Statistical Analysis Plan	
Clinical Investigation Plan Title	Low-fiel**D** magn**E**ti**C** **R**esonance imaging of pulmonar**Y** **P**arenchyma changes associated wi**T**h confirmed **SARS-CoV-2** infection in children and adolescents
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Confidentiality Statement All the information contained in this document is confidential.	

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1. Version History

Version	Changes	Authors, Title
v1.0	SAP generated	Ferdinand Knieling, M.D.
v.1.1	SAP under reviewed (08/08/2021)	Biostatistician

2. List of Abbreviations and Definitions of Terms

Abbreviation	Definition
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
COVID-19	Survival motor neuron 2
LF-MRI	Low-field magnetic resonance imaging
PCR	Polymerase chain reaction

3. Introduction

SARS-CoV-2 (Severe acute respiratory syndrome coronavirus type 2) is a new coronavirus and identified causative agent of COVID-19 disease. They predominantly cause mild colds but can sometimes cause severe pneumonia. The long-term consequences are still largely unexplained and misunderstood, especially in children and adolescents. The aim of this study is to assess the frequency of pulmonary parenchymal changes in pediatric and adolescent patients using low-field magnetic resonance imaging (LF-MRI) in the setting of proven past SARS-CoV-2 infection.

4. Study Objectives and endpoints

Determination of the frequency of lung parenchymal changes by low-field magnetic resonance imaging (LF-MRI) in past PCR-detected SARS-CoV-2 infection in pediatric and adolescent patients.

Hypotheses:

- Lung parenchymal changes in pediatric and adolescent patients with positive SARS-CoV-2 infection can be detected by LF-MRI
- The patients with changes do not show clinical symptoms

Primary Objective:

- Determination of the frequency of lung parenchymal changes by LF-MRI.

Secondary Goals:

- Determination of the frequency of positive SARS-CoV-2 antibodies.
- Determination of the anamnestic frequency of clinical respiratory symptoms.

Endpoints

Primary Endpoint

- Frequency of lung parenchymal changes

Secondary Endpoints

Correlation of imaging results/clinical course and:

- Antibodies against SarS-CoV-2
- Physical properties of single cells: Red blood cells - Deformation
- Physical properties of single cells: Red blood cells - Cells size [µm³]
- Physical properties of single cells: Red blood cells - Youngs modulus [kPa³]
- Physical properties of single cells: Leucocytes - Deformation
- Physical properties of single cells: Leucocytes - Cells size [µm³]
- Physical properties of single cells: Leucocytes - Youngs modulus [kPa³]
- Physical properties of single cells: Monocytes - Deformation
- Physical properties of single cells: Monocytes - Cells size [µm³]
- Physical properties of single cells: Monocytes - Youngs modulus [kPa³]

5. Investigation Plan

- This is a mono-centric, open-labeled, single arm study which aims to find the frequency of lung parenchymal changes in previous SarS-CoV-2 PCR-positive pediatric patients
- The study will include 58 patients.
- Each subject will be examined by blood samples and LF-MRI

Inclusion Criteria:

- (Past) Positive SARS-CoV-2 Infection (PCR proven)
- Age 5 to <18 years

Exclusion Criteria:

- Acute SARS-CoV-2 Infection and Isolation
- Quarantine
- Pregnancy
- Critical Illness
- No consent to LF-MRI
- General contraindications for LF-MRI, such as electrical implants, pace makers, perfusion pumps)

6. Determination of Sample Size

With a sensitivity of 90%, specificity of 85%, precision of ±0.15 and a prevalence of 0.3 with a confidence level of 95% for a 10% dropout, the number of cases was n=58 (including dropout).

7. Target parameters

LF-MRI will be utilized to generate lung parenchymal images. Blood samples are used to assess antibody and cell properties.

Primary Targets:

LF-MRT	Lung parenchymal changes

Secondary Targets:

Blood sample	SARS-CoV2-Antibody Mechanical cell properties (realtime deformability cytometry) Blood count
Clinical Features (via KIS)	Age Gender Weight Ethnicity Time of Infection (PCR result) Interval until LF-MRT Current medication Secondary Diagnoses Symptoms during COVID (e.g cough, shortness of breath, fever) Current symptoms (e.g cough, shortness of breath, fever)

7. Statistical Methods

7.1. Study Subjects

We will describe all screened and enrolled patients.

7.2. Clinical Investigation Plan (CIP) Deviations

Data will be analyzed according to the SAP; any further/additional/deviation from the SAP will be reported as such. Further post-hoc analysis will be performed, if necessary.

7.3 Analysis Sets

One analysis set will be created for study purpose.

7.4 General Methodology

Continuous variables are given as means and standard deviations; categorical variables are provided as numbers and percentages. If appropriate, descriptive statistics will be provided using Tables.

Frequency of lung parenchymal changes will be given as percentage of total population. Clinical symptoms will be correlated to extend of changes using correlations.

As appropriate, participants will be subjected to groups (subjects with lung phenotype/subjects without lung phenotyoe): Data are tested for normal distribution using Shapiro-Wilk test prior to inferential analysis. All variables (MRI/blood values) are compared between cohorts using t-tests. If the assumption of normal distribution is violated Wilcoxon signed-rank tests is used. All inferential tests are two-tailed, p values ≤ 0.05 are considered statistically significant. Bonferroni-Holm adjustment is used to control type I error, if appropriate. All analyses are performed using GraphPad Prism (Version 7.00 or newer, GraphPad Software, La Jolla, CA, USA) and/or IBM SPSS Statistics, version 25 or newer (IBM Corp., N.Y., USA).

7.5 Handling of Missing Data and Dropouts

No method of imputation will be used for missing data.

7.6 Safety Evaluation

During the study adverse events and serious adverse events will be monitored. The investigator is available for study subjects at any time, in case of any events.