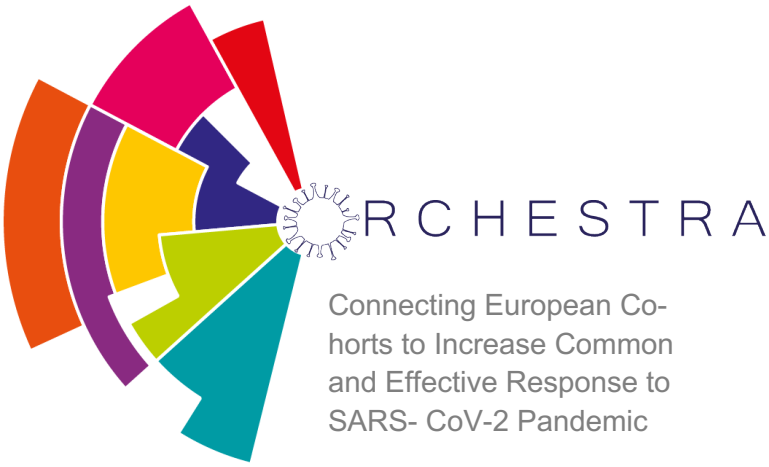

FOLLOW-UP OF COVID-19 LONG-TERM SEQUELAE

STUDY PROTOCOL

Università degli Studi di Verona (UNIVR)

ORCHESTRA has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101016167

Project Classification

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History of Changes

Version	Date	Created/Modified by
0.1	05.03.2021	Elisa Gentilotti (UNIVR), Fulvia Mazzaferri (UNIVR), Elda Righi (UNIVR), Evelina Tacconelli (UNIVR), Giorgia Tomassini (UNIVR)
0.2	07.06.2021	Lidia Del Piccolo (UNIVR), Maddalena Marcanti, Evelina Tacconelli (UNIVR)
0.3	12.07.2021	Gaia Maccarrone (UNIVR), Evelina Tacconelli (UNIVR), Giorgia Tomassini (UNIVR)

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2. TABLE OF CONTENTS

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3. PROTOCOL SUMMARY

3.1 Synopsis

Steering Committee	Evelina Tacconelli, UNIVR Elda Righi, UNIVR Fulvia Mazzaferri, UNIVR Maddalena Giannella, UNIBO Jade Gosh, AP-HP Cedric Laouenan, INSERM Janne Vehreschild / Luise Wolf, UHC Adriana Tami, UMCG Jesus Rodrìguez Baño, SAS Maria Luisa Moro, RER-ASSR Francine Ntoumi, FCRM Michele Mongillo, REG VEN (Elena Narne, Az. Zero) Surbhi Malothra, UANTWERPEN Alex Friedrich, UMGC Carlo Giaquinto, PENTA Paulo Roberto Abrão Ferreira, UNIFESP, Brazil Gabriel Levy Hara, UBA, Argentina Vidhya Krishnamoorthy, THSTI, India Mouli Natchu, CBCI, India
Principal investigator	Evelina Tacconelli, UNIVR
Co-principal investigators	Elisa Gentilotti, UNIVR
Study design	Multicenter, observational, prospective cohort study enrolling hospitalized and non-hospitalized patients with a diagnosed SARS-CoV-2 infection.
Study population	Patients of all age and any comorbidity with previous diagnosis of SARS-CoV-2 infection admitted to or treated as inpatients or outpatients in university and non-university hospitals or medical practices.
Inclusion criteria	Any age Any comorbidity

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	Laboratory confirmed SARS-CoV-2 infection by PCR diagnosis from nasopharynx, oropharynx, bronchoalveolar lavage, stool, or blood. Rapid tests are an acceptable alternative. Person (or attorney or deputy who has been authorized to make the decision for patients who lack capacity) consent to participate
Exclusion criteria	Lack of consent to participate
ORCHESTRA Partners	Università degli Studi di Verona (UNIVR); Alma Mater Studiorum – Università di Bologna (UNIBO); Institut National de la Sante et de la Recherche Medicale (INSERM); Servicio Andaluz de Salud (SAS); Consorzio Interuniversitario (CINECA); Luxembourg Institute of Health (LIH); Assistance Publique Hopitaux de Paris (AP-HP); Regione Emilia Romagna (RER-ASSR); Fundacion Privada Instituto de Salud Global Barcelona (ISGLOBAL); Ludwig-Maximilians-Universitaet Muenchen (LMU MUENCHEN); Universiteit Antwerpen (UANTWERPEN); Helmholtz Zentrum Muenchen Deutsches Forschungszentrumfuer Gesundheit und Umwelt GMBH (HMGU); Klinikum der Universitaet zu Koeln (UHC); Fondazione PENTA – for the treatment and care of children with HIV and related diseases - ONLUS (PENTA); Universitaet Stuttgart (USTUTT); Centre de Recherches Medicales DE Lambaréné (CERMEL); Regionalny Urad Verejneho Zdravotnictva so sidlom v Banskejbytrici(RAPH BB); Charité – Universitaetsmedizin Berlin (CHARITÉ); Academisch Ziekenhuis Groningen (UMCG);

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	Centre Informatique National de l'Enseignement Superieur (CINES); Universidad de Oviedo (UNIOVI); Universidad de Buenos Aires (UBA); Institutul National de Sanatate Publica (INSP); Regione del Veneto (REG VEN); Fondation Congolaise pour la Recherche Medicale (FCRM); Translational Health Science and Technology Institute (THSTI), Faridabad, Haryana, India; Catholics Bishops Conference of India, Society for Medical Education (CBCI), Bangalore, India; Escola Paulista de Medicina -Universidade Federal de São Paulo, Brazil.
Study rationale	The present study aims at harmonising follow-up strategies across the participating cohorts to allow a standardized collection of data on characteristics and determinants of COVID-19 long-term sequelae.
Primary objective	To describe characteristics of COVID-19 sequelae, including type, rate, and length through clinical, laboratory, and radiological assessments To investigate valuable, confounder-adjusted, associations between COVID-19 sequelae and COVID-19 severity, comorbidities, aetiology (SARS-CoV-2 variants), COVID treatment (including monoclonal antibodies), and trends in SARS-CoV-2 antibodies To describe the rate, the aetiology (SARS-CoV-2 variants), severity, and clinical determinants of COVID-19 re-infections
Secondary objective	To compare the time course of the immunological response of the population with sequelae with the immunological response of the population without sequelae. To investigate immunological patterns related to specific long-term sequelae. To investigate possible associations of SARS-CoV-2 variants with COVID-19 severity, time course of the immunological response, and long-term sequelae.

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	To describe the rate and severity of sequelae and immunological trends of COVID-19 in patients vaccinated against SARS-CoV-2. To describe the time course of intestinal and pulmonary microbiome after SARS-CoV-2 infection. To investigate possible associations of long-term sequelae with hypercoagulability To describe the relationship between risk perception and adherence to preventative measures over time, including vaccine acceptance. To describe the use of health care services among SARS-CoV-2 patients. To describe the relationship between risk perception of reinfection and the adherence to preventative measures over time after the SARS-CoV-2 infection, including vaccine acceptance. To describe the use of health care services among patients recovered from SARS-CoV-2. To identify human and viral genetic markers indicative of disease severity by doing genetic and epigenetic analysis in COVID-19 patients.
Time schedule	Patients will be followed-up for up to 18 months after the SARS-CoV-2 infection diagnosis.

3.2 Schedule of assessments (SoA)

Table 1 – Schedule of follow-up Assessments

	COVID-19 (2 weeks[1] ± 2 weeks)	**3 months[1] ± 1 month**	**6 months[1] ± 1 month**	**12 months[1] ± 1 month**	**18 months[1] ± 2 months**
Screening/baseline					
Inclusion criteria[1]					
Demographics[2]					
Healthcare setting[3]					
Length of hospital stay, days					
ICU admission					
Medical history[4]					
Treatment					
Comorbidity management[5]	X	X	X	X	X
Anti-COVID therapy[6]	X				
Antibiotic therapy[7]					

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Oxygen therapy[8]	X	X*	X*	X*	X*
SARS-CoV-2 vaccination[9]	X	X	X	X	X
Clinical assessment					
Relevant medical new events[10]	X	X	X	X	X
COVID-19 symptom[11]onset	X				
COVID-19 symptom end	X	X*	X*	X*	X*
COVID severity[12]	X				
SOFA score	X				
Vital signs[13]	X	X	X	X	X
Physical examination[14]	X	X	X	X	X
12-lead electrocardiography	X	X	X	X	X
6-minute walking test	X	X	X	X	X
DLCO (diffusing capacity for carbon monoxide)	X	X	X	X	X
Pulmonary function test[15]	X	X	X	X	X
Questionnaires					
Functional status[16]	X	X	X	X	X
Respiratory impairment[17]	X	X	X	X	X
Mental health[18]	X	X	X	X	X
Perceived risk of re-infection/admission/re-admission[19]	X	X	X	X	X
Adherence to main preventative non-pharmacological measures[20]	X	X	X	X	X
SARS-CoV-2 vaccination: acceptance/non-acceptance and reasons[21]	X	X	X	X	X
Imaging					
Lung ultrasound	X	X	X*	X	X*
X-ray	X	X	X*	X*	X*
High-resolution CT scan	X	X	X*	X*	X*
Cardiac ultrasound	X	X	X*	X	X*
Cardiac MRI [22]	X[21]	X[21]	X*	X[21]	X*
Biochemistry					
Blood tests[23]	X	X	X*	X	X*
Arterial blood gas test (pO_2/pCO_2/pH)	X	X	X*	X*	X*
Urine tests[24]	X	X	X*	X	X*
Immunology					
Immune - serology and type I IFNs autoantibodies	X	X	X	X	X
Immune - cytokine and chemokine	X	X [35]	X [35]	X [35]	X *
Immune - cellular	X	X [35]	X [35]	X [35]	X *
Microbiological tests-NP swabs					
Viral variant and metagenomics sequencing	X	X*,[34]	X*,[34]	X*,[34]	X*
EDTA whole blood					
Genetic and epigenetic analysis	X	X	X	X	X
Stool Sample (faeces or rectal swab)					
Metagenomic sequencing	X		X		X
Adjunctive variables for specific fragile populations					
HIV					
HIV-infection status[25]	X	X	X	X	X
HIV-Infection therapy[26]	X	X	X	X	X
Assessment of adherence to follow-up visits and antiretroviral therapy	X	X	X	X	X

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Elderly					
Cognitive status[27]	X	X	X	X	X
Pregnant women/new mother					
History of positive SARS-CoV-2 molecular test on amniotic fluid or breast milk[28]					
History of detection of micro-thrombotic disease on placenta tissue or umbelical cord tissue					
Children					
History of positive SARS-CoV-2 molecular test on amniotic fluid or breast milk[28]					
Biometric paramethers[29]	X	X	X	X	X
Transplant					
Transplant general information[30]					
Graft function[31]	X	X	X	X	X
Immunosuppressive regimen[32]	X	X	X	X	X
Onco-haematology					
Assessment of adherence to oncologic follow-up visits and therapy	X	X	X	X	X
Assessment of progression of the disease and relapse	X	X	X	X	X
Assessment of adverse events[33]	X	X	X	X	X

Footnotes

Modular data capture according to level of commitment (level I, level II, level III).

Level I	Assessments in level I are mandatory
Level II	Customized according to the feasibility of each cohort

* Reassessed only if outside the normal ranges at the previous assessment or if clinically indicated

1. Day 0: first positive SARS-CoV-2 test
2. Demographics: age (years), sex, ethnic group (African, Asian, European, Latin America...), education (no formal education, lower than college, college or higher), cigarette smoking (never-smoker, former smoker, current smoker), usual residence (home, long-term care facility, public dormitory, prison, homeless), current occupation (student, unemployed with no benefits, unemployed with benefits, employed, self-employed, informal worker)
3. Healthcare setting: (a) outpatient (b) non-intensive care unit (c) intensive care unit.
4. Medical history: cardiovascular diseases (hypertension, coronary artery disease, congestive heart failure), diabetes (without insulin, with insulin), chronic respiratory disease (asthma, chronic ob-

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structive pulmonary disease, obstructive sleep apnoea, restrictive lung disease, pulmonary hypertension), kidney disease (chronic with/without dialysis), liver disease other than cancer (HBV/HCV/HDV chronic viral hepatitis, other chronic disease, cirrhosis), metabolic disease, immunosuppressive conditions (solid organ transplant recipient, auto-immune diseases), cancer (solid cancer, haematological malignancies, type of primitive cancer/haematological malignancies, presence of metastases, if ongoing chemotherapy), mental or neurological disorders (psychiatric illness, anxiety disorder, mood disorder, psychotic disorder, Alzheimer disease, dementia other than Alzheimer, Parkinson's disease, myasthenia gravis, epilepsy, stroke (with/without residual deficits, neuromuscular disease, multiple sclerosis), muscular dystrophy, amyotrophic lateral sclerosis); TB co-infection; other opportunistic co-infection (specify) for HIV population

5. Comorbidity management: drug name and dose (to include only treatments taken regularly)
6. Anti-COVID therapy: drug name, maintenance dose, and duration
7. Antibiotic therapy: drug name, dose, duration, and type of treated infection
8. Oxygen therapy: nasal prongs, face mask, face mask with reservoir, high-flow nasal cannula, non-invasive ventilation, mechanical ventilation; numbers of O_2 (L/min) provided (maximum reached) and fraction of inspired O_2 (FiO_2) provided (maximum reached)
9. SARS-CoV-2 vaccination: vaccine name, date of administration
10. Relevant new medical events or worsening of previous conditions, including deep venous thrombosis, pulmonary embolism, infections (including a new SARS-CoV-2-infection during follow-up), malignancies (type of cancer, overall stage).
11. Symptoms: abdominal pain, ageusia/dysgeusia, anosmia, balance impairment, behaviour disorder, chest pain or chest tightness, confusion, cough, delirium, diarrhoea, disrupted sleep, dizziness, dyspnoea, fatigue, fever (including low-grade fever), headache, hypothermia, impaired cognitive status, lethargy, loss of appetite, mood affective disorder, myalgia, nausea/vomiting, palpitation, phlegm, runny nose, sore throat, stuffed nose, syncope, wheeze.
12. WHO Clinical Progression Scale
13. Vital signs: dead/alive, blood pressure, body temperature, heart rate, respiratory rate, peripheral oxygen saturation
14. Physical examination: BMI, abdominal examination, pulmonary examination, cardiac examination, neurological examination, peripheral vascular examination
15. Pulmonary function test: FEV_1, FVC, FEV_1/FVC, TLC, FRC, RV
16. Questionnaires to address the functional status: Post-COVID-19 Functional Status (PCFS) Scale, Global Physical Activity, Questionnaire (GPAQ), Barthel Index, Medical Outcome Study Short

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Form (MOS SF)-36 Score, EuroQol five-dimension five-level (EQ-5D-5L) questionnaire, Clinical Frailty Scale (CFS), Basic Activity of Daily Living (BADL).

17. Questionnaires to address the respiratory impairment: Saint George Respiratory Questionnaire (SGRQ), Transition Dyspnoea Index (TDI), mMRC (Modified Medical Research Council) Dyspnea Scale
18. Questionnaires to address the mental health: Hospital Anxiety and Depression Scale (HADS), Kessler Psychological Distress Scale (K10), Impact of Event Scale – Revised (IES-R), Resilience Scale for Adults (RSA)
19. Perceived risk of re-infection on a scale 0-10 (no risk- very high risk); perceived risk of admission/re-admission on a scale 0-10 (no risk- very high risk)
20. Frequency mask-wearing (type of mask); frequency hand washing; respect of social distance; avoidance of social gathering
21. Was the vaccine accepted? Why not accepted (lack of trust in efficacy and/or safety; not useful in the specific case; prefer someone else gets it before me)
22. Cardiac MRI only if abnormal cardiac ultrasound
23. Blood tests: White blood cell count, lymphocyte count, neutrophil count, platelets, sodium, potassium, creatinine, glucose, bilirubin, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transpeptidase, albumin, lactate dehydrogenase, ferritin, creatine kinase, fibrinogen, INR, partial thromboplastin time, D-dimer, NT-pro-BNP, troponin, C-reactive protein (CRP), procalcitonin, venous lactate
24. Urine tests: pH, concentration, protein, glucose, red blood, white blood cell count
25. CD4 lymphocyte count; HIV-viral load; AIDS status
26. HIV-therapy: drug name and dose (only ongoing treatment); previous switch to other regimens for virological failure
27. Questionnaires to address cognitive status: Cognitive Failure Questionnaire (CFQ), Mini-Mental State Examination, Clinical Dementia rating Scale, Montreal Cognitive Assessment (MOCA).
28. Results of SARS-CoV-2 molecular test on amniotic fluid
29. Weight, height/length, cranial circumference, BMI
30. Type of transplant (hearth, lung, kidney, liver, pancreas); single-combined; year of transplantation
31. Graft function: good, impaired, failure, rejection acute-chronic, recurrence of underlying disease, other
32. Immunosuppressive regimen: drug name and dose
33. According to Common Terminology Criteria for Adverse Events (CTCAE)

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34. At least one of three timepoints (month 3, month 6, month 12) is required to perform metagenomics analysis.
35. At least one of the three timepoints (month 3, month 6, month 12) is required.

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4. LIST OF ABBREVIATIONS

AOUI - Azienda Ospedaliera Integrata Universitaria
AP-HP - Public Assistance Hospital of Paris
BADL - Basic Activity of Daily Living
BMI - Body Mass Index
CBCI - Catholics Bishops Conference of India, Society for Medical Education , Bangalore, India;
CERMEL - Lambaréné Medical Research Center
CFQ - Cognitive Failure Questionnaire
CFS - Clinical Frailty Scale
CHARITÉ - University Medicine Berlin
CI - Confidance Interval
CINECA - Interuniversity Consortium
CINES - National IT Center for Higher Education confidence interval Cis
COVID-19 - COronaVIrus Disease 19
CRF - Clinical research form
CRO - Clinical Research Associate
CRP C-reactive protein
CTCAE - According to Common Terminology Criteria for Adverse Events
DLCO - Diffusion Lung CO
ECG - Electrocardiograph
eCRF - Electronic clinical research form
EQ-5D-5L - EuroQol five-dimension five-level
FCRM - Congolese Foundation for Medical Research
FDA – Food and Drug Administration
FEV - Forced Expiratory Volume
FRC – Functional Residual Capacity
FVC - Forced Vital Capcity
GHI - Good Clinical Practice,
GMBH HMGU - Helmholtz Zentrum Muenchen German Research Center for Health and Environment
GPAQ - Global Physical Activity, Questionnaire
HADS - Anxiety and Depression Scale
HCW - Health Care Workers
HIV - Human Immunodeficiency Virus
HMGU – Helmholtz Zentrum Munchen
ICF - Informal Consent Form
ICH - Conference on Harmonisation
ICMJE -International Committee of Medical Journal Editors
ICU - Intensive Care Unit
IES-R - Impact of Event Scale – Revised
INR - International Normalized Ratio
INSERM - Institut National de la Santé et de la Recherche Médicale
INSP - National Institute of Public Health
IQR - Interquartile Range
IRB – Institutional Review Board
IRB/IEC – Institutional review board/inidipendent ethics
ISGLOBAL-Barcelona Private Foundation Global Health Institut
K10 - Kessler Psychological Distress Scale
LCTF – Long term care facilities

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LEOSS - Lean European Open Survey on SARS-CoV-2 Infected Patients
LIH-Luxembourg Institute of Health
LMU MUENCHEN - Ludwig-Maximilians-University Munich
LTCF – Long Term Care
LURM Laboratorio Universitario di Ricerca Medica
mMRC - Modified Medical Research Council
MOS SF - Medical Outcome Study Short Form (MOS SF)-36 Score,
MRI - Magnetic Resonance Imaging
MRI - Magnetic Resonance Imaging
PCFS - Post-COVID-19 Functional Status Scale,
PCR- polymerase chain reaction
PENTA foundation – for the treatment and care of children with HIV and related diseases - ONLUS
USTUTT - University of Stuttgart
PI – Principal Investigator
RAPH BB - Regional Office of Public Health with its seat in Banská Bystrica
REG VEN-Veneto region
RER-ASSR Emilia Romagna region
RT-PCR - reverse transcriptase–polymerase chain reaction
RV-Residual volume
SAS-Andalusian Health Service
SGRQ - Saint George Respiratory Questionnaire
SoA - Schedule of Assessment
SOFA-Sequential[Sepsis-Related]Organ Failure Assessment Score
TDI - Transition Dyspnoea Index
THSTI - Translational Health Science and Technology Institute, Faridabad, Haryana, India;
TLC – Total Lung Capacity
UANTWERPEN - University of Antwerpen
UBA - Universidad de Buenos Aires
UHC - Clinic of the University of Cologne
UMCG - University Medical Center Groningen
UNIBO - University of Bologna
UNIOVI - University of Oviedo
UNIVR– University of Verona
WGS – Whole genome sequencing
WES – Whole exome sequencing
WP – Work Package

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5. BACKGROUND

The ongoing COVID-19 pandemic has created a global public health emergency that is challenging societies, health care systems and national economies worldwide [1]. Since the beginning of the pandemic, our knowledge of transmission dynamics [2], clinical presentation [3], long-term sequelae [4], and risk factors for disease progression [5-7] has been increasing steadily. Evidences on the efficacy of treatment strategies coming from clinical trials [8] have been obtained and preventive measures such as social distancing and vaccination campaigns, have been put in place. Nonetheless, there is still an urgent need for a more standardized research activity, connecting multiple countries and settings and focusing on the different aspects of this pandemic. High-quality data and biological samples collection coming from large multicentric cohorts are urgently required and have not yet been implemented in an international level. Evidence-based knowledge is required to enable rapid and effective decision-making, with particular attention to the transferability of the collected information.

COVID-19 can result in prolonged illness, even in young adults and children without underlying chronic medical conditions. In a telephone survey conducted by the Centers for Disease Control and Prevention among a random sample of 292 adults (≥18 years) who had a positive outpatient test result for SARS-CoV-2 by RT-PCR, 35% of 274 symptomatic respondents reported not having returned to their usual state of health 2 weeks or more after testing [9]. The burden of COVID-19 long-term sequelae and the exact underlying pathophysiology mechanisms remain unknown. Results from the follow-up of large cohorts are particularly needed to fully understand the characteristics and risk factors for SARS-CoV-2 infection long-term consequences.

From the point of view of the social science, this study will allow investigating two largely unexplored issues. On one hand, there is a need to dig into the determinants of adherence to preventative strategies, including non-pharmacological measures and vaccines. The lack of knowledge is particularly large among individuals who have experienced SARS-CoV-2 infection due to the possibility of re-infection and of infection transmission to others. On the other hand, there is a lack of knowledge on the use of health system resources SARS-CoV-2 infection implies across different settings and resources availability: length of hospital stay, frequency of follow-up visits, for example, have been projected at the start of the pandemic but the actual figures constitute unexplored information.

The present study is part of ORCHESTRA project, a three-year international research project aimed at tackling the coronavirus pandemic. ORCHESTRA provides an innovative approach to learn from the pandemic SARS-CoV-2 crisis, derive recommendations to further management of COVID-19 and be prepared for the possible future pandemic waves. The ORCHESTRA project aims to deliver sound scientific evidence for the prevention and treatment of the infections caused by SARS-CoV-2 assessing

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epidemiological, clinical, microbiological, and genotypic aspects of population, environment and socio-economic features. The project builds upon existing, and new largescale population cohorts in Europe (France, Germany, Spain, Italy, Belgium, Romania, Netherlands, Luxemburg, and Slovakia) and non-European countries (India, Perú, Ecuador, Colombia, Venezuela, Argentina, Brazil, Congo and Gabon) including SARS-CoV-2 infected and non-infected individuals of all ages and conditions. The primary aim of ORCHESTRA is the creation of a new pan-European cohort applying homogenous protocols for data collection, data sharing, sampling, and follow-up, which can rapidly advance the knowledge on the control and management of the COVID-19. ORCHESTRA will include SARS-CoV-2-negative individuals and thereby enable a prospective follow-up and an analysis of vaccination response. The cohort will involve all patients with SARS-CoV-2 infections including fragile individuals (children, elderly, transplanted, oncological, HIV infected, and those with Parkinson disease or rheumatologic disease) if followed up in one of the study Partners center. Within ORCHESTRA project, the Work Package 2 (WP2) aims to assess long-term sequelae in recovered COVID-19 individuals. The scope of the present protocol is to describe the procedures of the long-term follow-up of COVID-19 patients enrolled in ORCHESTRA cohorts.

6. STUDY RATIONALE

The present protocol, in accordance with the objectives of ORCHESTRA project - Work Package 2, aims at investigating the characteristics and determinants of COVID-19 long-term sequelae. This goal will be reached through the harmonization of follow-up strategies across the participating cohorts to allow a standardized collection of data on COVID-19 long-term sequelae. The result will be a platform including a set of data and biomaterials from large scale international cohorts, that will be uniformly recorded, prospectively tracked and analysed with the ultimate goal of providing evidence which will contribute to the optimization and improvement of the management of COVID-19 sequelae and to their prevention.

The follow-up will be organized in multiple levels of tests according to the capability of each cohort and will include questionnaires to collect demographic, epidemiological and clinical data, physical examination, radiological exams and biological sampling. The long-term follow-up will also allow the assessment of long-term immunological response to SARS-CoV-2 infection and its association to different treatment strategies, including monoclonal antibodies and vaccination.

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7. STUDY DESIGN

This is a multicenter, observational, prospective cohort study investigating COVID-19 sequelae in hospitalised and non-hospitalised patients up to 18 months after the diagnosis of SARS-CoV-2 infection. The present study is conceived, at the coordinating center of UNIVR, as an extension of the study "Biobanca associata a banca dati dei casi di COVID 19 gestiti presso l'Azienda Ospedaliera Universitaria di Verona" (COVID-19-VR), which was started in April 2020 at the Verona University Hospital to assess the long-term effects of COVID-19 on mental, respiratory, and functional status through the administration of questionnaires (CESC 2577). Patients will be recruited in multiple European and non-European countries, accounting for the participation of approximately 10000 individuals in the prospective follow-up data collection.

Recording of clinical data, administration of questionnaires, collection of biological samples and imaging will take place at fixed time-points to allow a comprehensive follow-up of COVID-19 patients. The follow-up will include two levels of assessments: the first one is mandatory, the second one will be customized according to the feasibility of each cohort. A detailed overview of the schedule of the study visits and the clinical variables to be recorded is shown in Table 1. An ad hoc database will be provided to each COVID-19 cohort involved to allow homogeneous and standardised data collection.

8. OBJECTIVES

Main objectives of the study are:

- To describe characteristics of COVID-19 sequelae, including type, rate, and length through clinical, laboratory, and radiological assessments
- To investigate valuable, confounder-adjusted, associations between COVID-19 sequelae and COVID-19 severity, comorbidities, aetiology (SARS-CoV-2 variants), COVID treatment (including monoclonal antibodies), and trends in SARS-CoV-2 antibodies
- To describe the rate, the aetiology (SARS-CoV-2 variants), severity, and clinical determinants of COVID-19 re-infections

Furthermore, data retrieved from COVID-19 cohorts will address the following objectives:

- To compare the time course of the immunological response of the population with sequelae with the immunological response of the population without sequelae.
- To investigate immunological patterns related to specific long-term sequelae.

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- To investigate possible associations of SARS-CoV-2 variants with COVID-19 severity, time course of the immunological response, and long-term sequelae.
- To describe the rate and severity of sequelae and immunological trends of COVID-19 in patients vaccinated against SARS-CoV-2.
- To describe the time course of intestinal and pulmonary microbiome after SARS-CoV-2 infection.
- To investigate possible associations of long-term sequelae with hypercoagulability
- To describe the relationship between risk perception of reinfection and the adherence to preventative measures over time after the SARS-CoV-2 infection, including vaccine acceptance.
- To describe the use of health care services among patients recovered from SARS-CoV-2.
- To identify human and viral genetic markers indicative of disease severity using WGS or WES followed by functional analysis of the most promising variants.

9. PATIENT COHORTS

A comprehensive and longitudinal research cohort will be established. A broad collection of fine-granular clinical and epidemiological data connected to collection of biological samples will help answer the most relevant questions in the context of the SARS-CoV-2 pandemic. By implementing an international cohort of SARS-CoV-2 infected patients, considering all age groups, with any comorbidity and socioeconomic background as well as any stage of disease severity and setting (in- and outpatients), answers regarding the epidemiology and optimal management of SARS-CoV-2 will be provided at local, regional, national and international level. Besides the comprehensive gathering of data and biological samples, representative sub-cohorts such as patients with specific comorbidities, adolescents or children can be analysed. Long-term follow-up data will further support both insight into pathophysiological mechanisms as well as improve individualized patient management. The information provided will help in adapting hospital management and public health strategies and thereby in reducing further socioeconomic damage. In long term perspective, the nation-wide collaboration of several stakeholders, the use and extension of existing and newly established infrastructures might serve as a fast response for coming global and national health challenges. The constitution of a “perpetual” cohort including not only population-based representatives but also substantial number of HCWs (high risk population in case of endemic event with high incidence of admission for infected individuals and therefore high risk of hospital spreading) and fragile population including pregnant women, children, elderly, immunocompromised subjects (transplant recipients, patients with onco-haematological malignancies or HIV-infection) and individual with neurological impairment enables to precisely define the target of vaccination trials based

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on severity of diseases in those population but also on the burden of diseases (in terms of delayed care as visits, chemotherapy or radiological assessment) in populations in need of periodic clinical evaluations. COVID-19 fragile population cohorts will be assessed in collaboration with ORCHESTRA – Work Package 4, focusing on the prevalence, clinical spectrum and therapeutic management of COVID-19 disease in established cohorts of fragile patients. Specific features of fragile populations to follow-up is reported in table 1.

Patients will be recruited within European and non-European cohorts participating to ORCHESTRA consortium. Data will be extracted according to the study protocol and the case report form in compliance with local regulatory rules.
A description of the cohorts is presented in the following sections.

9.1. University of Verona (UNIVR) – Italy

UNIVR will participate to the recruitment of COVID-19 general population and COVID-19 fragile populations: HIV positive, solid organ transplanted, oncological (both solid cancer and haematological neoplasms) patients and elderly people resident in LTCFs (General nursing home; residential home; specialized LTCFs; mixed LTCFs, other LTCFs; overall number of beds; ownership of the facility: public, for profit, not for profit). Estimated number of COVID-19 patients since the beginning of the pandemic: 66000

9.2. The French Covid-19 cohort

INSERM - Institut National de la Santé et de la Recherche Médicale

National French cohort which follows more than 3000 hospitalized patients across France The objectives of this cohort are to describe: 1) clinical features of illness; 2) treatment used and their outcome; 3) virus replication, excretion and evolution in multiple sites; 4) host responses including innate and acquired immune responses; 5) host genetic variants associated with disease progression/severity.

9.3. Lean European Open Survey on SARS-CoV-2 Infected Patients (LEOSS)

University Hospital of Cologne (UHC), Cologne- Germany

International cohort of more than 2,500 SARS-CoV-2 infected patients from 250 study sites all over the world, implemented by the University Hospital of Cologne (UHC), Cologne- Germany since March 2020.

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9.4. COVID-HOME study

University Medical Center Groningen (UMCG), Groningen, Netherlands

Prospective cohort study of non-hospitalised COVID-19 patients. The COVID-HOME study focuses on the community and household with early, systematic and prospective long-term follow-up The parameters collected in this study, such as viral load, antibody response, cytokine changes and clinical and laboratory parameters of patient evolution will allow the identification of independent factors/parameters determining evolution of disease, insight in other transmission routes than respiratory ones (such as sexual and faecal-oral), and immune response dynamics in non-hospitalised patients.

9.5. Fondation Congolaise pour la Recherche Médicale (FCRM) - Republic of The Congo

FCRM will participate to the enrolment of a cohort of recovered COVID-19 individuals to assess long-term sequelae.

9.6. ZIKAction

Led by Penta, the ZIKAction research consortium brings together 14 partners across South and Central America, the Caribbean and Europe with the complementary goals of 1) developing a multidisciplinary multinational ready to-act network capable of rapidly addressing any maternal and paediatric research need arising from (re-)emerging infectious diseases including Zika virus and 2) conducting an interdisciplinary programme of research studies within this network to address key knowledge gaps relating to ZIKV epidemiology, natural history and pathogenesis, with a particular emphasis on maternal and child health. ZIKAction is funded by the European Union's Horizon 2020 Programme. ZIKAction works closely with two other European Union-funded consortia, ZikaPLAN and ZIKAlliance, to establish a Latin American and Caribbean network. Within this research consortium, the SARS-oV-2 sub-study will address issues related to COVID-19 infection in pregnant women and children.

9.6. Servicio Andaluz de Salud (SAS) - Hospital Universitario Virgen Macarena (HUVM)

SAS is a public body providing healthcare services to the 8.4 million inhabitants in Andalusia, Spain. Hospital Universitario Virgen Macarena (HUVM) belongs to SAS and is a reference 900-beds hospital located in Seville. The Department of Infectious Diseases, Microbiology and Preventive Medicine has a vast experience in different fields of infectious diseases; it includes

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a multidisciplinary research group formed by basic scientists, clinical microbiologists, pharmacists and infectious diseases researchers performing basic and clinical research in this field. It hosts the coordination of the Andalusian Network for Clinical Research in Infectious Diseases (ANCRAID), the Spanish Network for Research in Infectious Diseases (REIPI) and is part of the Institute of Biomedicine of Seville (IBiS).

9.7. Universidade Federal de São Paulo (UNIFESP) - Escola Paulista de Medicina

A total of 1.652 patients diagnosed with COVID-19 confirmed by RT-PCR were hospitalized for at least 48 hours at Hospital São Paulo (HSP, UNIFESP), a 740-bed teaching hospital, located in the city of São Paulo, Brazil, between March 1st 2020 and April 30th 2021. Nearly 44.9% (N=723) of these patients were admitted to intensive care units and 1.152 were hospital discharged. UNIFESP will contribute with a retrospective and prospective cohort of COVID-19 patients admitted to HSP and followed-up every 3 months, providing demographic, epidemiological, clinical, imaging and laboratory data.

9.8. University of Bologna (UNIBO)

UNIBO has been involved in different research activities to tackle the diffusion of the virus. The Department of Medical and Surgical Sciences of UNIBO has been actively working, since the end of February 2020, to manage the COVID-19 pandemic both at clinical and research level in close collaboration with the Infectious Diseases Clinical Unit of the University Hospital S.Orsola-Malpighi and the Public Healthcare Provider governance of the Emilia-Romagna Region.

10. INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria:

- Any age
- Any comorbidity
- Laboratory confirmed SARS-CoV-2 infection by PCR diagnosis from nasopharynx, oropharynx, bronchoalveolar lavage, stool, or blood. Rapid tests are an acceptable alternative.
- Person (or attorney or deputy who has been authorized to make the decision for patients who lack capacity) consent to participate.

Exclusion criteria:

- Refusal to participate to the study.

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11. RECRUITMENT STRATEGY

Inpatients will be recruited by the study team at the treating hospital. Outpatients will be recruited by the study team in the emergency rooms, outpatient clinics, and through the coordination with regional primary care networks, who will offer the opportunity to enter the study to patients with active or previous COVID-19. This allows a broad recruitment of cases and the rapid inclusion of additional study centers in regional hotspots.

Patients will be followed up for up to 18 months after the SARS-CoV-2 infection diagnosis. The recruitment can take place at any time between the SARS-CoV-2 infection diagnosis and the end of the 18-month follow-up, provided that the patient ensures at least one follow-up visit. Discharged inpatients as well as recovered outpatients will be invited to participate by a phone call (which will be repeated once in case of no reply). The screening/baseline data collection (including the informed consent process) can be carried out on the same day of a follow-up visit.

Before the first patient is recruited at a location, the responsible investigator ensures that all legal and regulatory requirements are met. Patients can revoke their consent to participate in the study without restriction at any time and at their own request, without giving reasons and without any consequences for their future treatment. In case of loss to follow-up, the previous follow-up assessments will be included in the data analyses. Patients will be recruited until January 2023 and followed-up until June 2023, allowing a partial follow-up for participants whose SARS-COV-2 infection occurred after January 2022.

12. STUDY PROCEDURES

The assessments of this study are designed to improve the COVID-19 patient care in the context of routine clinical care. Considering the lack of standardized follow-up pathways for COVID-19 patients, the selection of assessments has been based on available evidence and according to the definition of good clinical practice.

The organization of follow-up visits will be up to each center, according to the facilities and logistics of outpatient monitoring.

There will be a modular data capture according to the level of commitment of each cohort.

- Level I: Assessments in level I are mandatory
- Level II: Customized according to the feasibility of each cohort

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The modular schedule of assessments is presented in Table 1. Overall, the follow-up will last 18 months. Day 0 corresponds to the time of the first positive SARS-CoV-2 test. The screening/baseline data collection (including the informed consent process, as detailed in section 14.3) can be carried out on the same day of a follow-up visit (as detailed in section 11). Procedures conducted as part of the participant's routine clinical management at the time of SARS-CoV-2 active infection and obtained before the signature of the informed consent form may be recorded, provided that the procedures meet the protocol-specified criteria.

Subsequent follow-up visits will occur at the following time-points: 3, 6, 2, 18 month(s). During each time-point, epidemiological data collection (SARS-CoV-2 vaccination status), treatment data collection (comorbidity management), clinical assessments (relevant medical new events, COVID-19 symptom assessment, physical examination, vital signs, 6-minute walking test), and the administration of questionnaires on functional status, respiratory impairment, and mental health will be performed as part of level I (mandatory) assessments, as well as the assessment of the SARS-CoV-2 immunological status (SARS-CoV-2 antibodies). A SARS-CoV-2 molecular test will be repeated only if it turned out positive at the previous follow-up visit.

Self-administered questionnaires on symptoms, as listed in Table 1, will be completed daily by the participant to provide timely data on the symptom length. The participant will assign a score based on symptom severity, which will be recorded daily in the self-administered diary: absent (0), mild (1), moderate (2) and severe (3).

As detailed in Table 1, level II (imaging, biochemistry, pulmonary function tests, electrocardiography) will be performed according to the capability of each cohort and will be based on the clinical evaluation, according to the participant's healthcare status. Imaging and biochemistry tests will be reassessed only if outside the normal ranges at the previous follow-up visit or if clinically indicated. During the follow-up visit at month 12, cardiac and lung ultrasounds, blood and urine tests will be offered as part of the participant's care.

COVID-19 biological samples (including blood, naso-pharingeal swabs, urine, and stool) will be collected and stored in the biobank of each participating center (according to local ethic commission recommendations). The Azienda Ospedaliera Integrata Universitaria (AOUI) of Verona developed a COVID-19-VR registry for biobanking COVID-19 biological samples to allow further research and national/international collaborations. As per protocol approved by the hospital Institutional Review Board (IRB 2577CESC), biological samples of COVID-19 patients are stored in the LURM (Laborato-

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rio Universitario di Ricerca Medica). Test to be performed in a centralised laboratory (genomic, transcriptomic, cytokine, viral analyses, PBMCs and genetic and epigenetic see Table 2) will be sent, following international regulation, to the University of Antwerp, INSERM, University of Bologna or HMGU. A dedicated protocol will be developed for expeditions procedures. Nasal swabs collected prospectively for diagnosis of SARS COVID-2 will be collected in the local laboratory and sent out to University of Antwerpen and INSERM. Stool simple for microbiological analysis will be sent out to University of Bologna (see Table 2). Whole blood samples for epigenetic and genetic analysis will be sent out to UNIBO, INSERM and Helmholtz Zentrum Munchen (HMGU). In-depth human genetic analysis will be conducted using WGS or whole exome sequencing (WES) followed by functional analyses of the most promising variants. Genome-wide methylation analyses of COVID-19-positive patients in addition to a small number of control patients will enable differentiation of inherited and acquired genomic regulatory features through COVID-19 infection, which result in severe disease or an efficient clearing of infection through immune responses.

Table 2 – Samples collected at SARS-CoV-2 diagnosis and at follow up.

Sample	Aliquot	Sample type	Volume	Storage solution	Storage temp. (°C)	Shipping temp. (°C)	Task	Comment	Partner
PBMC	1 or 2 samples	PBMC		0.5 ml FBS/DMSO 20%	-70°C or below or liquid nitrogen	Dry ice	Characterisation of T-cell immune response		UANT-WERPEN
Blood	1	EDTA plasma, but heparin plasma or serum can also be used, if EDTA plasma is absolutely unavailable	350 µL	EDTA plasma has to be processed according to the protocol provided before freezing (preferably at -80°C directly)	Short term at -20 °C, long term at -70°C or below	Dry ice	Cytokinome analysis	Please process and freeze within 2 hours.	UANT-WERPEN

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	2	Se-rum/plasma	200 µL	NA	-20°C, long term at -70°C or be-low	Dry ice	Auto-anti-bodies against type I IFNs	If available	INSERM
	3	Serum	100 µL	NA	-20°C, long term at -70 ° or below	Dry ice	Antibodies detection		INSERM; UANT-WERPEN
Whole blood	1	Extracted DNA or whole blood	4 µg if DNA, 2 ml if whole blood	NA	-20°C/ -80°C	Dry ice	NGS of COVID-19 cohorts	DNA could be ex-tracted lo-cally or at HMGU.	INSERM/UN-IBO
	2	DNA or whole blood	750 ng in 45 µL if extracted DNA; otherwise 1 aliquot	TE buffer or wa-ter if extracted DNA	-20°C	Dry ice	Illumina EPIC DNA methylation	DNA could be ex-tracted lo-cally or at HMGU.	HMGU
NP swab	1	NP swab	400 µL	TRIzol; RNA later; DNA/RNA shield	-70°C or be-low	Dry ice	Characterisa-tion of viral markers Respiratory microbiome dynamics		UANT-WERPEN-INSERM
Stool sample or rec-tal swab	1	Stool (faecal swab if stool is unavaila-ble)	1-2 g	RNA later if possible, other-wise frozen.	+4°C (up to 24 h) long term at -70°C or be-low	Dry ice	Intestinal microbiome profiling		UNIBO

13. STATISTICAL ANALYSIS

Sample size calculation. The number of inclusions will depend on the progress of the SARS-CoV-2 pandemic, which is unknown at this time. Therefore, the number of patients who will be included cannot be determined in advance. However, depending on the timing of the project and the included cohorts, the expected enrollment is 10000 subjects.

We will carry out comprehensive descriptive analyses taking into account sociodemographic factors and clinical courses. The frequency distributions of the characteristics will be given in absolute and relative numbers, median plus interquartile range (IQR) or mean values plus 95% confidence interval (CIs). Associations with specific treatment strategies, disease severity patterns and laboratory results will be analysed using chi-square tests, t-tests or Mann-Whitney tests, depending on the data. To evaluate po-tential risk factors, multivariate regression models will be carried out. Outcome time analyses using Cox proportional-hazards regression models with time-dependent covariates will be performed to examine

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factors associated with each endpoint (including death). In addition, we will use cumulative incidence functions, such as the Fine-Gray subdistribution hazard regression model, to account for competing events (i.e., relocation, discharge against medical advice, etc.). For missing values, a different strategy to understand the causes and the significance for the analysis will be developed and a graduated procedure for dealing with censorship and imputations via linked regressions will be developed. The significance level is defined with a p-value <0.05. All statistical analyses will be carried out with STATA, Python and/or R statistics software by trained staff (epidemiologists, statisticians) using the latest analysis methods.

14. REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

14.1 Regulatory and ethical aspects

The study protocol is designed and will be conducted to ensure adherence to the principles and procedures of Good Clinical Practice and to comply with Italian laws, as described in the following documents and accepted, with their signature, by the study investigators: 1. ICH harmonized tripartite guidelines for good clinical practice 1996.2. Directive 91/507 / EEC, The Rules Governing Medicinal Products in the European Community. 3. Legislative Decree No. 211 of 24 June 2003.4. Legislative Decree n.200 November 6, 2007.5. D.M. 21 December 2007.6. AIFA Determination March 20, 2008. All essential clinical documents will be kept to demonstrate the validity of the study and the integrity of the data collected.

All the document and protocol, protocol amendments, ICF, and other relevant documents must be submitted by the principal investigator to the ethical committee of the promotor center and reviewed and approved by the ethical comittae before the study is initiated. The protocol will also be submitted to the local ethics committees of the participating centers by the centers themselves.

14.2 Financial disclosure

Finance and insurance are addressed in the Investigator and/or CRO agreements, as applicable.

14.3 Informed consent process

Participant's informed consent/assent must be obtained and documented in accordance with local regulations, ICH-GCP requirements, and the ethical principles that have their origin in the principles of the

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Declaration of Helsinki. Prior to obtaining informed consent, information should be given in a language and at a level of complexity understandable to the participant in both oral and written form by the Investigator (or designee).

Each participant will have the opportunity to discuss the study and its alternatives with the Investigator. Prior to participation in the study, the ICF should be signed and personally dated by the participant, or his/her legal representative.

The participant or his/her legal representative must receive a copy of the signed and dated Informed Consent form. As part of the consent process, each participant must consent to direct access to his/her medical records for study-related monitoring, auditing, IRB/IEC review, and regulatory inspection. If the ICF is amended during the study, the Investigator must follow all applicable regulatory requirements pertaining to the approval of the amended Informed Consent form by the IRB/IEC and use of the amended form.

The participant may withdraw their consent to participate in the study at any time.

14.4 Data protection

The participant must be informed that his/her personal study-related data will be used by the promotor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

Participants will be assigned a unique identifier by the Promotor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The electronic case report form (eCRF) will be provided using RedCap® software of the University Hospital of Verona. Investigators from participating study sites log into the system with username and a safe password including letters, numbers, and symbols.

Investigators will be informed about handling of their personal data and their IP and location during the registration process.

The data collection could be performed also retrospectively after a patient case has been completed (treatment is finished or patient's death). This process will be compliant with all applicable European

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and German federal data protection regulations, including EU directive 2016/679 and the German DS-GVO.

14.5 Data collection

Patient data will be collected through a specific eCRF created for the study, which will not contain personal data suitable for identifying the patient. The clinical and outcome information collected in relation to the study will be limited to the objectives of the study, taking care to reduce as much as possible the burden for the patient and for the enrolling clinical center. Only the enrolling clinical center will have access to the patient's identity and will be able to contact him if the coordinating center needs to have further information or for follow-up checks during or after the closure of the study being analyzed and reporting. For each patient, a special unique digital identification code (barcode) will be provided. The code will consist of a three-character part to identify the recruiting center and a second part consisting of 5 digits to identify the enlisted person. The code will be associated with the patient by the clinical center, which will keep a copy of it in the patient file and in the medical record. Each patient enrolled will have a specific study file with all ethical and clinical documentation. The manual for compiling the eCRF will be provided in the Trial Master File.

The data will be entered directly into the patients' eCRF and the related clinical and outcome information will be digitized in a specific database that will be developed using the RedCap® data capturing platform. The system has been successfully used in the network to which the centers are part of SOLIDARITY. RedCap®, installed on protected servers of the Integrated Hospital of Verona, is periodically updated and allows you to create accounts for users, control their access for data entry, limit user privileges (access only to the data of your center) and closure of the validity of the account at the end of the study.

14.6 Data quality assurance

All participant data relating to the study will be recorded on printed, or eCRF unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF or paper CRF. The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF or paper CRF. The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

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The Promotor is responsible for the data management of this study including quality checking of the data.

14.7 Source Document

All source documents must be accurate, clear, unambiguous, permanent, and capable of being audited. They should be made using some permanent form of recording (typing, printing, optical disc). They should not be obscured by correction fluid or have temporary attachments (such as removable self-stick notes). Source documents are original records in which raw data are first recorded. These may include hospital/clinic/general practitioner records, charts, diaries, x-rays, laboratory results, pharmacy records, care records, ECG or other printouts, questionnaires, or video, for example. Source documents should be kept in a secure, limited access area.

14.8 Study and site closure

The study start date is the date the clinical study will be open for the recruitment of participants. The first step for recruitment is informed consent and will be the start date of the study.

The study centers will be closed upon completion of the study. A center will be considered closed when all required study documents and materials have been collected and a study closing visit has been performed in the center.

14.9 Publication policy

The PI is responsible for the final publication of data. Authors must satisfy all of the following ICMJE authorship criteria: 1. Substantial contributions to conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND 2. Drafting the work or revising it critically for important intellectual content; AND 3. Final approval of the version to be published; AND 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Data collection, general supervision of the research group, or overseeing the conduct of the study alone does not justify authorship. Publications will be planned by the PI and the scientific and statistical committees. Publication of partial or

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local data must be approved by the PI. A detailed publication policy agreement will be developed by Partners at the beginning of the study.

14.10 Amendments or any other modification

Modifications to the protocol will be made as amendment. No other modality is allowed. Any modification will be recorded in the "Clinical Study Report" Archiving documents. The principal investigator is responsible for archiving and storing the essential documents during all the period of study according by current legislation and GCP.

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