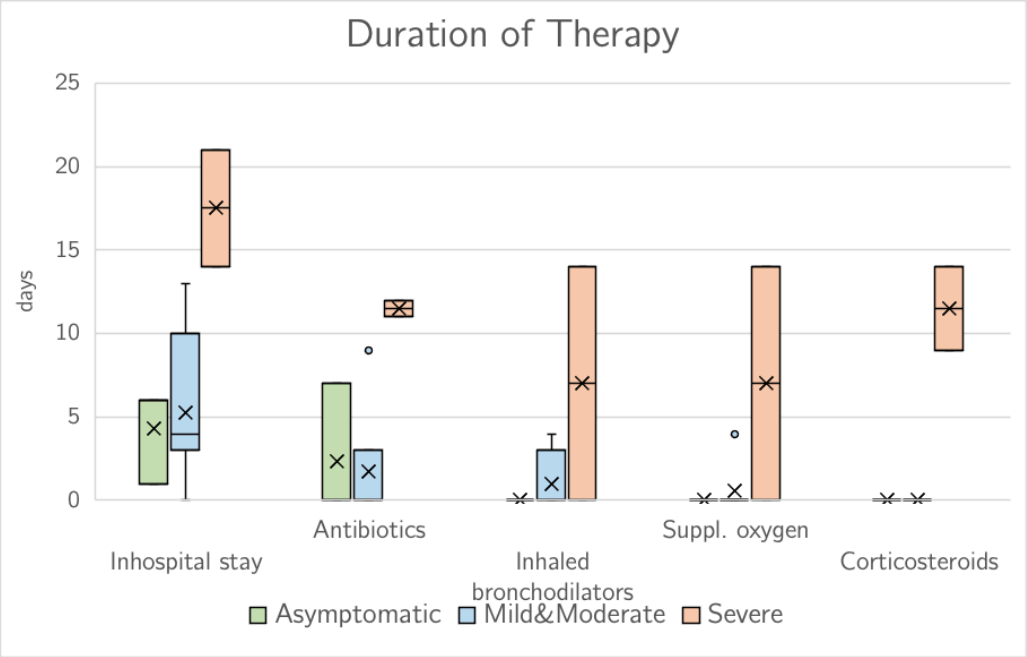

Figure 4.15 boxplot showing the duration of therapeutic measures in days that were applied within the different groups.

Laboratory values – Figure 4.16 shows that inflammatory parameters in the group "Severe" were significantly elevated. CRP reached a maximum of 20.6 mg/dl and a mean value of 17.4 mg/dl on admission day in "Severe", compared to a mean of 0.17 mg/dl in "Mild & Moderate". IL-6 reached a maximum up to 494 pg/ml and a mean of 298.3 pg/ml in "Severe" compared to a mean of 4.54 pg/ml in "Mild & Moderate". Procalcitonin with mean=12 ng/ml in "Severe" compared to mean=0.08 ng/ml in "Mild & Moderate". Haemoglobin values were decreased in "Severe" 10.25 g/dl, which already fell below the minimum range of 10.8 g/dl, whereas the "Mild & Moderate" were still within the range with 11.8 g/dl.

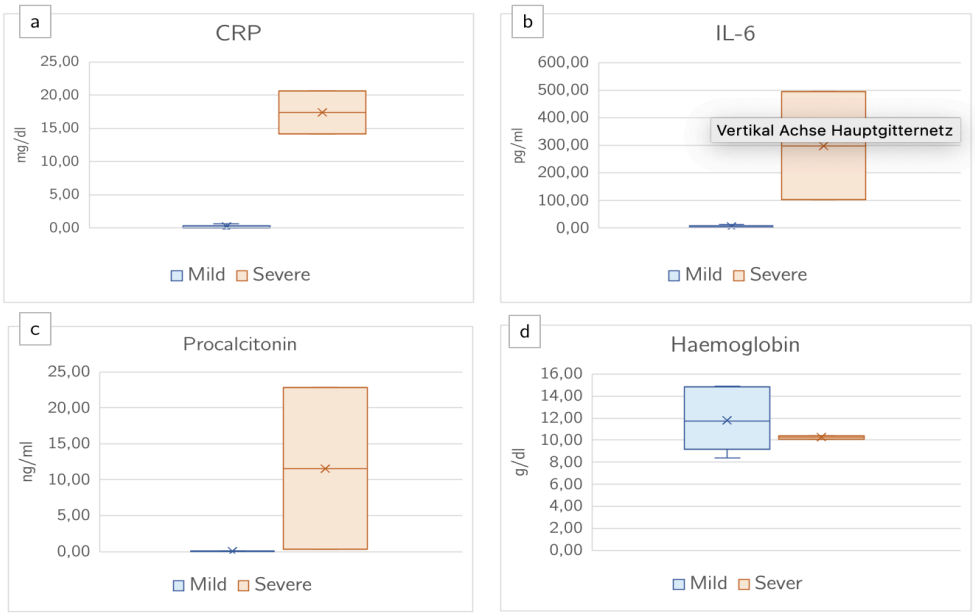

Figure 4.16 Boxplots showing selected laboratory values from admission day of the groups "Mild & Moderate" and "Severe", namely: CRP, IL-6, Procalcitonin and Haemoglobin.

Laboratory values compared to normal ranges – As some reference values range widely within the age groups, Table 4.9 illustrates how many children (in percent) had values within their reference range, comparing "Mild & Moderate" with "Severe". Colour contrasts mark a big difference between the groups. The table only gives information of whether reference ranges have been exceeded, not of its extent. Values with big differences were CRP, IL-6, Troponin, GOT, Ferritin and Quick. Table 4.10 shows the corresponding means and standard deviations in absolute numbers.

	Asymptomatic	Mild and Moderate	Severe
Laboratory Values			
CRP		80%	0%
IL-6		67%	0%
Procalcitonin		33%	50%
Troponin		0%	100%
GOT		100%	0%
GPT		100%	50%
LDH		67%	50%
Harnstoff		100%	100%
Ferritin		100%	0%
Quick		100%	0%
pTT		100%	100%
Fibrinogen		0%	0%

Table 4.9 a heat map showing the percentage of patients with laboratory values on admission day outside/inside the individual reference range, where 100% means that all values are within the reference range and 0% means that all values are outside the reference range. Values of the "Asymptomatic" group are not listed due to the lack of blood samples. Values listed: CRP (C-reactive Protein), IL-6 (Interleukin-6), GOT (Glutamate-Oxaloacetate-Transaminase), GPT (Glutamat-Pyruvat-Transaminase), LDH (Lactatdehydrogenase), pTT (Partial-Thromboplastin-Time).

	Mild&Moderate (n=7)		Severe (n=2)	
	Mean	**SD**	**Mean**	**SD**
CRP (mg/dl)	0,17	0,21	17,4	4,53
IL-6 (pg/ml)	4,54	4,37	298,3	276,83
Procalcitonin (ng/ml)	0,08	0,02	11,6	15,89
GPT (U/l)	20,60	10,26	44,5	37,48
LDH (U/l)	260,75	69,02	513,0	366,28
Harnstoff (mg/dl)	31,50	9,19	21,0	5,66
Creatinin (mg/dl)	1,15	1,51	0,6	0,21
Hemoglobin (g/dl)	11,82	2,73	10,3	0,21
Thrombocytes (g/l)	301,86	80,01	303,0	234,76

Table 4.10 showing the Mean and SD (standard deviation) of selected laboratory parameters to display the absolute value range of movement.

5. Discussion

This chapter discusses the key findings and methods used, following the structure of the research objectives, which were defined at the beginning of this thesis. The objectives are reviewed and answered where possible, while open-ended questions are highlighted as an impulse for further research.

5.1. The impact of COVID-19 on paediatric care

Objective 1: To what extent was the paediatric hospital of the LMU in Munich, Germany affected by the COVID-19 outbreak during the first wave of the SARS-CoV-2 pandemic (March-May 2020)? Specifically, how did the number of (a) infectious diseases diagnosed, (b) surgeries performed and (c) patients at the A&E reported at the hospital differ compared to previous years?

Answer: Significantly fewer patients were seen at the Dr. von Hauner Children's Hospital during the first wave of the pandemic (March to May 2020): the total number of **patients visiting the A&E decreased by 43.4%** (P=.017), while the number of **children with infectious diseases decreased by 74.2%** (P=.013) and **surgical operations decreased by 34%** (P=.007) compared to the previous year 2019. Only 12 children were hospitalised due to COVID-19 in the first wave (and were included in this study). In context of nationally overrun and exhausted health care system, these findings indicate that children were substantially less impacted by the first wave of the pandemic in comparison to older age groups (approx. 70% less than other age groups). Key reasons to explain these findings include: (i) children were less susceptible to severe courses of COVID-19 (discussed in Chapter 5.1.2), (ii) children were less frequently ill (also from other diseases) during the first wave of the pandemic (discussed in Chapter 5.1.3), (iii) guardians were more concerned to report/present children at the hospital due to a heightened risk of infection (discussed in Chapter 5.1.4), and (iv) elective

surgeries were postponed due to a fear of overloaded healthcare system (discussed in Chapter 5.1.5).

5.1.1. Methodology and design

Study type – Data collection for this thesis was carried out as a non-randomised, observational cohort study during the first wave. The method of gathering information and processing samples for each cohort member was suitable, since only a small number of children satisfied the inclusion criteria. The cohort was clustered according to the severity of manifestations of infection.

In principle, non-randomised observational studies are capable of observing causal relationships between cause and target variables. However, these types of studies may be susceptible to bias, as cohorts are not randomised blindly. One of the primary challenges faced as part of research for this thesis, was the small cohort size (n=12), making it difficult to draw meaningful, statistical relationships. The small cohort size itself, was however a meaningful data point to answer the first research question.

Cooperation – To extend the catchment area and maximize the cohort size within a reasonable geographic area (city of Munich), a multicentre approach with cooperation of LMU and TU (details in Chapter 3.1.2) was chosen. By involving more institutions, certain aspects of data collection were more complex and challenging, e.g.: relatively long communication channels with a heightened risk of losing information, and various clinical documentation systems and laboratory standards making data collection more difficult. In retrospect, given the small number of children that were hospitalised due to COVID-19 in Munich, the cooperation across hospitals was essential.

Patient selection – The willingness to participate in the study was high amongst the majority of children and guardians. This was likely due the extensive media coverage of COVID-19 in 2020. As described in the patient journey (Chapter 3.1), children that were suspected to have been infected by COVID-19 (i.e. showing symptoms including fever, respiratory or gastrointestinal symptoms) were asked to participate in the study.

Contrary to what we expected, the number of children that were hospitalised due to COVID-19 and/or other infections/diseases declined dramatically in the first wave – a remarkable finding. This meant, that recruiting a homogeneous control group (e.g. with other viral or bacterial infections) to compare with those infected with COVID-19 proved very difficult. Ultimately, a valid control group could not be obtained and only 12 children of the in total 63 patients recruited for this study fulfilled the inclusion criteria. Regardless of having a valid control group or not, it was decided to continue researching the effect COVID-19 had on children, as very little was known about the disease at the time. An exciting topic for further research would be to investigate the long-term effects experienced by the cohort recruited for this thesis, as these were among the first to have hosted the virus.

5.1.2. Children suffered less severe courses of COVID-19

Children were under-proportionally infected – A nationwide comparison of infection figures for all age groups, shown in Figure 4.1 (a), was made to answer the question of the children's involvement in the pandemic. The analysis showed that the age group from 0 to 19 years was approx. 70% less affected than the older age groups during the first wave (March to May 2020). Naturally, the question arises: what can explain, why children were so significantly less impacted? One could assume, that children had less exposure to the virus when compared to adults, as they likely travelled less and tended to meet with the same, selected group of children and adults [46]. On the other hand, children probably also kept less distance amongst each other while playing together or during sports, which would have increased the risk of transmission.

Children demonstrated low virus transmissibility – As we know today, after extensive research following the first wave, groupings within kindergartens and schools acted as sources of infection [47] [48] [49]. Although data concerning the viral load detected in different age groups in 2020 is not fully consistent, and tracing the root/cause/place of infection remains challenging, there seems to be sufficient evidence to show that especially in the age group $<$ 12 years of age, the viral load and chance

of infection or transmissibility of the virus was significantly lower than in adults [50] [51] [52] [53]. For example: Hippich et al. reported that in a cohort of 11,000 children in Bavaria the transmission between SARS-CoV-2 positive family members and children occurred in only 35% of cases [54], while student-to-student virus transmission in kindergartens and schools was reported to be limited when certain prevention measures were in place [55] [56].

Children were tested less often – A further explanation as to why the number of infections for children was significantly lower than for other age groups may have been that children were tested significantly less frequently than adults. At the peak of the first wave in April 2020, PCR-SARS-CoV-2 testing was performed in 26,302 children aged 0 to 15 years (the RKI does not provide information about 0 to 18 years old), and in 507,608 persons above 16 years [57]. Averaged over the population living in Germany in the respective age groups, this means that adults were tested ca. 3-4 times more frequently than children [58]. Given the low hospitalisation rates of children infected with COVID-19, and the rather mild course of disease in children when compared to adults, this probably means that many infected children remained undiagnosed. Hippich et al. reported figures indicating that SARS-CoV-2 antibodies were found in approximately 6 times more children (0.61%) than were reported positive by the health department (0.16%) [54]. For comparison, in April 2020 the nationwide SeBluCo-study found a seroprevalence of approximately 1.3% in the population over the age of 18 years [59].

Children less frequently hospitalised due to COVID-19 – While the reported number of infections in children was significantly lower than in adults, and it is not definitively clear whether this was solely due to the number of undiagnosed cases or an intrinsic ability of children to fight the virus, what is clear is that the number of children that were hospitalised during the first was extremely low. In fact, in April 2020 a maximum of 26 children were reported by the DGPI to have been hospitalised at the same time due to COVID-19 as shown in Figure 4.1 (b). The low hospitalisation rates may have been due to the high number of asymptomatic or mild courses of disease

within infected children. The reported share of children that experienced no symptoms during infection ranged from approx. 15% [60], to 35% or 45% [61].

Less ACE-2 receptors in children – A reason for the difference in the severity of the course of disease between children and adults, and associated hospitalisation rates, may be the amount of ACE2 receptors in the respiratory epithelium. These are considered to be the entry point for the virus into the host cell [62]. The number of ACE2 receptors increases with age and offers the virus more of a target in the elderly. Bunyavanich et al. have shown that the nasal gene expression of ACE2 receptors were at the lowest level in the age group 0 to 10 years, followed by children 11 to 17 years, before young adults aged 18 to 24 years. Highest expression was found in adults aged 25 years and older [63], which may support the trends seen in Figure 4.1 (a).

Age-dependent ACE2 variance – In addition to increased intrusion, the blocked ACE2 receptor may also lose its tissue-protective function in severe respiratory failure. The tissue-protective function of ACE2 could be shown in models with mice that got severe acute lung injury - induced through acid-aspiration, or sepsis. Mice with loss of ACE2 had worse oxygenation, increased inflammatory cell infiltration, and developed more lung oedema and hyaline membrane formation than wild-type mice. Furthermore lung function and pulmonal oedema of these *knockout* mice improved again after injection of human recombinant ACE2 (rhuACE2) [64]. An evolving imbalance between ACE and its counterpart ACE2 of the renin-angiotensin system (RAS) of the lung appears to be important to the pathogenesis of ARDS: upon lung injury, Ang I is converted to Ang II by ACE, activating the AT1 receptor, which leads to vasoconstriction and therefore exacerbation of the lung injury. At the same time, SARS-CoV-2 blocks the counter-regulatory ACE2 and down-regulates it, which loses its protective effect by not being able to re-transform Ang II [65]. In an animal study with rats, it was shown that in the group of older animals, compared to the younger ones, the balance in the RAS shifted from the protective ACE2 towards an increased release of ACE with enhanced inflammatory response and lung damage [66]. This could be one of the reasons why children are less likely to develop ARDS or have a severe

course of disease than adults [67]. Yet another clinical study, using BAL analyses of different age groups, failed to find a significant difference in lung RAS activity between children and adults, as has been shown in animal models. They suggest other underlying pathophysiological mechanisms [68].

IFN-I antibodies – A further factor increasing the risk for a severe course of COVID-19 may be defects in the type I interferon immunity. Interferons (IFN) function as cytokines in the innate and adaptive immune system to fight viruses [69]. Casanova et al. report that neutralising autoantibodies (IgG) block IFN, and thus prevent them from targeting SARS-CoV-2. Approximately 10% of patients that developed life-threatening COVID-19 were found to have these antibodies. They can also be found in uninfected people and likely present the cause and not the consequence of a severe COVID-19 disease [70] [71]. For example, they occur in almost all patients with auto-immune polyendocrinopathy syndrome type I (APS-1) [72]. Again supporting the results of Chapter 4.1, that children are less likely to develop a severe course and require hospitalisation, these studies showed that type I IFN antibodies occurred in only 0.5% of people under 60 years, and are rising to 4% at age 70 and up to 7% by age 85 [73].

Age-dependant pre-existing-conditions – A further reason for the significantly lower hospitalisation rates in children compared to adults may be the lower prevalence of pre-existing conditions. Factors that increase mortality rates in adults infected with COVID-19 include (without ranking): heart diseases, COPD, obesity, Down Syndrome, cancer diseases, Diabetes mellitus, liver - and kidney disease, immunosuppressive medication and smoking [74] [75]. These are all factors, which prevail more frequently in elder age groups than younger ones, for example: the prevalence of coronary heart disease (acute or chronical coronary syndrome) in over 75-year-olds is 16-24%, whereas in 18- to 44-year-olds it is only 0.2-0.4%; the prevalence of COPD and Diabetes mellitus also increases with age [76] [77], and smoking (the main cause of COPD) is significantly less common among children than in adults [78]. Furthermore, overweight is less common among children $<$18 years of age, 15.8% compared to 53% among $>$18 year olds in Germany [79] [80].

5.1.3. Children were less ill in the first wave (also from other diseases than COVID-19)

Children were less ill during the first wave – In the Dr. von Hauner Children's Hospital the number of children diagnosed with pre-selected ICD-10 codes like respiratory or gastrointestinal infectious diseases decreased in the months March to May by 74.2% compared to 2019 and by 62.2% compared to 2018 (Figure 4.2 and Table 4.1). Although this seemed surprising at first, given the generally overloaded health system in Germany during the first wave, similar trends were seen elsewhere in Germany: a significant decrease of 37% in the number of cases compared to 2019 was also noted in paediatric practices in a large study in western Germany during calendar week 12 through 18 of the first wave [81].

Children were exposed to less sources of disease – The risk of contracting all kinds of disease causing agents - respiratory, gastrointestinal and others - is much lower if children cannot get them at school, day-care centres or through contacts [82]. This assumption is further supported by nationwide figures on infectious diseases, such as influenza or RSV-infections. Monitoring infectious diseases, the LGL collects randomly conducted samples taken by general practitioners from patients with symptoms. A total number of 38 and 71 influenza positive samples were sent to the LGL (according to the old and new reference definition, respectively) for the 2020/2021 season in Germany, compared to 23,500 and 35,000 sent in cases in the previous season 2019/2020. Returns were also noted in RSV infections, with only 1 RSV-positive out of 119 sent in swabs taken from children with acute respiratory symptoms [83]. Collected data from the "Kassenärztliche Vereinigung" in Germany showed a decrease of respiratory infections of any kind by more than 50% in the age group of 3 to 5 years in the 2nd quarter of 2020 compared to the previous year 2019 and a decrease of 77% in infectious intestinal diseases and streptococcal-angina [84].

5.1.4. Guardians were hesitant to present children at hospital due to a heightened concern for infection

Many medical facilities reported that patients avoided going to the hospital even in emergencies out of fear of infection. The number of patients in the A&E department at the Klinikum Rechts der Isar fell by 40% compared to the same period in the previous year [85]. Likewise the AOK (Allgemeine Ortskrankenkasse) reported a 28% drop in heart attacks admitted to hospital and a 15% drop in strokes in mid-March 2020 [86]. This indicates a growing hesitation towards seeking medical help during this time. For young patients, this avoidant behaviour can have severe consequences, e.g. if serious underlying diseases in childhood are diagnosed or treated too late. Another study suggested however, that the number of treatment appointments for chronic diseases in children was similar to the number before the first wave [84]. An additional side-effect that was reported showed that the rates of childhood vaccinations declined in the first wave, ultimately having a negative impact on health prevention. [87] [88].

5.1.5. Elective surgeries were postponed

Less surgeries performed – The decline in children visiting the hospital during the first wave may have also been due to a change in policies regarding elective surgeries: For example, many elective operations were cancelled. This leads us to the third part *'Surgery'* in Figure 4.2: Averaged over the first wave, there were 34% (P=.007) fewer operations in the paediatric hospital compared to 2019 and 24% (P=.025) fewer than in the 2018. Compared to the 41% decrease in non-paediatric figures from the "Berufsverband der Deutschen Chirurgen" during the first wave, this is a rather small decrease in surgeries [89]. This may be due to the fact that surgeries in children more often have an acute indication such as appendicitis, bone fractures or testicular torsion. Furthermore, it often is less justifiable to postpone operations that require surgery for a longer period than it would be in adults.. In conclusion we could see that the surgical section of the Dr. von Hauner Children's Hospital has experienced the least change

during the first wave in contrast to the number of patients admitted to A&E and of the patients with ICD-10 infections.

5.2. Manifestation of COVID-19 in children

Objective 2: How did SARS-CoV-2 infections manifest in children? Were only relatively mild cases reported? And if not, what did severe manifestations look like?

Answer: The manifestation of SARS-CoV-2 infections in the cohort recruited for this study ranged from an absence of symptoms and good health in 3 children, to mild symptoms for up to approximately 6 days with fever, cough and rhinitis in 59% of the cohort. Additionally, 2 children developed life-threatening hyperinflammatory syndromes with symptoms such as acute respiratory distress syndrome and multiorgan failure. The following section first discusses the methodology used to investigate how COVID-19 manifested in children, and then discusses the course of disease in asymptomatic, mild and severe cases.

5.2.1. Methodology: Data sources and cohort criteria

The clinical survey – Key elements of assessing the course of disease within children included a clinical survey, laboratory testing, the patients file and discharge letters. In principle, the clinical survey was well suited as a tool to assess symptoms of the children in the period before hospital admission. However, the information provided by the patients and guardians in the survey did not always align with the information found in the patients file. Patients and guardians struggled to reconstruct the exact timing of beginning and end of symptoms, and had to make vague estimates (e.g. providing date ranges of more than 3 days). Furthermore, the diagnosis of symptoms such as headache, loss of smell and taste or sore throat, in particularly young children who could not express themselves proved difficult. These difficulties were not unexpected and are inherently associated with clinical surveys. Nevertheless, the data collected was insightful, and could be used for analysis albeit not accurate to the day but rather indicative of approximate time-ranges.

Laboratory testing – Collecting samples from children for scientific purposes is commonly associated with particular challenges: for example, staff and parents are

often reluctant to draw blood from children unless absolutely necessary. In the context of this study, the reluctance to draw blood led to substantial gaps in the data set of clinical chemistry. In some cases, laboratory parameters could not be measured as the blood volumes drawn were too small. In addition, blood for this study was only drawn in cases where there were other medical indications to draw blood. However, even for cases where there was a medical indication, blood was not always provided for this study as there were many parallel studies ongoing at the hospital. These types of difficulties are common within paediatric research.

Classifications – To classify the cohort recruited for this thesis in a meaningful way, two existing classification systems were combined (shown in Table 3.3): a classification by severity made by the World Health Organisation (WHO) adapted for children, and the NIH COVID-19 treatment guidelines which also includes an "Asymptomatic" category. The WHO classification was used as it provided precise and detailed definitions for the more serious forms of the disease, while the NIH guideline provided a definition for asymptomatic courses of disease. Further classifications were reported by e.g. the Robert-Koch-Institute (only for adults, [90]) and the Chinese Journal of Paediatrics ([91]), however were deemed to not add any further detail or benefits and were therefore not included in this study. Due to the relatively small cohort size recruited for this study, the 5 categories established by combining the WHO and NIH classifications were clustered into 3: category 1 "Asymptomatic"; category 2 a combination of "Mild" and "Moderate", and category 3 a combination of "Severe" and "Critical".

5.2.2. Asymptomatic COVID-19 infection

Incidental diagnosis – There was a wide range of disease severity in the cohort recruited for this study, from asymptomatic to severe. It was surprising to see that the cohort included asymptomatic cases, as only hospitalised children were considered in this study. Two cases were admitted for social reasons and one because of his epilepsy. It has been reported that SARS-CoV-2 can trigger neurological symptoms, thereof

approx. 1.7% epileptic seizures [92]. Although a viral cause for the epilepsy in the patient may be possible, it is more likely that the patient did not reliably take his regular antiseizure medication and the infection was more of a coincidence.

Cohort description – The asymptomatic cohort (n=3) in our study included only boys, with an average age of 12 years. This however cannot be generalized, and reported large-scale hospital screenings indicate that sex does not generally lead to a difference in course of disease (reported cohort consisting of 52.2% male and 47.5% female) [93].

Cycle threshold not considered – The Ct (cycle threshold) values for the cohort recruited for this thesis were not analysed and documented for all samples in the early stages of recruiting as it was not yet the norm to document these values. As the first wave progressed, the importance of measuring the Ct values became more evident (with increasing international research), however for those cases where they were not reported they could also not be reconstructed retrospectively. Studies comparing the Ct (cycle threshold) values of asymptomatic and symptomatic children found, that the Ct values of the asymptomatic children were significantly higher [93]. In some studies low Ct values are related to increased mortality and infectivity [94].

5.2.3. Mild and Moderate COVID-19 infection

Diverse cohort – The children of the group "Mild & Moderate" are particularly interesting because of the heterogeneity within the group. There was a large span in the age of children, ranging from an infant of 6 months up to young adults of 18 years, and also the pre-existing conditions (PEC) varied significantly across the group: 29% presented without any pre-existing conditions, 29% reported only mild PECs such as pollen allergies, and 43% had severe pre-existing illnesses.

2 cases with medicinal immunosuppression – Two of the children in the "Mild & Moderate" group were immunocompromised due to chemotherapy or Mycophenolat-Mofetil therapy after an organ transplantation. Surprisingly, despite

immunosuppression and severe chronical illness, these two children only showed fever for a few days, hardly any respiratory symptoms and seemed fairly resilient to the virus. Even in comparison with other children of the group "Mild & Moderate" they presented the mildest manifestation of infection, whereas, overall, an increased risk is assumed for immunocompromised patients [95]. Another study even suggests that in the asymptomatic cases, the immune response was weaker with low IgG levels and thus had a positive influence [96]. With regards to hyperinflammatory syndromes, it has been reported that immunosuppression could have a certain protective effect [97].

Case with liver transplantation – The child (Patient ID COV007) in our cohort which had a liver transplantation due to his Alagille syndrome showed mild rhinitis and cough for one day and fever for four days, but no further symptoms. There are different statements in the literature regarding COVID-19 in liver transplanted patients. Some studies say that more pneumonia and respiratory distress occur in SOP (solid organ transplant) than in non-organ transplanted patients [98] and that SOP patients generally have a higher risk of a severe COVID-19 outcome [99]. On the contrary, a multicentre study found that the incidence of COVID-19 in children with chronic liver disease like the Alagille-Syndrome is higher than in the general population, but does not represent an increased risk of severe COVID-19 [100]. Others have not found a higher COVID-19 incidence to the overall population [101].

Case with chemotherapy – The other immunosuppressed patient in the "Mild & Moderate" group (Patient ID COVS005) received chemotherapeutical treatment (Cyclophosphamid) due to her ALL-B while tested positive for SARS-CoV-2. She showed no respiratory, gastrointestinal or other symptoms. The only symptom she reported of was fever the day before hospital admission, which may be led back to her chemotherapy. During her hospital stay she was free of symptoms and fever. Little literature is available on childhood acute lymphoblastic leukaemia and COVID-19 at the time of the first wave [102]. A study in paediatric oncology centres in Poland suggests that most cancer patients are not at increased risk of severe COVID-19 progression. However, patients who tested positive were more likely to have their

chemotherapy interrupted, which is a disadvantage in the treatment of the underlying cancer disease [103].

Neurological symptoms – While most children presented with fever and respiratory syndromes, some showed neurologic symptoms like headaches. Notable was the neurological damage of one girl (Patient ID COVS007) developing a facial nerve palsy with painful trigeminal neuralgia, likely associated with her SARS-CoV-2 infection (other causes could be ruled out). A large review study found, that 1,2% of all neurological symptoms in COVID-19 appear to be neuralgia [92]. Therefore, patients who present with neuralgia as their main symptom like this girl, should be tested for SARS-CoV-2, even though it should still be considered a diagnosis of exclusion.

Cohort representative of literature findings – The symptoms and courses of diseases observed in the "Mild & Moderate" group are largely consistent with those reported in literature: COVID-19 in the majority of children tends to manifest in a mild course with mostly fever, respiratory and gastrointestinal symptoms [102] [104].

5.2.4. Severe COVID-19 infection

Mortality in children with COVID-19 is very low: in Germany only three COVID-19 deaths in the group under 20 years of age were reported to the RKI up until May 19th 2020, (0.0004% of all reported COVID-19 death cases in Germany) [105]. Nevertheless, critical and life-threatening illness can be possible: approximately 10% of the children hospitalised due to COVID-19 in Germany had to be treated at a paediatric intensive care unit (PICU) [106] [107] [108].

Hyperinflammatory syndromes – In the cohort recruited for this study, two children were classified as "Severe"; both children were in serious health condition during their hospital stay and developed virus-triggered hyperinflammatory syndromes. One child (Patient ID COVS009) developed a paediatric inflammatory multisystem syndrome (PIMS), the other child (Patient ID COV006) developed a secondary haemophagocytic lymphohistiocytosis (sHLH) (detailed information is given in 1.1.3).

PIMS or Kawasaki Disease – A patient (Patient ID COVS009) was diagnosed in her discharge letter with "*Kawasaki Disease or multiinflammatory syndrome with association of COVID-19*". The clinical profile of the patient matched nearly all paediatric inflammatory multisystem syndrome (PIMS) criteria (details to her symptoms in Chapter 4.2.4), while also fulfilling some of the very similar Kawasaki Disease (KD) criteria: young age, fever, erythema, exanthema and increased inflammatory parameters.

Considerations for PIMS – The girl was admitted to hospital at the beginning of May, at the peak of the PIMS cases. That appeared with a delay of 31 days to the peak of the positive PCR tested SARS-CoV-2 infections. The incidence was 322 per 100,000 children under 21 years positive for SARS-CoV-2 and 2 per 100,000 children under 21 years diagnosed with PIMS in New York [109]. That is why we also consider a high

probability of PIMS in our child (COVS009) in the temporal context. This child was not tested positive for SARS-CoV-2 but had a positive SARS-CoV-2 IgG serology. In large studies 87% of the PIMS patients had a positive IgG serology [110], far more than a positive SARS-CoV-2 PCR test [109] [111]. Additionally, the inflammatory parameters are generally assumed to be more elevated in PIMS than in KD [112] and were especially high in the patient (CRP at 20,6 mg/l, and the IL-6 at 102,5 ng/l). Furthermore, gastrointestinal symptoms such as nausea, end-grade meningismus, acute renal insufficiency and, above all, the positive SARS-CoV-2 antibody serology, evidence of a previous viral exposure, indicate the patient suffered from PIMS.

Considerations against Kawaski disease – One of the main diagnostic criteria of KD are skin lesions on the trunk, extremities and mucous membranes. These were present in this girl but could also be due to her Epidermolysis bullosa. Echocardiography was normal and showed no signs of coronary complications.

In light of the considerations detailed above, the case would likely be classified as PIMS nowadays. Despite the many similarities, KD and PIMS should be seen as two separate diseases [112]. Importantly, viral triggers for hyperinflammation are suspected in both [113]. It is suggested by the DGPI that PIMS should not be considered as an independent syndrome from COVID-19, but as a hyperinflammatory condition/ complication in the context of SARS-CoV-2 infection [114].

Course of disease PIMS – Studies showed that very heterogeneous degrees of PIMS disease severity were possible, ranging from clinically stable patients to life threatening conditions with circulatory shock [107]. Fortunately, apart from a minimal pericardial effusion, our patient's cardiac function was normal. A major difference between PIMS and severe COVID-19 seems to be that cardiovascular problems predominate in PIMS whereas respiratory problems predominate in COVID-19 [115]. Mortality in PIMS is with 1.7% higher than in COVID-19 [116]. Our patient was cardiorespiratory stable and mainly needed anti-inflammatory therapy. Dufort et al. published in the NEJM that 80% of their PIMS-patients were admitted to the PICU, 70% received intravenous

immunoglobins, 64% received systemic glucocorticoids and 48% received both [109]. Our patient received 2x IVIG a 2g/kg and 9 mg prednisolone for 14 days, which is compatible with the guidelines [114]. Under this therapy she recovered and could be discharged home after 14 days of inhospital stay.

Case study with sHLH – A patient (Patient ID COV006) developed a severe course of disease (described in detail in Chapter 4.2.5), and fulfilled the criteria for a SARS-CoV-2 triggered secondary haemophagocytic lymphohistiocytosis (sHLH), with a H-score resulted in a value of 200 (indicating above 93% sensitivity and 86% specificity). A study from hospitals in Shanghai reported a mortality rate of children with sHLH and multiorgan failure of 46% [117].

Intensive medical care – For this patient, intensive medical care was essential, given the critical state of health. Treatment followed according to the recommendations of the DGPI and the AWMF pCAP (paediatric community-acquired pneumonia) guidelines during his moderate phase of illness: he received oxygen (1l/min to 4l/min), antipyretics as needed and intravenous fluid substitution. He also received NaCl and Salbutamol inhalations for which there is no evidence of efficacy. Due to bacterial superinfection (haemophilus influenza, streptococcus pneumoniae), which occur in about 30% of all pCAP, he was treated according to guidelines with antibiotics [118] [119]. After exacerbation and his critical state of health, further treatment options had to be considered.

Further treatment options – Antivirals like Redmdesivir, Lopinavir/Ritonavir and (Hydroxy-) Chloroquine were used for therapy in adults in clinical trials, however, the effectiveness of (Hydroxy-) Chloroquine and Lopinavir/Ritonavir in vivo has not been proven [114] [120]. Remdesivir is currently approved by the EMA (European medicines agency) for treatment from 12 years of age and 40 kg body weight [121]. In individual cases, however, it can also be given to younger children within the setting of a clinical

trial after carefully weighing up the risks. If chosen to be applied, the medication should be given as early as possible in the antiviral phase, if possible, within the first 7 days after the onset of symptoms, in exceptional cases within up to 10 days. However, these recommendations have been published only after we have threated this patient. Remdesivir therapy was not started in our child until the 9th day after the initial onset of symptoms, during which the antiviral phase (II) may have already been overtaken by the hyperinflammatory phase (III). Retrospectively, the child might have benefited from an earlier administration of Remdesivir, although the effect of this antiviral has not yet been conclusively proven [120]. The use of systemic glucocorticoids is recommended when there is evidence of hyperinflammation. A CRP of above 15 mg/dl and other factors (described in Chapter 4.2.5) justified giving Prednisolon on day 9 after symptom onset. It has been reported that the use of systemic glucocorticoids in COVID-19 has reduced the risk of mortality, as well as the duration of hospitalisation and the need for ventilation and is therefore recommended by the WHO for severe and critical COVID-19 [122].

Combined Prednisolon and IVIG – In combination with Prednisolon, a single dose of intravenous immunoglobulins (IVIG) can be applied for hyperinflammatory syndromes [123]. This combination of therapies apparently showed a positive effect, as the child's health improved from day 9 on. Inflammation values dropped again, the fever decreased, blood values and respiration normalised.

Recovery – The child recovered after its severe illness and could be discharged home in good health after a hospital stay of 21 days. He showed no residual symptoms even at the follow-up several weeks later. However, this would not have been possible without intensive and diligent medical care. This case clearly shows that a SARS-CoV-2 infection can also take a very severe course in childhood and must be taken seriously. Extensive research into further, not only causal therapy options and drug efficacy, especially in paediatrics, is essential to prevent tragic outcomes and severe courses of disease.

5.3. Clinical and laboratory parameters as predictors for severe COVID-19

Objective 3: Can clinical or laboratory parameters be used as predictors for a severe course of COVID-19 in children?

Answer: The following factors were observed within the recruited cohort and may be associated with having a higher risk of developing a severe course of COVID-19 (concordant with findings in the literature [9] [124] [125]): (i) younger age (mean=2 years), (ii) congenital heart disease (PFO, ASD II and AVSD), and (iii) Down Syndrome as risk factors for developing severe COVID-19. Additionally, the following clinical parameters were observed in children with a severe course of disease: highly elevated levels of IL-6, IL-2, CRP and Procalcitonin, occurrence of emesis, longer duration of fever, development of hyperinflammatory syndromes (sHLH, PIMS), prolonged duration of hospitalisation and treatment with IVIG and corticosteroids. It should be noted, that given the small cohort size (associated to the low hospitalisation rates of children in the first wave), the findings presented in this thesis must be interpreted with caution and can only act as reference point when comparing to other trends seen in literature. The cohort size was insufficient to provide statistically significant predictors for developing a severe course of COVID-19.

5.3.1. Methodology: Data analysis

Small cohort size – Due to the small cohort size (n=12), we decided not to perform multiple statistical tests as to avoid producing inconclusive or biased values and instead only provide the description of our observations.

Incomplete data – Gaps in clinical documents presented a challenge: values that were not explicitly documented as "absent" were considered as "NA" (not available). Bias thus arose in the mean values, since a result of e.g. 100%, gives the impression that all children exhibit a certain characteristic, whereas it was only 100% of the children from whom the information was available. The amount of missing information

could have been reduced by adding a second patient survey on day of discharge to also capture the symptoms during the hospital stay. The survey that was used, covered only the time from symptom onset to hospital admission. All subsequent events were collected from the clinical documentation, which largely left out sensitive information for the study. Quality of data would have approved by a survey, considering a second period.

5.3.2. Sociodemographic risk factors for severe COVID-19

Age – The children in the "Severe" group were younger (mean=2 years), this is surprising, as regarding the whole population (including adults), higher age is usually mentioned as a risk factor for severe courses [126]. A Chinese study from the early phases of the pandemic showed that most of the severe and critical paediatric COVID-19 patients were <1 year old (32%), followed by the age group 1-5 years. If we consider only the critically ill, even 50% of the group were <1 year old [127]. In Germany, the infection rate was highest among children 12 to 17 years of age, but the hospitalisation rate was highest among infants [128]. Chinese epidemiologists also reported that children under the age of six years had higher risk for a severe course of disease [9].

Obesity – Chao et al. showed that obesity (>30 kg/m^2) in children was associated to PICU admission (30%), although not significantly [106]. Differences in the BMI could be observed, but the weight of both children in the "Severe" group were within age average (BMI mean: 16 kg/m^2). Out of the entire cohort, only one 3-year-old girl (COV022) had a BMI minimally above the age average (20 kg/m2), all others were normal or even slightly underweight. In studies for adults obesity and overweight are seen as a risk factor for ICU admission [67] but only severe obesity is linked to higher mortality [129]. Not only obesity but also patients with underweight (BMI <18.5 kg/m^2) had a higher risk for hospitalisations [130].

Smoking – Smoking may be linked to a worse course of disease, although opposing opinions have been reported [131] [132] [133] [134] [135]. Assuming that the children in

our cohort were non-smokers, we do not know whether they grew up in a smoking household and may have smoked passively. It would have been interesting to include this aspect in the survey. We know that COPD and Asthma bronchiale are risk factors in adults [136] [137]. There are indications that Asthma in children could even have a protective effect [138]. The data in this regard is currently not yet sufficient to answer the question clearly [139]. However, in our cohort, there were no known pre-existing lung diseases.

Socio-economic status & ethnicity – In groups with lower socio-economic status, there is more tobacco use and obesity than in groups with higher status (in Germany and other western countries) [140] [141] [142]. Poorer health and higher mortality are generally associated with lower education and lower income [143]. A New York study showed that of all boroughs, the Bronx - a district with the poorest population and also the highest rate of people of colour - had most hospitalisations and deaths due to COVID-19 [139]. In Milwaukee County 78% of COVID-19 deaths were African American citizens, whereas they make up only 28% of the population living there [144]. It has been found that non-Hispanic Blacks, Hispanics, Asians or native Americans have a higher risk of hospitalisation and poor outcome than non-Hispanic Whites living in North America [109] [145]. The child with Patient ID COV006 in the "Severe" group that developed sHLH was also of Central African descent. Although we included the parents' country of origin in the survey shortly after the study started, this item was left out by most participants.

HLA – However, some African countries counted fewer COVID-19 cases and deaths than statistically predicted [146] [147]. The Major Histocompatibility Complex (MHC), specifically the HLA, appears to play a role in the manifestation and susceptibility of COVID-19 and might even have protective effects within some populations [148] [149]. It is reported that there are HLA variants in Afro-American populations that have a protective effect in viral control [150] [151]. Casanova et al. link certain HLA constellations to a slowing of disease progression in HIV patients [152]. HLA dependent regulation on the adaptive immune system is also known in other viral diseases like

hepatitis B and C [150] [152]. HLA thus seems to play a major role controlling disease severity in viral infections [153].

5.3.3. Pre-existing conditions as risk factors for severe COVID-19

Pre-existing conditions – Frequently discussed risk factors for severe COVID-19 involve pre-existing conditions. Both children of the "Severe" group had cardiovascular (PFO, ASD II and AVSD) and other pre-existing conditions (Down Syndrome, Epidermolysis bullosa). Children with congenital heart diseases (CHD) generally have a higher risk of mortality and morbidity from respiratory infections of the lower respiratory tract [154]. Cardiovascular diseases are also considered a leading diagnosis in COVID-19 hospitalisations, ICU admissions and deaths [7] [155]. Epidermolysis bullosa and Down Syndrome (DS) both occur with chronic immune dysregulation [156] and will be discussed in detail.

Down Syndrome – It has been reported, that patients with DS have a higher prevalence of pneumonia and prolonged hospital stays in other viral diseases like RSV or H1N1-influenza infections [157] [158] [159]. Hospitalised patients with DS additionally have a higher level of severity of COVID-19 than age-matched controls and have higher risk to require mechanical ventilation and develop sepsis [124]. They also show increased mortality compared to COVID-19 patients without DS [160] [161]. A generally promoted pro-inflammatory cellular state in patients with DS is discussed to cause cytokine storms, leading to an excessive immune reaction [156]. Patients with DS should therefore be considered as of high risk developing severe COVID-19 and PIMS [162]. In DS, genetic alterations on chromosome 21 have been discovered, which are mainly related to an overactivity of the immunoregulatory IFN (Interferon) pathway [156]. Furthermore, immune cells in DS are particularly sensitive to IFN stimulation, which ultimately trigger a cytokine storm resulting in elevated levels of inflammatory markers like CRP, IL-6, TNF-alpha and other cytokines and chemokines

[160] [163]. Huggard et al. wrote shortly before the SARS-CoV-2 pandemic started "The extent of immune dysregulation in DS is substantial, spanning the innate and adaptive systems, anomalies in T and B cells, abnormal monocyte phenotype, neutrophil chemotaxis, circulating cytokines, and suboptimal antibody responses which contribute to a phenotype at risk of increased infections, poorer clinical outcomes and chronic inflammation." [164].

Down Syndrome: Congenital heart disease – In addition to genetics, other predisposing factors that are more prevalent in patients with Down Syndrome (DS) can be a reason for poorer outcomes. It has already been described that congenital heart disease (CHD) is a risk factor for higher mortality and more severe SARS-CoV-2 infections [125] [165]. In a paediatric clinic at Columbia University for example, 3 out of 5 patients hospitalised with pulmonary hypertonia had DS and AVSD (atrioventricular septal defect) [166]. The prevalence of any CHD in DS is about 50%, which can be explained by overexpression of CHD-associated genes or gene mutation on chromosome 21 [167] [168].

To summarize the effect of Down Syndrome as risk factor for severe COVID-19: on the one hand, patients with DS are at risk due to their altered, auto-inflammatory immune system and on the other hand, there is an increased prevalence of CHD, obesity and diabetes, each of which is associated with a poor outcome for COVID-19 in the general population [156] [169].

Epidermolysis bullosa – Increased prevalence or severity of COVID-19 infections in children with EB has not yet been reported. The data available on this subject remains sparse at the time of publication [170]. Since the open dermal lesions in EB get frequently bacterially colonised/infected [171], there was a consideration that the lesions could provide an enlarged entry portal for SARS-COV-2, putting the patients at increased risk. However, this has not yet been validated [171]. An increased risk was also feared with regard to the often necessary immunomodulating therapy for patients with autoimmune bullous diseases, but so far this has not been confirmed in the

literature [172]. Until now, only a possible link between COVID-19 and increased risk for developing autoimmunity was suspected [173]. However, it is interesting that elevated cytokines and IL-6 upregulation with increased acute phase protein production were also described in patients with EB [174]. Therefore, patients could be more vulnerable developing a hyperinflammatory syndrome induced by SARS-CoV-2, as it was the case in our child (COVS009). Furthermore, EB can be further considered as an "Inborn error of immunity", which manifest themselves in increased susceptibility to infectious diseases [175].

Susceptibility for immunodysregulation – As mentioned earlier, hyperinflammation, and respectively a cytokine storm, due to uncontrolled production of pro-inflammatory mediators - like IL-1, IL-6, TNF-alpha and IFN, is recognized as the cause of life-threatening COVID-19 cases [176] [177]. Zhou et al. wrote "Initial reports about COVID-19 suggested a pathogenic role of the immune system in the disease, damaging the lungs in a cytokine-storm provoked by CD4+ T-lymphocytes and monocytes" [178]. The cytokines are produced by multiple immune cells like the T- and B-lymphocytes, dendritic cells, macrophages, and natural killer-cells, causing massive hyperinflammatory, further leading to ARDS [177] [179]. In patients with life-threatening COVID-19 infections, specific genetic alterations were identified and linked to the severity of the course [179]. It is assumed that monogenetic inborn errors of immunity (IEIs) play a key role in developing severe COVID-19 [180]. Antiviral response is disrupted through certain IEIs, resulting in greater susceptibility to EBV, HPV, human rhinoviruses (HRN), influenza, HSV-encephalitis or SARS-CoV-2 [181]. Especially the integrity of pulmonary epithelial cells could be disturbed by IEIs [182]. Investigating immunogenetic differences in patients with severe COVID-19 versus asymptomatic or mild disease, showed that there were genetic defects influencing the TLR (toll-like receptor) genes and the type I IFN cell-intrinsic immunity in the severely ill [71]. As a result, one could conclude that people who are predisposed to cytokine storms due to their genetic constellation, as is the case with DS [169], are at particular risk for developing severe and critical COVID-19. This was also the case with our two

patients COV006 and COVS009 in the "Severe group", both of whom had developed hyperinflammatory syndromes, which by definition corresponded once to sHLH and once to PIMS. This predisposition has been reported in the literature for familial HLH [183], as well as for PIMS [184]. Data on exactly which groups of people are susceptible to cytokine storms in COVID-19 infection are a topic of current research and essential for identifying vulnerable groups [176].

5.3.4. Laboratory parameters associated with severe COVID-19

CRP, procalcitonin & leukocytes – As aforementioned, cytokine storms are associated with a severe course of COVID-19 disease and higher mortality. The laboratory parameters of our cohort reflect their disease severity and are largely congruent with the literature. No blood was taken from our asymptomatic children due to lack of indication. Therefore, we compared the "Mild & Moderate" group with the "Severe" group. A difference could be seen in the inflammatory parameters between the two groups. Mean CRP was elevated in the "Severe" group (17.4 mg/dl), whereas the average of the "Mild & Moderate" group had normal values (0.17 mg/dl). The same was the case for Procalcitonin: "Severe" group had strongly elevated Procalcitonin (11.6 ng/ml), while the "Mild & Moderate" group was at normal levels (0.08 ng/ml). Elevated CRP and procalcitonin levels are associated with higher risk of PICU admissions [106]. The leukocytes, however, stayed except for initially slightly elevated values within the normal range. Kim et al. report that leukocytopenias are seen in cytokine storms and often correlate with severity, which is believed to be due to a transport into secondary lymphatic organs [176].

IL-6 levels – The biggest difference in laboratory parameters between the "Mild & Moderate" and "Severe" group was seen in the IL-6 levels, where the severely ill children showed an average value of 298 pg/ml and the children from the "Mild & Moderate" group showed values of 4.5 pg/ml. Increased IL-6 levels in critically ill paediatric patients have been described several times [185]. In adult patients, IL-6 levels were also significantly higher in fatal SARS-CoV-2 infections [186] and in ICU-admitted patients

[178]. Elevated Il-6 levels can be directly associated with hyperinflammation [187] and can be seen as independent risk factor for mortality [117]. This laboratory parameter clearly reflects the difference in disease severity between the two groups.

Further cytokines – As aforementioned, two children in the recruited cohort had hyperinflammatory syndromes with increased cytokines such as IL-2 receptors, IFN, TFN-alpha, etc. In a further study it would be interesting to compare these cytokine parameters to the entire cohort, however these were only determined in very few cases. In paediatric HLH patients, elevated IL-2 receptor levels may be observed and can be decisive in the diagnostic [188]. The girl with PIMS (Patient ID COVS009) also had very elevated IL-2 receptor levels (2720 U/ml). Further cytokines and chemokines associated with severe COVID-19 are, among others: IL-7, IL-8, IL-10, granulocyte colony-stimulating factor (G-CSF), TNF-alpha, endothelial growth factor (VEGF) and monocyte chemoattractant protein-1 (MCP-1) [125] [189] [190].

Hemoglobin – The two severely ill children both showed anaemia, while the children in the "Mild & Moderate" group showed normal levels of hemoglobin (Hb). Additionally, PIMS patients have been reported to have elevated D-Dimer and Troponin [109]. The child with sHLH (Patient ID COV0006) also showed elevated D-Dimer und Ferritin, whereas Troponin-T stayed normal. In the literature all three values: Troponin T, Ferritin and D-Dimer are associated with more severe disease manifestations [191].

Cycle threshold – As aforementioned (see Chapter 5.2.2), the Ct (cycle threshold) values for the cohort recruited for this thesis were not analysed and documented for all samples in the early stages of recruiting as it was not yet the norm to document these values.

5.3.5. Clinical parameters associated with severe COVID-19

Fever – Fever was the symptom that all patients in the "Severe" group as well as all patients in the "Mild & Moderate" group showed during their infection. However, the fever persisted longer in the two children with severe illness - 14 days compared to an average of 2 days in the "Mild & Moderate" group. In total, it took about 19 days for the two severely ill children to become symptom-free, while the children in the "Mild & Moderate" group had no more symptoms after an average of 6 days. Accordingly, the length of hospital stay was also shorter in the "Mild & Moderate" group, with an average of 5 days compared to 18 days in the "Severe" group.

Emesis – Emesis occurred in both severely ill children, but in none of the others. It has been reported, that particularly patients under the age of 1 year, suffering from a severe course of COVID-19, exhibit emesis [192].

Respiratory symptoms – Cough occurred in all children in the "Mild & Moderate" group during their infection, but persisted for only 3 days, whereas the child with sHLH suffered from shortness of breath for 21 days and cough for 24 days and even developed ARDS. However, the child with PIMS had neither cough nor shortness of breath. Comparing with the literature, fever (51%) and cough (47%) appeared in most children that showed symptomatic COVID-19 [192]. Only 1.9% of hospitalised paediatric patients in Germany developed ARDS. Children with severe COVID-19 were dominated by fever, and usually by respiratory symptoms like cough and dyspnoea [102] [185]. In contrast to typical COVID-19, respiratory symptoms were less significant in PIMS, instead, they had more gastrointestinal symptoms [116] [193]. Therefore, only one child in the "Severe" group received an oxygen treatment and inhalative bronchodilators for 14 days. However, the duration of therapy was longer in the "Severe" group, with an average of 12 days compared to an average of 2 days in each of the other two groups. Corticosteroids and IVIG were given exclusively to the two children with PIMS and sHLH for 9 to 14 days. In a multicentre study in the United States, only 38% of hospitalised PIMS children required respiratory support, whereas 81%

received IVIG and 63% received steroids [162], even though some studies question the benefit of this therapy [194].

Other symptoms – Some symptom-complexes, such as "headache", "abdominal pain", "sore throat" or "anosmia & ageusia" could not be adequately assessed among the younger children (<3 years) and therefore difficult to be interpreted. The results give the impression that the children with severe disease were less likely to have these symptoms than those in the "Mild & Moderate" group, yet it was simply due to the younger children in the "Severe" group who were unable to provide a response to this.

Summary: symptoms and medical interventions – To summarise, we could associate prolonged fever, as well as prolonged hospitalisation with severe COVID-19 infection. These findings are also reported by paediatric studies from Wuhan, where severely ill patients had a 10 days hospital stay and critically ill patients a 20 days hospital stay [185]. Likewise, the need for corticosteroids and IVIG treatment could be associated with severe COVID-19. However, these are not the reasons for the severe disease, but the consequence of it and therefore must not be considered as "risk factors".

6. Conclusions & Outlook

Context – The novel coronavirus SARS-CoV-2 started to spread across the world in 2020, inducing an often life-threatening lung disease named COVID-19. Amidst the first wave (March to May 2020), the impact this disease would have on people was largely unknown, and especially for children the course of the disease was unclear. Initially, it seemed that children were particularly resistant against the virus (compared to older age-groups), however, cases of severe hyperinflammatory syndromes could be observed in a limited number of children and dedicated research to investigate the disease in this age group had to happen.

Objectives – The research conducted for this thesis aimed to: (1) investigate to what extent the paediatric hospital of the LMU Munich (Dr. von Hauner Children's Hospital) was affected during the first COVID-19 wave (March to May 2020), (2) provide a description of the manifestation of COVID-19 in hospitalised children and (3) analyse whether predictors for a severe course of the disease could be identified.

(1) **Impact of COVID-19 on paediatric care** – During the first COVID-19 wave (March to May 2020) significantly fewer patients were seen at the Dr. von Hauner Children's Hospital compared to previous years: Compared to 2019, the number of patients in A&E decreased by 43%, while the number of children with infectious diseases decreased by 74% and the number of surgeries performed decreased by 34%. Reasons for this significant reduction in patient numbers may be: the reduction of transmission pathways of other infectious diseases through social distancing, avoidance of seeking medical care for fear of infection, and change in hospital policies related to elective surgeries and interventions.

(2) **Manifestation of COVID-19 in children** – The manifestation of SARS-CoV-2 infections ranged from the complete absence of symptoms (i.e. asymptomatic) to life-threatening diseases. Of the cohort recruited for this thesis (n=12, all with positive SARS-CoV-2 -PCR test and/or -serology), 25% were found to be in a healthy condition, 53% displayed mild symptoms such as fever,

cough and/or rhinitis for a maximum of 6 days, and 17% developed life-threatening hyperinflammatory syndromes including acute respiratory distress syndrome and multiorgan failure.

(3) **Predictors for severe COVID-19** – Within the recruited cohort (detailed statistical analysis could not be performed at n=12), the following factors were observed to be potentially associated with having a higher risk of developing a severe course of COVID-19 in children: young age (mean=2 years), pre-existing conditions including congenital heart diseases (PFO, ASD II and AVSD) or Down Syndrome. Additionally, the following clinical parameters were observed in children with a severe course of disease: highly elevated levels of IL-6, IL-2, CRP and Procalcitonin, occurrence of emesis, longer duration of fever, development of hyperinflammatory syndromes (sHLH, PIMS), prolonged duration of hospitalisation and treatment with IVIG and corticosteroids.

It should be noted, that given the small cohort size (associated to the low hospitalisation rates of children in the first wave), the findings presented in this thesis must be interpreted with caution and can only act as reference point when comparing to other trends seen in literature.

Further Research – While the research presented in this thesis contributed to creating clarity in the very early phases/first wave of the COVID-19 outbreak, it by no means was sufficient to fully understand how COVID-19 manifests in children. Many open questions remain (at the time of publication), including: why are many children better equipped to fight the virus than adults? Why does the immune system of some children react in a way that the reaction itself becomes life-threatening? What is the exact pathogenesis of COVID-19 (in particular for SARS-CoV-2 triggered hyper-inflammatory syndromes)? What patient groups/attributes are particularly at risk for developing a severe course of illness? Research on immunological processes and genetic characteristics may help give answers to these questions, in a time where undoubtedly the urgency to do so is at an all-time-high.

Zusammenfassung & Ausblick (German translation)

Kontext - Das neuartige Coronavirus SARS-CoV-2 begann sich im Jahr 2020 weltweit zu verbreiten und löste eine oft lebensbedrohliche Lungenkrankheit namens COVID-19 aus. Während der ersten Welle (März bis Mai 2020) war weitgehend unbekannt, welche Auswirkungen diese Krankheit auf die Menschen haben würde, und insbesondere bei Kindern war der Krankheitsverlauf unklar. Zunächst schien es, als seien Kinder besonders resistent gegen das Virus (im Vergleich zu älteren Altersgruppen), doch wurden bei einer begrenzten Anzahl von Kindern schwere hyperinflammatorische Syndrome beobachtet, die gezielte Forschungsarbeiten in dieser Altersgruppe notwendig machten.

Ziele - Die im Rahmen dieser Arbeit durchgeführten Untersuchungen zielten darauf ab: (1) zu untersuchen, inwieweit die Kinderklinik der LMU München (Dr. von Haunersche Kinderklinik) während der ersten COVID-19-Welle (März bis Mai 2020) betroffen war, (2) die Manifestation von COVID-19 bei hospitalisierten Kindern zu beschreiben und (3) zu analysieren, ob Prädiktoren für einen schweren Verlauf der Erkrankung identifiziert werden konnten.

(1) **Auswirkungen von COVID-19 auf die pädiatrische Versorgung** - Während der ersten COVID-19-Welle (März bis Mai 2020) wurden im Dr. von Haunerschen Kinderspital deutlich weniger Patienten vorstellig als in den Vorjahren: Im Vergleich zu 2019 sank die Zahl der Patienten in der Notaufnahme um 43%, die Zahl der Kinder mit Infektionskrankheiten um 74% und die Zahl der durchgeführten Operationen um 34%. Gründe für diesen signifikanten Rückgang der Patientenzahlen könnten sein: die Verringerung der Übertragungswege anderer Infektionskrankheiten durch soziale Distanzierung, die Vermeidung der Inanspruchnahme medizinischer Versorgung aus Angst vor Ansteckung und die Änderung der Krankenhauspolitik in Bezug auf elektive Operationen und Eingriffe.

(2) **Manifestation von COVID-19 bei Kindern** - Die Manifestation von SARS-CoV-2-Infektionen reichte vom völligen Fehlen von Symptomen (d. h. asymptomatisch) bis zur lebensbedrohlichen Verläufen. Von der für diese Arbeit rekrutierten Kohorte (n=12, alle mit positivem SARS-CoV-2 -PCR-Test und/oder -Serologie) befanden sich 25% in einem symptomlosen Zustand, 53% zeigten leichte Symptome wie Fieber, Husten und/oder Rhinitis für maximal 6 Tage, und 17% entwickelten lebensbedrohliche hyperinflammatorische Syndrome einschließlich akutem Atemnotsyndrom und Multiorganversagen.

(3) **Prädiktoren für schweres COVID-19** - In der rekrutierten Kohorte (eine detaillierte statistische Analyse konnte bei n=12 nicht durchgeführt werden) wurden die folgenden Faktoren beobachtet, die potenziell mit einem höheren Risiko für die Entwicklung eines schweren Verlaufs von COVID-19 bei Kindern verbunden sind: junges Alter (Mittelwert=2 Jahre), Vorerkrankungen einschließlich angeborener Herzerkrankungen (PFO, ASD II und AVSD) oder Down-Syndrom. Darüber hinaus wurden bei Kindern mit schwerem Krankheitsverlauf folgende klinische Parameter beobachtet: stark erhöhte IL-6-, IL-2-, CRP- und Procalcitonin-Werte, Auftreten von Erbrechen, längere Dauer des Fiebers, Entwicklung von hyperinflammatorischen Syndromen (sHLH, PIMS), längere Dauer des Krankenhausaufenthalts und Behandlung mit IVIG und Kortikosteroiden.

Es sei darauf hingewiesen, dass die in dieser Arbeit vorgestellten Ergebnisse angesichts der geringen Kohortengröße (in Verbindung mit den niedrigen Hospitalisierungsraten der Kinder während der ersten Welle) mit Vorsicht zu interpretieren sind und nur als Anhaltspunkt für den Vergleich mit anderen in der Literatur beobachteten Trends dienen können.

Weitere Forschungsarbeiten - Die in dieser Arbeit vorgestellten Forschungsergebnisse haben zwar dazu beigetragen, Klarheit über die sehr frühen Phasen bzw. die erste Welle des COVID-19-Ausbruchs zu schaffen, sie reichten

jedoch bei weitem nicht aus, um vollständig zu verstehen, wie sich COVID-19 in Kindern manifestiert. Viele Fragen sind (zum Zeitpunkt der Veröffentlichung) noch offen, darunter: Warum sind viele Kinder besser gegen das Virus gewappnet als Erwachsene? Warum reagiert das Immunsystem einiger Kinder so stark, dass die Reaktion selbst lebensbedrohlich wird? Wie sieht die genaue Pathogenese von COVID-19 aus (insbesondere bei durch SARS-CoV-2 ausgelösten hyperinflammatorischen Syndromen)? Welche Patientengruppen sind besonders gefährdet, einen schweren Krankheitsverlauf zu entwickeln? Die Erforschung immunologischer Prozesse und genetischer Merkmale kann dazu beitragen, Antworten auf diese Fragen zu finden und haben zweifellos eine Dringlichkeit wie nie zuvor.

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8. List of tables and figures

8.1. List of figures

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Figure 1.3 Taxonomy of the coronaviruses with beta-coronaviruses, that are able to infect human cells: hCoV-HKU1 and hCoV-OC43 (usually causing the common cold) and SARS-CoV and MERS-CoV (causing respiratory disease), based on [14]..... 5

Figure 1.4 The 3 phases of infection, with progression of severity depending on the state of immune reaction, according to days since symptom start; based on [26]. 9

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Figure 4.1 (a) A heatmap showing the average number of confirmed SARS-CoV-2 infections in Germany per 100,000 inhabitants and by age group during the first wave of the pandemic (data extracted from RKI [42]), and (b) the rise and fall of the number of nationwide infections within the age group 0-19 alongside the number of children hospitalised with COVID-19 in orange (data extracted from DGPI, only considering normal ward [43]) and recruited as part of this study in blue..... 25

Figure 4.2 shows the numbers of 3 different events at the Dr. von Hauner Children's Hospital, comparing the last 3 years from February to July: (a) The number of patients in thousands who presented at A&E. (b) The number of patients in thousands with an infection according to ICD-10 (the included ICD-10 diagnoses are listed in the text below). (c) The number of surgeries performed. 28

Figure 4.3 Type and duration of symptoms in relation to the time of hospitalisation in the "Mild & Moderate" cohort. Patients COV001, COVS007 and COV009 can be found in Table 4.4 above - exemplary for three different/ascending grades of health preload. *COV022 is not shown in the graph. Note: fever in COV009 appeared on day 1 and day

5, in between he was largely within normal temperatures and is counted as "2 days of fever" in further calculations. 37

Figure 4.4 Type and duration of symptoms in relation to the time of hospitalisation in the 'Severe' cohort. (a) COV006 represents the child with severe COVID-19, he shows a long period of illness (24 days) with mainly respiratory symptoms, but also fever and fatigue. (b) COVS009 represents the child with suspected PIMS, which prominently appeared with high fever for 13 days, respiratory symptoms were of subordinate role. 40

Figure 4.5 (a) chest ray posterior-anterior recorded in sitting position on day 0 of hospital admission, showing confluent infiltrates on both sides leading to bronchopneumonia with ventilation dysfunction. (b) computed tomography recorded on day 3 with features of an atypical pneumonia, with marked deterioration compared to the recording of the 0. 43

Figure 4.6 showing clinical parameters, treatment and phases of disease of a patient with severe COVID-19 according to the days of hospital stay. Phase I is not shown in the graph as it appeared before hospital admission. For note: the patient received a single IVIG dose on the same day when Remdesivir and Prednisolon were started. Values within the reference range are coloured in green, above/under the reference in red. Reference values: body temperature (<37°C), lymphocytes (18-67 %), CRP (0-1.1 mg/dl). CMV (Continuous mandatory ventilation), CRP (C-reactive protein), IVIG (intravenous immunoglobulins), PICU (paediatric intensive care unit). Parts of this figure (therapeutics, virology) have already been published in [1]. 44

Figure 4.7 (a) chest X-ray from the 9TH day of treatment with bilateral shadowing, (b) chest X-ray from the 20TH day of treatment showing regressive reduction in transparency in the middle and lower lobes and complete regression of shading in the apical right middle lobe. 49

Figure 4.8 bar chart visualising the sex distribution in the three groups in percent. 51

Figure 4.9 bar chart showing the percentage of the cohort with: complete STIKO vaccination status, cases where the index case is known (person that directly transmitted the infection), where bacterial coinfection during illness and previous febrile infections occurred within the last months before hospital admission. Respiratory infections were not reported and are therefore not shown. 51

Figure 4.10 bar chart providing an overview of which symptoms occurred at any time during the illness (yes/no). By definition, the "Asymptomatic" group is not shown. 52

Figure 4.11 Bar chart showing the average of therapeutical measurements such as anti-infectives, systemic corticosteroids, bronchodilatators and supplemental oxygen in the three groups. 52

Figure 4.12 bar chart showing the average of reported pre-existing conditions. "Others" include Down Syndrome, Alagille Syndrome, iron deficiency anaemia. 53

Figure 4.13 (a) boxplot (from bottom to top consisting of: minimum, lower quantile, mean (x), median, upper quantile, maximum), showing the age distribution of the three groups and (b) showing the differing Body-Mass-Index in kg/m² of the three groups. 54

Figure 4.14 boxplots showing the duration of symptoms in days, comparing the groups "Mild & Moderate" and "Severe". 55

Figure 4.15 boxplot showing the duration of therapeutic measures in days that were applied within the different groups. 56

Figure 4.16 Boxplots showing selected laboratory values from admission day of the groups "Mild & Moderate" and "Severe", namely: CRP, IL-6, Procalcitonin and Haemoglobin. 57

8.2. List of tables

Table 3.1 showing the entire cohort recruited: (I) one COVID-19 group and two control groups containing (II) participants with any illnesses except COVID-19 and (III) healthy participants from the surgery department. Green highlighted: COVID-19 patients aged 0-18 years make the study cohort for this thesis, the others will not be thematised any further, but are listed here for completeness. 18

Table 3.2 shows the number of all patients that were recruited in the cooperating institutions. As mentioned above only 12 children met the inclusion criteria for this thesis. 19

Table 3.3: Categorisation of COVID-19 severity from (1) asymptomatic to (3) critical according to criteria adapted from NIH [34] and WHO [35]. ARDS (acute respiratory distress syndrome) 20

Table 3.4 Sources used for generating primary and secondary data. 20

Table 4.1 The performed T-test (paired two samples for means) provides the mean, standard deviation (SD) of the number of (A) patients, that were admitted to the A&E, (B) patients, that presented with predefined ICD-10 infections and (C) operations performed in the hospital averaged over the months MARCH-MAY and additionally the *P*-values (*P*(T<=t) two-tail) of 2018 and 2019 comparing to 2020. To avoid the bias of multiplicity through multiple testing the level of significance is adjusted with the Bonferroni Correction to $P'=.0083$ by dividing the usual level of significance to adjust (A=.05) through the number of tests performed (N=6) → $\alpha n = 0.056 = 0.0083$. 29

Table 4.2 shows the cohort divided into three groups "Asymptomatic", "Mild and Moderate" and "Severe" according to their clinical presentation, giving an overview to general characteristics, symptoms, pre-existing conditions, therapy and specific information. For note: The figures refer to the available data, missing information was not included in the percentage. 31

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Table 4.4 Characteristics and health care information from the hospital stay of study participants with mild symptoms as a proxy for 3 different degrees of pre-existing conditions. (Hb) means Haemoglobin, (-) means decrease and (!) increase of the

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Table 4.6 showing SIRS criteria with references (extracted from the AWMF-register [45]) and how our patient fulfilled 3 out of 4 SIRS criteria with involvement of the cardiovascular-, respiration- and blood-system. *Horowitz ratio/Oxygenation index = paO2/FiO2 47

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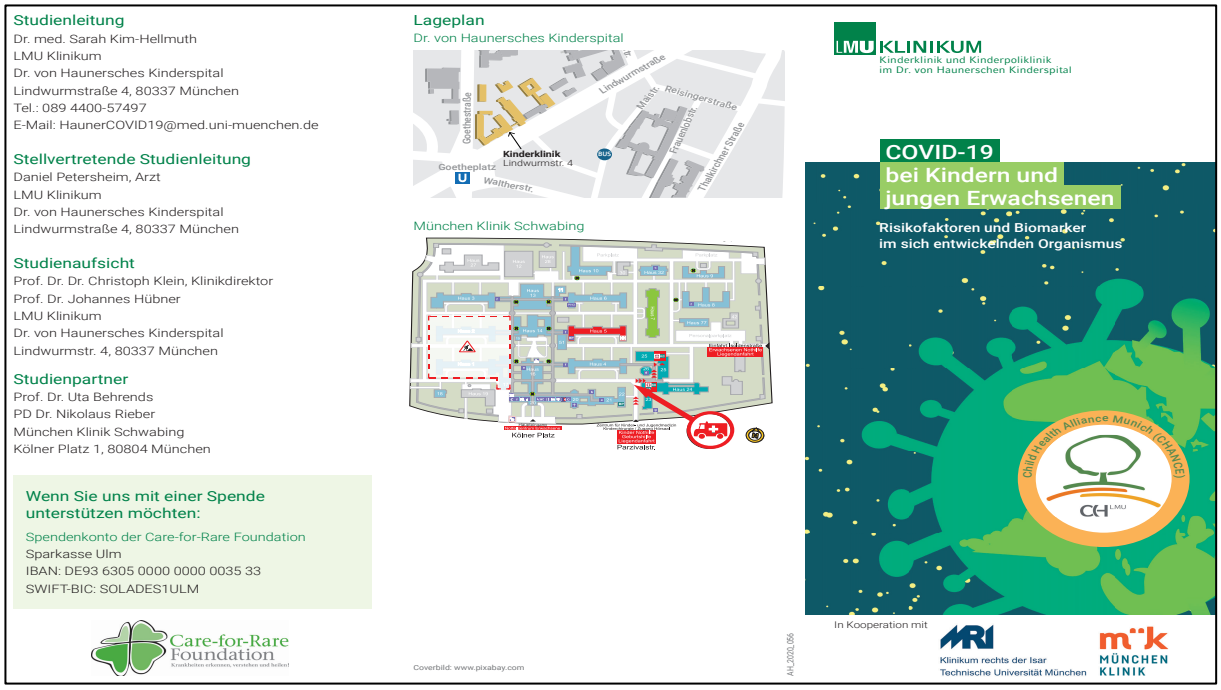
Table 4.8 comparing the average (mean value) and SD (standard deviation) of the three different groups of severity. 54

Table 4.9 a heat map showing the percentage of patients with laboratory values on admission day outside/inside the individual reference range, where 100% means that all values are within the reference range and 0% means that all values are outside the reference range. Values of the "Asymptomatic" group are not listed due to the lack of blood samples. Values listed: CRP (C-reactive Protein), IL-6 (Interleukin-6), GOT (Glutamate-Oxaloacetate-Transaminase), GPT (Glutamat-Pyruvat-Transaminase), LDH (Lactatdehydrogenase), pTT (Partial-Thromboplastin-Time). ... 58

Table 4.10 showing the Mean and SD (standard deviation) of selected laboratory parameters to display the absolute value range of movement. 59

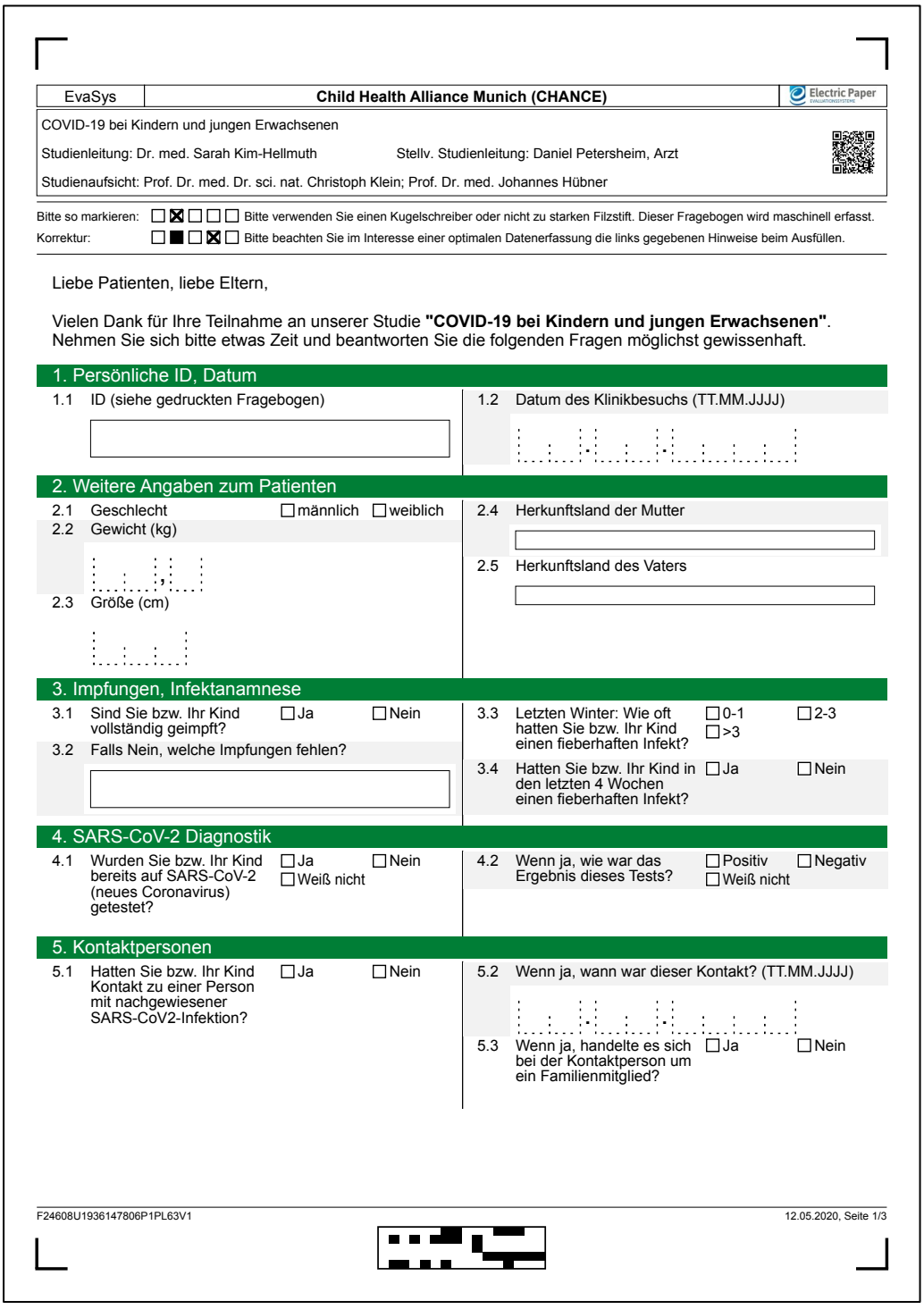
9. Appendix

A1 Study flyer



Annex 1: Informative and catchy flyer to raise awareness for the study.

A2 Clinical survey



EvaSys | **Child Health Alliance Munich (CHANCE)** | Electric Paper

COVID-19 bei Kindern und jungen Erwachsenen

Studienleitung: Dr. med. Sarah Kim-Hellmuth Stellv. Studienleitung: Daniel Petersheim, Arzt

Studienaufsicht: Prof. Dr. med. Dr. sci. nat. Christoph Klein; Prof. Dr. med. Johannes Hübner

Bitte so markieren: ☐ ☒ ☐ ☐ ☐ Bitte verwenden Sie einen Kugelschreiber oder nicht zu starken Filzstift. Dieser Fragebogen wird maschinell erfasst.

Korrektur: ☐ ■ ☐ ☒ ☐ Bitte beachten Sie im Interesse einer optimalen Datenerfassung die links gegebenen Hinweise beim Ausfüllen.

Liebe Patienten, liebe Eltern,

Vielen Dank für Ihre Teilnahme an unserer Studie **"COVID-19 bei Kindern und jungen Erwachsenen"**. Nehmen Sie sich bitte etwas Zeit und beantworten Sie die folgenden Fragen möglichst gewissenhaft.

1. Persönliche ID, Datum

1.1 ID (siehe gedruckten Fragebogen)

1.2 Datum des Klinikbesuchs (TT.MM.JJJJ)

2. Weitere Angaben zum Patienten

2.1 Geschlecht ☐ männlich ☐ weiblich

2.2 Gewicht (kg)

2.3 Größe (cm)

2.4 Herkunftsland der Mutter

2.5 Herkunftsland des Vaters

3. Impfungen, Infektanamnese

3.1 Sind Sie bzw. Ihr Kind vollständig geimpft? ☐ Ja ☐ Nein

3.2 Falls Nein, welche Impfungen fehlen?

3.3 Letzten Winter: Wie oft hatten Sie bzw. Ihr Kind einen fieberhaften Infekt? ☐ 0-1 ☐ 2-3 ☐ >3

3.4 Hatten Sie bzw. Ihr Kind in den letzten 4 Wochen einen fieberhaften Infekt? ☐ Ja ☐ Nein

4. SARS-CoV-2 Diagnostik

4.1 Wurden Sie bzw. Ihr Kind bereits auf SARS-CoV-2 (neues Coronavirus) getestet? ☐ Ja ☐ Nein ☐ Weiß nicht

4.2 Wenn ja, wie war das Ergebnis dieses Tests? ☐ Positiv ☐ Negativ ☐ Weiß nicht

5. Kontaktpersonen

5.1 Hatten Sie bzw. Ihr Kind Kontakt zu einer Person mit nachgewiesener SARS-CoV2-Infektion? ☐ Ja ☐ Nein

5.2 Wenn ja, wann war dieser Kontakt? (TT.MM.JJJJ)

5.3 Wenn ja, handelte es sich bei der Kontaktperson um ein Familienmitglied? ☐ Ja ☐ Nein

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Annex 2.1: Clinical survey filled out by study participants (1/3).

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6. Symptome

Welche Symptome bestehen/bestanden bei Ihnen bzw. Ihrem Kind?

6.1	**Fieber**	☐ Ja	☐ Nein
6.2	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.3	**Husten**	☐ Ja	☐ Nein
6.4	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.5	**Atemnot**	☐ Ja	☐ Nein
6.6	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.7	**Halsschmerzen**	☐ Ja	☐ Nein
6.8	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.9	**Schnupfen**	☐ Ja	☐ Nein
6.10	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.11	**Durchfall/dünner Stuhl**	☐ Ja	☐ Nein
6.12	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.13	**Erbrechen**	☐ Ja	☐ Nein
6.14	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.15	**Bauchschmerzen**	☐ Ja	☐ Nein
6.16	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.17	**Müdigkeit/ Abgeschlagenheit**	☐ Ja	☐ Nein
6.18	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.19	**Kopfschmerzen**	☐ Ja	☐ Nein
6.20	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14
6.21	**Geruchs-/ Geschmacksverlust**	☐ Ja	☐ Nein
6.22	Wenn ja, seit wie vielen Tagen?	☐ heute ☐ 2 ☐ 5-6 ☐ 11-14	☐ 1 ☐ 3-4 ☐ 7-10 ☐ >14

6.23 Bestehen/bestanden weitere Symptome und wenn ja, welche?

6.24 Wurden die Symptome bereits behandelt (inkl. "Hausapotheke")? ☐ Ja ☐ Nein

6.25 Wenn ja, wie? (Medikament, Dosis, wie lange gegeben?)

7. Vorerkrankungen

7.1 Bestehen bei Ihnen bzw. Ihrem Kind Vorerkrankungen? ☐ Ja ☐ Nein

7.2 Falls ja, bitte Zutreffendes ankreuzen:

- ☐ Lungenerkrankung
- ☐ Typ-2 Diabetes mellitus
- ☐ Autoimmunerkrankung/ rheumatische Erkrankung
- ☐ Nierenerkrankung/Niereninsuffizienz
- ☐ Allergien
- ☐ Herzerkrankung/Herzfehler
- ☐ Neurologische/neuromuskuläre Erkrankung
- ☐ Andere
- ☐ Typ-1 Diabetes mellitus
- ☐ Bekannter Immundefekt
- ☐ Hämato-onkologische Erkrankung

Annex 2.2: Clinical survey filled out by study participants (2/3).

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7. Vorerkrankungen [Fortsetzung]

7.3 Weitere Informationen (Name der Erkrankung, Schweregrad, Zeitpunkt der Erstdiagnose, Therapie etc.)

8. Medikamente

8.1 Nehmen Sie bzw. Ihr Kind regelmäßig Medikamente ein? Wenn ja, welche und wie oft?

8.2 Besteht eine medikamentöse Immunsuppression? ☐ Ja ☐ Nein

8.3 (Vom Studienpersonal auszufüllen: Medikationsplan wird aus vorherigen Arztbriefen entnommen) ☐ Ja ☐ Nein

9. Weitere Angaben

9.1

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Annex 2.3: Clinical survey filled out by study participants (3/3).

A3 Excel sheet excerpt

Patient code/ Pseudonym	DOB (dd.mm.yyyy)	Age	Age group	date inclusion	date of hospital arrival	date of hospital discharge	days since admission	days of inhospital stay	inpatient (y=1, n=0)	Sex	Sex (% Male)	weight (kg)	height (cm)	BMI	country of origin (mother)	country of origin (father)	Is your child completely vaccinated?	How many febrile infections last winter? (1-4 >0-1; 2->2-3; 3-> >3	Febrile infection s within the last 2 weeks?	Have you already been tested for SARS-CoV-2?	SARS Test result	Index case known	Index case specif
COV001	14.09.19	0	1 (0 - 2)	31.03.20	31.03.20	02.04.20	1	3	1	m	1,0	7,2	68,0	15,6	Deutschland	Deutschland	1,00	0,00	0,00	1	1	1	father
COV002	11.05.91	28	6 (18+)	31.03.20	31.03.20	02.04.20	1	3	1	f	0,0	65,0	170,0	22,5	Deutschland	Deutschland	1,00	0,00	0,00	1	1	1	boyfr
COV003	03.11.04	15	5 (14 - 18)	02.04.20	31.03.20	02.04.20	3	3	1	f	0,0	58,0	174,0	19,2	Deutschland	Deutschland	1,00	1,00	1,00	1	1	1	Conta
COV004	17.10.07	12	4 (10 - 14)	02.04.20	28.03.20	02.04.20	6	6	1	m	1,0	46,0	165,0	16,9	NA	NA	NA	NA	NA	1	1	1	moth
COV005	25.09.14	5	3 (5 - 10)	02.04.20	29.03.20	07.04.20	5	10	1	f	0,0	17,0	106,0	15,1	Romania	Romania	1,00	3,00	0,00	1	1	0	NA
COV006	16.02.17	3	2 (2 - 5)	02.04.20	30.03.20	20.04.20	4	21	1	m	1,0	12,7	89,0	16,0	Kongo	Kongo	1,00	2,00	0,00	1	1	0	Kein bekan
COV007	29.06.15	4	2 (2 - 5)	06.04.20	06.04.20	11.04.20	1	6	1	m	1,0	15,0	112,0		Deutschland	Deutschland	NA	NA	NA	1	0	0	NA
COV008	02.12.02	17	5 (14 - 18)	08.04.20	07.04.20	11.04.20	2	4	1	f	0,0	75,0	**175,0**	24,5	NA	NA	1,00	NA	NA	1	1	1	Fathe kontak
COV009	29.01.02	18	6 (18+)	08.04.20	02.04.20	15.04.20	7	13	1	m	1,0	65,0	188,0	18,4	Deutschland	Deutschland	NA	NA	NA	1	1	0	NA
COV015	26.08.19	0	1 (0 - 2)	29.04.20	27.04.20	30.04.20	3	3	1	m	1,0	10,1	72,0		Deutschland	na	1,00	1,00	NA	1	0	0	NA
COV016	25.07.99	20	6 (18+)	29.04.20	29.04.20	29.04.20	1	0	0	f	0,0	50,0	168,0		Deutschland	Deutschland	1,00	2,00	0,00	1	0	0	NA
COV017	15.04.11	9	3 (5 - 10)	29.04.20	29.04.20	04.05.20	1	6	1	m	1,0	33,0	150,0		Deutschland	Deutschland	1,00	1,00	0,00	1	0	0	NA
COV018	27.12.06	13	4 (10 - 14)	02.05.20	02.05.20	06.05.20	1	6	1	m	1,0	44,0	155,0		Tschechien	Tschechien	1,00	1,00	0,00	1	0	0	NA
COV019	15.01.19	1	1 (0 - 2)	04.05.20	04.05.20	11.05.20	1	8	1	m	1,0	9,2	72,0		Kenia	Nigeria	1,00	2,00	0,00	1	0	0	NA
COV020	11.08.88	31	6 (18+)	06.05.20	06.05.20	06.05.20	1	0	0	f	0,0	55,0	172,0	18,6	Deutschland	Deutschland	1,00	1,00	0,00	1	1	1	NA
COV021	29.09.08	11	4 (10 - 14)	07.05.20	06.05.20	07.05.20	2	1	1	f	0,0	72,0	155,0		Holland	Türkei	1,00	2,00	0,00	1	0	0	NA
COV022	15.07.16	3	2 (2 - 5)	12.05.20	12.05.20	12.05.20	1	0	1	f	0,0	19,2	98,0	20,0	Afghanistan	Afghanistan	1,00	1,00	0,00	y (pos. am	1	1	father
COV005	30.06.05	14	5 (14 - 18)	30.04.20	29.04.20	05.05.20	2	6	1	m	1,0	65,0	170,0	22,5	NA	NA	1,00	1,00	0,00	1	1	0	NA
COV007	15.01.03	17	5 (14 - 18)	12.05.20	11.05.20	15.05.20	2	4	1	f	0,0	70,0	174,0	23,1	NA	NA	1,00	1,00	1,00	1	1	1	father
COV009	24.01.19	1	1 (0 - 2)	22.05.20	08.05.20	22.05.20	15	14	1	f	0,0	8,7	72,0	16,8	NA	NA	1,00	NA	NA	1	0	NA	NA

Annex 3: Extract from the Excel spreadsheet used for data collection.

A4 Patients information

LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN

Patienten/-Probandeninformation für Erwachsene und Erziehungsberechtigte zur Studie:

COVID-19 bei Kindern und jungen Erwachsenen
–
Identifizierung individueller Risikofaktoren und Biomarker im sich entwickelnden Organismus

Patientenetikett:

Liebe Patient, lieber Proband, liebe Eltern!

Wir möchten Sie und/oder Ihr Familienmitglied über unsere Studie zur Charakterisierung der COVID-19 Erkrankung bei Kindern und jungen Erwachsenen informieren. Bitte lesen Sie diese Informationen aufmerksam. Sie können im Anschluss alle offenen Fragen mit einem Arzt oder dem Studienteam besprechen. Vielen Dank!

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Direktor der Klinik: Prof. Dr. Christoph Klein
Das Klinikum der Universität München ist eine Anstalt des Öffentlichen Rechts
Vorstand: Ärztlicher Direktor: Prof. Dr. med. Karl-Walter Jauch (Vorsitz), Kaufmännischer Direktor: Markus Zendler
Stellvertreter des Pflegedirektors: Marcus Huppertz, Vertreter der Medizinischen Fakultät: Prof. Dr. med. dent. Reinhard Hickel (Dekan)
Institutionskennzeichen: 260 914 050, Umsatzsteuer-Identifikationsnummer gemäß §27a Umsatzsteuergesetz: DE813536017

Annex 4: Patients information for clarification about the study (p. 1/6)

A5 Declaration of consent

LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN | CAMPUS INNENSTADT | KINDERKLINIK UND KINDERPOLIKLINIK IM DR. V. HAUNERSCHEN KINDERSPITAL

Einverständniserklärung zur Studie:
COVID-19 bei Kindern und jungen Erwachsenen – Identifizierung individueller Risikofaktoren und Biomarker im sich entwickelnden Organismus

Patientenetikett:

Ich habe den Aufklärungs- und Einwilligungsbogenbogen mit Informationen über die oben genannte Studie erhalten, gelesen und verstanden. Ich wurde über Zweck, Art, Umfang und Aussagekraft der geplanten Untersuchungen aufgeklärt. Ich hatte ausreichend Gelegenheit, offene Fragen zu besprechen. Ich habe auch eine Kopie dieses Aufklärungs- und Einwilligungsbogens erhalten. Die Teilnahme an dieser Studie ist freiwillig.
Mit meiner Unterschrift willige ich in meine Studienteilnahme oder die meines Kindes ein und gebe mein Einverständnis, dass mir oder meinem Kind Blut, ein Rachenabstrich oder eine Stuhlprobe entnommen und mit Hilfe der oben beschriebenen Methoden untersucht wird.
Ich bin damit einverstanden, dass meine Daten in anonymsierter Form in einer wissenschafltichen Zeitung veröffentlicht werden. Dabei wird aus den Daten nicht hervorgehen, wer an dieser Untersuchung teilgenommen hat. Meine persönlichen Daten unterliegen dem Datenschutzgesetz.

Bitte ankreuzen:

Ich bin damit einverstanden für weitere Fragen oder im Falle von besonderen Ergebnissen vom Studienteam auch später noch kontaktiert werden zu können.

ja nein

Telefonnummer:_____ E-mail:_____

Ich bin damit einverstanden, dass neben den Genotypisierung häufiger genetischer Varianten auch Exom- bzw. Genom-Sequenzierungen durchgeführt werden. Wenn ich „nein“ ankreuze bin ich nur mit der Genotypisierung häufiger genetischer Varianten einverstanden.

ja nein

Wenn „ja“, bin ich ferner über die Möglichkeit, dass in seltenen Fällen sogenannte genetische Zufalls- oder Zusatzbefunde erhoben werden, informiert worden. Ich bitte um Mitteilung von Zufallsbefunden, sofern sich aus ihnen praktische Konsequenzen ableiten lassen.

ja nein

Ich bin damit einverstanden, dass ggf. Teile meiner Bioproben oder Daten in pseudonymisierter Form an wissenschaftliche Kooperationspartner im In- und Ausland, zu Zwecken der Erforschung von COVID-19 weitergegeben und in für diesen Zweck etablierte internationale Datenbanken eingebracht werden. Ich bin darüber informiert worden, dass die Datenweitergabe primär an Zielländer erfolgt, deren Sicherheits- und Datenschutzstandards, denen in der Europäischen Union gleichwertig sind. Es ist mir bekannt, dass dies jedoch nicht in allen Fällen gewährleistet werden kann.

ja nein

Direktor der Klinik: Prof. Dr. Christoph Klein
Das Klinikum der Universität München ist eine Anstalt des Öffentlichen Rechts
Vorstand: Ärztlicher Direktor: Prof. Dr. med. Karl-Walter Jauch (Vorsitz), Kaufmännischer Direktor: Markus Zendler
Stellvertreter des Pflegedirektors: Marcus Huppertz, Vertreter der Medizinischen Fakultät: Prof. Dr. med. dent. Reinhard Hickel (Dekan)
Institutionskennzeichen: 260 914 050, Umsatzsteuer-Identifikationsnummer gemäß §27a Umsatzsteuergesetz: DE813536017

Annex 5 The declaration of consent had to be subscribed from both parents/legal guardians (p. 1/2)

A6 Ethics proposal

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

ETHIKKOMMISSION BEI DER LMU MÜNCHEN

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29.07.2020/Hb/sh

Projekt Nr: **20-263** (bitte bei Schriftwechsel angeben)

Nachträgliche Änderungen;

Studientitel: COVID-19 bei Kindern und jungen Erwachsenen – Identifizierung individueller Risikofaktoren und Biomarker im sich entwickelnden Organismus
Antragsteller: Dr. med. Sarah Kim-Hellmuth, Dr. von Haunerschen Kinderspital, Klinikum der Universität München, Lindwurmstr. 4, 80337 München,
Untersucher: Dr. med. Sarah Kim-Hellmuth, Klinikum der Universität München, Kinderklinik und Kinderpoliklinik im Haunerschen Kinderspital, Lindwurmstr. 4, 80337 München

Sehr geehrte Frau Dr. Kim-Hellmuth,

besten Dank für die Übersendung der geänderten Unterlagen.

Die Ethikkommission (EK) äußert keine Einwände gegen das Amendment, allerdings ist auf einen tiefen Rachenabstrich bei den gesunden Kindern zu verzichten. Bitte stattdessen einen Mundabstrich vornehmen, um die Belastungen zu minimieren.

Das Votum ist gültig bis zum 30.07.2025 (5 Jahre).

Sofern das Votum über diesen Zeitraum hinaus benötigt wird, bitten wir, der EK unaufgefordert die durchgeführten Forschungen mit den Registerdaten /Bioproben zusammenfassend darzulegen und eine Verlängerung des Votums mindestens 3 Monate vor Fristende zu beantragen.

Mit freundlichen Grüßen

[redacted]

Prof. Dr. W. Eisenmenger
Vorsitzender der Ethikkommission

Mitglieder der Kommission:
Prof. Dr. W. Eisenmenger (Vorsitzender), Prof. Dr. R. M. Huber (stellv. Vorsitzender), Prof. Dr. C. Wendtner (stellv. Vorsitzender), Prof. Dr. H. Angstwurm, Dr. G. Atzeni, Prof. Dr. S. Böck, J. Eckert, Prof. Dr. B. Emmerich, Prof. Dr. S. Endres, Prof. Dr. R. Fischer, Prof. Dr. R. Gärtner , Prof. Dr. O. Genzel- Boroviczény, Prof. Dr. K. Hahn, Prof. Dr. N. Harbeck, Dr. B. Henrikus, Prof. Dr. C. Heumann, Prof. Dr. R. Hohlfeld, Prof. Dr. A. Holstege, Prof. Dr. V. Klauss, Dr. F. Kohlmayer, Dr. K. Kühlmeyer, Prof. Dr. J. Lindner, Prof. Dr. S. Lorenzl, Prof. Dr. U. Mansmann, Prof. Dr. G. Marckmann, Dr. V. Mönch, Prof. Dr. H. Mudra, Prof. Dr. R. Penning, Prof. Dr. J. Peters, Prof. Dr. K. Pfeifer, Dr. R. Ratzel, Prof. Dr. H. Scharday, Prof. Dr. M. Schmauss, Prof. Dr. U. Schroth, Prof. Dr. O. Steinlein, PD Dr. G. Stüben, Dr. B. Vogl, Prof. Dr. H. Waldner, PD Dr. U. Wandl, Prof. Dr. M. Wörnle, Dr. A. Yassouridis, Dr. C. Zach

Annex 6 The ethics proposal for the study

A7 PIMS Criteria

Cirteria for PIMS		
Criteria	**COVS009**	
Age < 21 years	1 year	✓
the need for hospitalisation	14 days	✓
fever for at least 24 hours of over 38°C	12 days	✓
>2 organ system dysfunctions (i.e. cardial, renal, etc.)	renal insufficency	
laboratory evidence of an inflammatory response (elevated IL-6, CRP, ESR, etc.)	CRP 206 mg/l, Il-6 102.5 ng/l	✓
association with a SARS-CoV-2 infection	anti-SARS-CoV-2 IgG	✓

Annex 7 COVS009 fulfilling 5/6 criteria points for PIMS according to the CDC [28].

A8 Murray lung injury score

The Murray lung injury scale				
1. Chest roentgenogram score			COV006	
No alveolar consolidation		0		
Alveolar consolidation confined to 1 quadrant		1		
Alveolar consolidation confined to 2 quadrants		2		
Alveolar consolidation confined to 3 quadrants		3		
Alveolar consolidation in all 4 quadrants		4	Large-scale consolidation in all segments	4
2. Hypoxemia score (in mmHg)				
PaO2/FiO2	>300	0		
PaO2/FiO2	225-299	1		
PaO2/FiO2	175-224	2		
PaO2/FiO2	100-174	3		
PaO2/FiO2	<100	4	65.2	4
3. PEEP score (in cm H2O)				
PEEP	<5	0		
PEEP	6–8	1		
PEEP	9-11	2		
PEEP	12-14	3	12	3
PEEP	>15	4		
Level of lung injury				
0 = no lung injury, 0.3-2.5 = mild to moderate lung injury, >2.5 = severe lung injury			**3,7 → Severe lung injury**	11/3

Annex 8 The Murray lung injury scale based on [195].

A9 HScore

HScore for secondary HLH by clinical parameter		
Criteria	**Points**	**COV006**
Temperature		
<38.4°C	0	
38.4-39.4°C	33	38.5°C
>39.4°C	49	
Organomegaly		
None	0	-
Hepatomegaly or splenomegaly	23	
Hepatomegaly and splenomegaly	38	
Number of cytopenia		
Cell lineage		
One lineage	0	
Two lineages	24	Platelet count 73 G/l WBC 4,75 G/l
Three lineages	34	
Triclycerides (mg/dL)		
<132,7	0	
132,7-354	44	151 mg/dL
>354	64	
Fibrinogen (mg/dL)		
>250	0	
≤ 250, >400	30	455 mg/dl
Ferritin (ng/ml)		
<2000	0	
2000-6000	35	
>6000	50	7499 ng/ml
Serum aspartate aminotransferase (IU/L)		
<30	0	
≥30	19	108 IU/L
Hemophagocytosis on bone marrow aspirate		
No	0	-
Yes	35	
Known immunosuppression		
No	0	-
Yes	18	

Total HScore of 200

HScores greater than 169 have 93% sensitivity and 86% specificity for HLH

Annex 9 HScore for secondary HLH extracted from [196] added up with clinical parameters of our patient COV006

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Curriculum vitae