Algemene gegevens / General Information

Programma / Programme	:	**COVID-19 Programma**
Subsidieronde / Subsidy round	:	**Bottom-up ronde COVID-19 aandachtsgebied 1**
Projecttitel / Project title	:	**Identificatie van de pathogenese van COVID-19 pathologie in de Nederlandse bevolking en ontrafeling van verschillen in pathogenetische mechanismen tussen hoog- en laag-risico groepen.**
Projecttaal / Project language	:	**Engels / English**
Geplande startdatum / Planned start date	:	**13-07-2020**
Geplande duur / Planned duration	:	**24 maanden / months**
Datum indienen / Date of application	:	**16-06-2020**
Projecttype / Project type	:	**Toegepast onderzoek**
Vervolg eerder ZonMw-project / Continuation previously funded project ZonMw	:	**Nee / No**

Projectleden / Project members

5.1.2e
Functie / Position: 5.1.2e *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e @erasmusmc.nl

Erasmus MC
5.1.2e
Department of Pathology
Postbus 2040
3000 CA ROTTERDAM

5.1.2e
Functie / Position: 5.1.2e *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e @erasmusmc.nl

Erasmus MC
5.1.2e
Department of Pathology
Postbus 2040
3000 CA ROTTERDAM

5.1.2e
Functie / Position: 5.1.2e *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e @erasmusmc.nl

Erasmus MC
5.1.2e
's-Gravendijkwal 230
3015 CE ROTTERDAM

5.1.2e
Functie / Position: 5.1.2e *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e @umcg.nl

Universitair Medisch Centrum Groningen
Longziekten
Postbus 30001
9700 RB GRONINGEN

5.1.2e
Functie / Position: 5.1.2e | *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e@erasmusmc.nl

Erasmus MC
Radiologie, Nucleaire Geneeskunde
Postbus 2040
3000 CA ROTTERDAM

5.1.2e
Functie / Position: 5.1.2e | 5.1.2e | *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e@radboudumc.nl

Radboudumc
Pathology
Postbus 9101
6500 HB NIJMEGEN

5.1.2e
Functie / Position: 5.1.2e | *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e@lumc.nl

Leids Universitair Medisch Centrum
Department of Internal Medicine
Thrombosis and Hemostasis
Albinusdreef 2
2333 ZA LEIDEN

5.1.2e 5.1.2e 5.1.2e
Functie / Position: 5.1.2e | *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e@erasmusmc.nl

Erasmus MC
Viroscience
Postbus 2040
3000 CA ROTTERDAM

5.1.2e
Functie / Position: 5.1.2e | *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e@umcg.nl

Universitair Medisch Centrum Groningen
Pathologie en Medische Biologie
5.1.2e
Postbus 30001
9700 RB GRONINGEN

5.1.2e
Functie / Position: 5.1.2e | *Opleiding / Education:*
Studierichting / Subject:
T: 5.1.2e | *F:* | *E:* 5.1.2e@amsterdamumc.nl

Amsterdam UMC - locatie VUmc
Pathologie
Neuropathology
Postbus 7057
1007 MB AMSTERDAM

Projectgegevens / Project information

Aandachtsgebieden / Focus

1.1 Thema's aandachtsgebied 1

- Risicoanalyse en prognostiek
- Virus, immuniteit, immuunrespons en pathogenese

1.3 Setting
- Ziekenhuiszorg

Samenvatting / Summary

COVID-19 wordt gekenmerkt door een opvallende variatie in uiting en ernst van ziekte tussen patiënten, en is sterk tijdsafhankelijk. Het is tot dusverre onvoldoende duidelijk welke weefselreacties en pathogenetische processen aan deze variatie ten grondslag liggen. Dit beperkt zowel de ontwikkeling van specifieke diagnostische middelen als ook van gepersonaliseerde therapie voor individuele patiënten. Studie van het weefsel van door COVID-19 betroffen patiënten kan in dit opzicht verhelderend zijn, omdat in het weefsel een onderscheid kan worden gemaakt tussen direct door het virus veroorzaakte beschadiging, auto-immuniteit en de inmiddels vanuit de kliniek bekende stollingsfenomenen, die mogelijk allen een andere behandeling behoeven.
Wij stellen voor om het in Nederland beschikbare en geschikte weefsel voornamelijk van obducties, maar ook van resectiepreparaten, en zo mogelijk biopten van met SARS-CoV-2 geïnfecteerde patiënten te organiseren in een virtuele biobank waarin deze vragen kunnen worden gesteld en beantwoord. Dit gebeurt op initiatief van het Dutch COVID-19 Pathology Consortium, dat reeds enkele weken actief is op het gebied van de informatievoorziening t.a.v. van COVID-19 in de pathologiepraktijk.
Specifieke vragen die worden gesteld zullen betrekking hebben op 1) immuunreacties in het weefsel die voorspellen hoe de ziekte zich zal gaan ontwikkelen , wat de onderliggende pathogenetische mechanismen zijn en wat een mogelijke behandeling hiervoor kan zijn; 2) de relatieve aanwezigheid van voor COVID-19 belangrijke eiwitten in het weefsel, specifiek in relatie tot bekende risicogroepen; 3) de betrokkenheid van het centraal zenuwstelsel bij COVID-19; en 4) de lange termijn effecten van COVID-19 op het weefsel van overlevers van de acute fase van de ziekte.
Deze data zullen naar verwachting leiden tot verbeterd begrip van de ontstaansgeschiedenis en variabiliteit van deze ziekte en leiden tot gepersonaliseerde behandeling, en derhalve hopelijk overleving.

Trefwoorden / Keywords

COVID-19; pathologie; histopathologie; pathogenese; risicofactoren; obductie; immuunhistochemie; proteomics; diagnostiek; biomarkers; prognose; predictie; gepersonaliseerde behandeling

Samenwerking / Collaboration

Samenwerking tussen onderzoek en praktijk / Cooperation between research and practice:

Ja / Yes

Financiële gegevens / Financial data

ZonMw budget

	Jaar / Year								
Kostenpost	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	**Totaal / Total**
Personeel	5.1.1c		0	0	0	0	0	0	253.512
Materieel			0	0	0	0	0	0	200.900
Implementatie			0	0	0	0	0	0	25.000
Apparatuur			0	0	0	0	0	0	0
Overig			0	0	0	0	0	0	20.000
Totaal / Total	**249.706**	**249.706**	**0**	**0**	**0**	**0**	**0**	**0**	**499.412**

Co-financiering / Cofinancing

Naam co-financier / Name of cofinancier	Bedrag / Amount	Status

Bijzondere gegevens / Additional information

Vergunningen / Permits

	Verklaring nodig / Statement required?		Status verklaring / Statement status		
	Ja / Yes	Nee / No	Verkregen / Acquired	Aangevraagd / Applied	Nog niet aangevraagd / Not applied yet
METC	X		X		
DEC		X			
WBO		X			

Onderschrijvingen / Assents

	Ja / Yes	Nee / No	N.v.t. / N.A.
Code biosecurity / Code Biosecurity			X
Code openheid dierproeven / Code Transparency of Animal Testing			X

Andere vergunningen / Other permits

AANVRAAGFORMULIER
UITGEWERKTE SUBSIDIEAANVRAAG
– BOTTOM-UP RONDE

COVID 19 programma

Deadline voor indiening: 15 juni 2020 (14:00 u)

LEES ALSTUBLIEFT ALLE INSTRUCTIES IN BIJLAGE "TOELICHTING INDIENING SUBSIDIEAANVRAAG" VAN DE OPROEPTEKST ZORGVULDIG!

Wanneer u het formulier heeft ingevuld:

1. **Zet het formulier om naar een PDF file en controleer de details**
2. **Upload het complete formulier als een bijlage bij uw indiening in Projectnet (Let op: dit zijn twee verschillende links, gebruik maar 1 van de 2!)**

ProjectNet: Aandachtsgebied 1 (voorspellende diagnostiek en behandeling)
ProjectNet: Aandachtsgebied 2 (zorg en preventie)

BASISGEGEVENS (voorpagina)

NAAM VAN DE HOOFDAANVRAGER:

5.1.2e

ORGANISATIE:

Erasmus MC

ENGELSE PROJECTTITEL:

COVID-19 Pathogenesis in Dutch high- and low-risk groups.

NEDERLANDSE PROJECTTITEL:

Identificatie van de pathogenese van COVID-19 pathologie in de Nederlandse bevolking en ontrafeling van verschillen in pathogenetische mechanismen tussen hoog- en laag-risico groepen.

ONDERZOEKSVOORSTEL **max 8 pagina's A4** (inclusief literatuurreferenties)	(voorpagina met basisgegevens niet meegerekend - font type Arial 10 pts)

1. PROBLEEMSTELLING, URGENTIE EN DOELSTELLING(EN)

Risk factors for fatal COVID-19 include older age and comorbidities, such as respiratory disorders, cardiovascular disease, diabetes mellitus and obesity. The outcome of the disease remains difficult to predict for individual patients, and may include permanent damage, prolonged recovery and chronic disease. Also, to date, there are no evidence-based effective treatment options for COVID-19. Thus, while some may benefit from antiviral and immune-potentiating treatment in the viral response phase, others could benefit from immune suppression against an ensuing cytokine storm.

Histopathology, including ancillary techniques, has the unique characteristic of revealing well-described disease patterns, which in other diseases have proven to be ameliorated by targeted therapy. Harnessing this information from autopsies / surgical specimens / biopsies, and carefully correlating this information with clinical and imaging parameters, has the potential to confirm and expand on current hypotheses regarding the pathogenesis of the disease and underlying risk factors[1]. Results of our study are expected to have a significant accelerating impact on the development of treatment strategies and biomarkers (e.g. serology / radiology / function tests) that will serve as surrogates for disease subphenotype, progression, and stage of COVID-19. We will enable personalised prognosis, monitoring and therapy prediction, leading to a decrease in disease burden and societal impact.

To that end, we will study tissue from Dutch COVID-19 patients in a nationwide multi-centre retrospective and prospective cohort study, focussing on histopathological and molecular tissue phenotyping, annotated by clinical, serological, and radiological data. This will enable mechanistic insights into the differences in pathogenesis and underlying immunological and tissue regenerative response patterns within the timeframe of the proposed project (2 years). The specific aims of this project are to:

Work package 1: Delineate the mechanism of SARS-CoV-2 entry and of SARS-CoV-2 infection, incl. viral distribution and histopathology correlation, in relation to known risk factors, **to enable understanding and prevention of infection by SARS-CoV-2 in at-risk populations**;

Work package 2: Expand on current knowledge of COVID-19 pathogenesis in lungs, extra-pulmonary organs and systemic disease, **to generate biomarkers and leads for therapy of acute infection**;

Work package 3: Analyse sequelae of COVID-19, esp. secondary infections, fibrosis and pulmonary hypertension, to **predict and treat late complications of COVID-19** in individual patients.

WP1: *Problem*

SARS-CoV-2 infection and its often-dire consequences are dependent on a range of risk factors, including a range of comorbidities, such as respiratory disorders, cardiovascular disease, diabetes mellitus and obesity. It is unclear how these relate to the risk to contract the disease and its severity. Moreover, the pathogenesis of SARS-CoV-2-associated lung damage is poorly understood, because SARS-CoV-2 infection is new in the human population and its clinical and pathological presentations differ considerably from those of other respiratory virus infections.

Urgency

Risk groups are much more likely to contract, and die from, COVID-19 than other respiratory virus infections[2]. It is highly urgent to better understand the underlying mechanisms of infection and the pathogenesis of SARS-CoV-2-associated DAD and pulmonary thrombosis in order to better differentiate between different disease categories of COVID-19, provide primary prevention and to develop treatment that is better tailored to the pathogenesis-associated predisposing factors.

Objectives

To identify *molecular mechanisms* that mediate viral entry, propagation, and tissue pathology in the airways, lung parenchyma, and pulmonary blood vessels.

WP2: *Problem*

In general, mortality rates of COVID-19 are between 0.5 - 2%, but if hospital admission is required, this rate can increase to 28%[3]. No evidence-based treatment for COVID-19 is yet available, and adequate biomarkers for disease endotypes are lacking.

Urgency

COVID-19 cases leading to hospital admission, transfer to ICU and prolonged periods of mechanical ventilation and in some cases a fatal outcome, have placed a great strain on healthcare systems, individuals and societies worldwide. There is an urgency for the development of markers and predictors of severity of disease as well as individualized mechanism-based treatments for severe COVID-19 to alleviate suffering, provide secondary prevention (leading to a reduction in hospital stay and mortality) and reduce impact on healthcare systems and societies as a whole.

Objectives

To comprehensively characterize disease pathogenesis, pathophysiology, disease endotypes and the related *immune response* in lungs and other organs of deceased COVID-19 patients in order to accelerate the development of biomarkers and personalized therapies for severe acute COVID-19.

WP3: *Problem*

We now know that some COVID-19 patients develop secondary infections[4] or show prolonged recovery after having survived the acute viral phase of the disease, and it is likely that permanent organ damage will persist in a subset of individuals[5].

Urgency

It is important to identify survivors of COVID-19 who are at risk of medium- to long-term sequelae of the disease. This will allow for more accurate prognosis, and in some cases, tertiary prevention or treatment in at-risk groups.

Objectives

To retrospectively and prospectively analyze tissue from COVID-19 survivors, in close collaboration with clinical registries and radiological biobanks, *to investigate the scale and spectrum of the sequelae of COVID-19 pathology* (super-infections and otherwise), and generate prediction models for medium- to long-term complications of the disease.

2. LOPEND ONDERZOEK

Biopsies and autopsies in COVID-19 patients have greatly contributed to our understanding of the disease[1]. This has yielded a relatively consistent range of disease response patterns, but also demonstrated the variability of response (1) between patients, (2) dependent on time after infection, and (3) within patients. These differences are likely to correspond to different clinical presentation, radiological appearance, disease progression, response to treatment and outcome of COVID-19, and are likely to reflect different stages / types of pathogenesis, and as such are likely to benefit from different types of specific treatment.

The surface receptor angiotensin-converting enzyme 2 (ACE2) and the associated proteases, transmembrane protease serine 2 (TMPRSS2), furase and Cathepsin L (CTSL), have previously been identified as **mediators of SARS-CoV cellular entry**[6]. Single-cell RNA-seq (scRNA-seq) has been employed to assess the cell-type-specific expression of *ACE2*, *TMPRSS2*, and *CTSL*, identifying specific subsets of epithelial cells as putative targets of viral infection in the respiratory system[7,8]. Meta-analysis of scRNA-Seq datasets has shown cell-type specific effects on expression of *ACE2*, *TMPRSS2*, and *CTSL* by age, smoking status and gender: clinical covariates of COVID-19 severity[9]. All single-cell RNA seq data of these receptors to date are based on cells from normal tissue donors, and urgently require validation at the protein level. We have previously shown that expression of the ACE2 receptor is low to absent in the bronchial epithelium of healthy individuals and more clearly expressed in the alveolar epithelium (W.Timens)[10,11], whereas ACE2 staining in nasal and bronchial epithelium has been reported by others[12]. However, the expression patterns of the SARS-CoV-2 receptors and activating proteases have not been extensively evaluated in individuals with risk factors for COVID-19, including pre-existing chronic lung disease such as asthma, smoking-related lung disease (COPD), lung fibrosis, obesity or as a function of age or sex. Notably, children have been affected less than adults by COVID-19, in spite of preliminary data showing that young children might be more susceptible to the virus[13].

As the relevance of differences in expression of SARS-CoV-2 receptors and activating proteases for differences in susceptibility to COVID-19 and variation in target organ involvement remains largely unknown, **co-localization of expression with histopathological abnormalities is likely to provide significant advances in our understanding of COVID-19 pathogenesis**. Lungs of fatal cases of COVID-19, often show diffuse alveolar damage (DAD)[8], similar to other viral infections of the lung, such as SARS-CoV, MERS-CoV[14,15] and influenza[16]. However, a unique feature of SARS-CoV-2 pathology is the abundant presence of thrombi in alveolar capillaries and small pulmonary arteries. Ackermann et al.[17] found that alveolar capillary microthrombi were nine times as prevalent in COVID-19 as in influenza. Although it has been claimed that coronavirus particles can been detected by transmission electron microscopy (EM) in the cytoplasm of endothelial cells associated with these thrombi[17,18], there is no evidence from human studies or from non-human primate models[15,19] that endothelial cells are permissive for SARS-CoV-2 replication, and so far there are no strong hypotheses regarding the pathogenesis of the coagulant effect of COVID-19 to guide therapy[20]. Moreover, clinical identification of patients who are at risk of, or are in process of, developing such microvascular complications, has thus far remained elusive. As thrombo-embolic events seem to be prevalent in this disease[21], there may also be a role for anticoagulation. As such, interference in the bradykinin-kallikrein pathway has also been speculated to be an attractive strategy[22]. However, as the pathogenesis of neither DAD nor pulmonary thrombosis from SARS-CoV-2 infection have been fully elucidated and many treatments remain empirical.

In addition to pulmonary manifestations, **neurological symptoms and neuroimaging findings have been reported in COVID-19**. The symptoms include anosmia, ageusia, impaired consciousness and encephalopathies. The most common neuroimaging findings include white matter microangiopathy, acute or subacute ischemic infarct, and acute hemorrhage[23]. Critically ill COVID-19 patients with persistent

mentally depressed status also show patchy demyelinating lesions and acute hemorrhagic necrotizing encephalopathy involving the thalami and medial temporal lobes[24,25]. In one case, virus was isolated using polymerase chain reaction (PCR) on cerebrospinal fluid (CSF), but in-depth studies of brain tissues are still lacking[26,27]. In a preliminary cohort of COVID-19 patients 5.1.2e, in 8 out of 10 cases the pathology showed similarities to both paraneoplastic encephalitis and entities such as acute demyelinating encephalomyelitis (ADEM)[28] and neuroceliac disease/gluten ataxia. This suggests a parainfectious, immunologically mediated cause, which requires confirmation.

The **range and extent of sequelae of COVID-19 pneumonia**, such as secondary infection and fibrosis, is as yet unknown. In patients with severe influenza any indication of Aspergillus is highly suggestive of influenza-associated pulmonary aspergillosis[29]. As COVID-19 is known to cause epithelial lysis and hemorrhagic infarction, predisposing to aspergillus colonization, the same may hold true for COVID-19. However, in COVID-19 a larger proportion of patients might be colonized, as COVID-19 patients with evidence for aspergillosis have survived without antifungal therapy[30]. Furthermore, proven aspergillosis cases in COVID-19 (CAPA) have been reported while serum tests remained negative[31]. It is therefore critical to gain insights into the histopathology of Aspergillus in lung tissue of COVID-19 patients and to correlate findings with Aspergillus diagnostic results.

Moreover, COVID-19 infection is likely to result on permanent organ damage following a severe viral infection phase, for instance akin to lung fibrosis following SARS/MERS[32], and possibly including chronic thrombo-embolic pulmonary hypertension (CTEPH) but is as yet unknown what the risk factors and prevalence of clinically significant disease will be due to the limited follow-up available thus far.

It is essential to **fill in the gaps in our knowledge in order to initiate timely and targeted antiviral, anti-inflammatory, anticoagulant, or even antifibrotic therapy**. So far, many of the treatments lack a solid pathogenetic basis as individual endotyping / subphenotyping solely based on clinical and radiological parameters has thus far proved elusive. The unique aspect of our project is that we will study the complete process in a real patient, annotated by detailed clinical and radiological data. **This is why we feel strongly convinced that with (1) the highly multi-disciplinary nature of the proposed research, (2) the numbers afforded by the proposed nationwide effort and (3) the focus on specific phases of the disease, pathogenetic mechanisms and organ systems, we are ideally placed to make a significant contribution to the (inter)national body of knowledge.**

3. PLAN VAN AANPAK (ONDERBOUW KEUZES)

WP 1. Mechanisms of SARS-Cov-2 entry and infection.

WP 1.1. Pathogenetic factors explaining susceptibility in high-risk groups 5.1.2e i.c.w. GRIAC-PIs 5.1.2e

1. **What is the relation between current smoking, sex, age, BMI, steroid use, or pre-existing lung disease such as asthma and COPD, and level of SARS-CoV-2 receptor protein & activating protease expression in the upper and large and small lower airways and in the alveoli?**
2. **Is SARS-CoV-2 receptor protein expression in the airways correlated with presence of specific immune cells: what is the potential impact of low-grade inflammation as present in smokers on viral transmission, replication and clearance?**
3. **Can we link genetic variants to SARS-CoV-2 receptor & activating protease protein expression?**

A large lung tissue and biopsy biobank is available at the pathology department at the UMCG, with extensively clinically characterized patients, and in addition already available molecular characterization with respect to DNA and RNA expression analysis (combined as eQTL, microRNA expression, immune cell and extracellular matrix (ECM) phenotyping, RNA sequencing and proteomics). Through our international collaborations (below), we will also be able to include airway biopsies of infants and preschool children that is absolutely unique in the world. This will allow analysis of the known SARS-CoV-2 receptor/entry molecules and the networks they are involved in. eQTL data will allow direct coding relations (cis-eQTL) as well as indirect coding effects (trans eQTLS) that may enable detection of new spatial and functional relationships at the cellular level in airways and lung tissue. Known as well as newly detected SARS-CoV-2 relevant molecules/proteins will subsequently be analyzed by immunohistochemistry (IHC, when relevant in a multiplex way), in particular to detect relationships and differences in levels of expression related to main general risk factors: age, BMI, gender, and smoking, asthma and COPD, and steroid use. To study COVID receptor protein expression in large and small airways and alveolar epithelium we will use:

A. Pre-Existing Biopsy cohorts:

Healthy individuals; NORM n=96 (current and never-smoking, age, gender, BMI)[33]

1. Asthma patients; RAS-COA-REPAIR n=100 (current, ex-and never-smoking, age, sex, steroid use, BMI)[34]; Helsinki Infant and Preschool children Biopsy cohort (n=50, severe wheeze, including passive smoking, age, sex[35] and fatal asthma (n=20)[36].
2. COPD patients; GLUCOLD n=100 (current and ex-smoking, age, gender, steroid treatment, BMI)[37].

B. Pre-existing Lung tissue biobank:

1. Controls n=30 (current, ex -and never-smoking, age, gender, BMI)

2. Mild-Moderate COPD n=30 (current -and ex-smoking, age, gender, BMI)
3. Severe COPD n=30 (all ex-smokers, age, gender BMI)

We will perform:

1. IHC protein expression for ACE2, TMPRSS2 and Cathepsin L (other markers may be added) in biopsies and lung tissue and determine differences in large and small airways and alveolar cells with respect to: Sex; Age; BMI; Current smoking; Steroid treatment, response, withdrawal; Presence of Asthma and/or COPD, other diseases/comorbidities.
2. Correlate ACE2, TMPRSS2 and Cathepsin L protein expression with specific immune cell counts in bronchial biopsies, for which the data are already available.
3. Correlate ACE2, TMPRSS2 and Cathepsin L protein expression with gene expression in bronchial biopsies of same cohorts, use cell type deconvolution methods to correct for cell type composition & link to relative expression per cell type.

We expect to determine:

1. **Effects of known risk factors on level of SARS-CoV-2 receptor protein & activating protease expression in the upper and large and small lower airways and in the alveoli;**
2. **The effect of low-grade inflammation in smokers on SARS-CoV-2 receptor protein expression in the airways showing the potential impact on viral transmission, replication and clearance;**
3. **Linkage of genetic variants to SARS-CoV-2 receptor & activating protease protein expression.**

WP 1.2. Correlation of viral distribution and histopathology in human tissues 5.1.2e .

1. **What is the role of SARS-CoV-2 infection in diffuse alveolar damage (DAD)?**
2. **What is the role of SARS-CoV-2 infection in pulmonary thrombosis?**

We hypothesize that DAD associated with COVID-19 is initiated by SARS-CoV-2 infection of the primary target cells in the pulmonary alveoli, type I and type II pneumocytes, similar to H5N1 influenza[16], and could be aggravated by pre-existing conditions that predispose to an excessive immune response, e.g. obesity[38]. We also hypothesize that pulmonary thrombosis is caused by SARS-CoV-2-induced endocytosis of ACE2 on pulmonary blood vessels, resulting in upregulation of angiotensin II (Ang-II), resulting in vasoconstriction, platelet activation, thrombosis, and vascular inflammation[17,39]. Such a mechanism would be aggravated by pre-existing conditions that result in enhanced ACE2 expression on endothelial cells, e.g. hypertension and previous stroke[39].

To test the hypothesis that DAD is initiated by excess production of pro-inflammatory cytokines by SARS-CoV-2 infected pneumocytes, we will use cases of COVID-19-related DAD determine the number and types of cells in the respiratory tract that are permissive for SARS-CoV-2 infection by IHC and/or in situ hybridization (ISH), dividing them in early or late phases of DAD. Subsequently, we will determine both levels of SARS-CoV-2 and levels of pro-inflammatory cytokines, by use of both PCR and next generation sequencing (NGS), in clinical and postmortem samples of sputum, throat swabs, nasal swabs, and blood, and postmortem samples at different levels of the respiratory tract, for those cases in which DAD is diagnosed. To test the hypothesis that pulmonary thrombosis is initiated by SARS-CoV-2-induced endocytosis of ACE2 on pulmonary blood vessels, we will identify surgical and autopsy COVID-19 cases with pulmonary thrombosis, and determine the expression of SARS-CoV-2, and expression and up- or downregulation of ACE2, Ang-II, P-selectin, E-Selectin, ICAM-1, and other activation markers of endothelial cells, using EM, ISH, IHC, PCR, and tissue multiplex gene expression profiling (see WP 2.1).

We expect to unravel:

1. **Mechanisms by which SARS-CoV-2 can cause DAD in COVID-19;**
2. **Mechanisms by which SARS-CoV-2 can cause pulmonary thrombosis in COVID-19 pneumonia.**

WP 2. Viral pathogenesis of COVID-19.

WP 2.1. Pulmonary: Profiling of lung tissue for prognosis and treatment (5.1.2e

1. **Does immune profiling and high-throughput protein expression analysis correspond with patient-specific clinical characteristics, to enable the development of prognostic and therapeutically predictive biomarkers?**

To comprehensively characterize disease pathogenesis and the related immune response, we will histopathologically characterize lung tissue (according to known COVID-19 tissue response patterns, including fibrosis) and clinically categorize deceased COVID-19 patients according to WHO standards. These features will be correlated with viral expression and tissue multiplex gene expression profiling in formalin-fixed paraffin-embedded (FFPE) samples using:

1. NanoString nCounter Technology for immune-related genes and fibrosis-related genes combined with 10 COVID-19 Spike-In genes;
2. Spatial analysis of immune cells infiltration using the novel NanoString GeoMax Digital Spatial Profiling (DSP) technique;
3. Liquid Chromatography Mass Spectrometry-Based (LC-MS) Proteomics (i.c.w. 5.1.2e Erasmus MC).

Data will be fed through an existing bio-informatics pipeline (on FAIR principles) and correlated with histopathological patterns, as well as clinical and radiological (incl. Functional Respiratory Imaging

(FRI) by FLUIDDA and Thirona 5.1.2e [40,41] **parameters to:**

1. **Generate mechanistic insights into the pathogenesis of different endotypes of COVID-19 pneumonia, esp. the 'vascular' type with extensive thrombotic/thrombo-embolic events;**
2. **Provide leads for circulating / lavage (BAL) biomarker development;**
3. **Determine novel and/or patient and phase-specific treatment based on defined pathophysiological mechanisms and associated clinical parameters and patient characteristics, such as age, the presence of obesity, hypertension and diabetes mellitus (according to ICD code);**
4. **Predict potential complications such as aspergillus infection and fibrosis (see below).**

WP 2.2. Extrapulmonary: Damage pattern of COVID-19 in the central nervous system (5.1.2e

1. **Can SARS-CoV-2 enter the CNS, and if so, via which route?**
2. **How does immune-related damage in SARS-Cov-2 compare to histologically similar conditions (paraneoplastic encephalitides, ADEM, gluten ataxia, multiple sclerosis and viral encephalitides)?**
3. **Which innate and adaptive mechanisms are operative in COVID-19 brains?**

We will comprehensively analyze tissues from the CNS, focusing on virus entry via cranial nerves (olfactory and trigeminal nerve) and the circulation, and study related immune mechanisms. We will compare the histology of COVID-19 and neurological conditions of established origins to pinpoint pathogenetic mechanisms. Specifically:

1. Different parts of the CNS, including the CSF, will be analyzed for the presence of viral RNA or proteins (i.c.w 5.1.2e Erasmus MC), resp. by quantitative PCR (qPCR) / ISH / subgenomic RNA, and IHC. Since virus-specific intrathecal antibodies are indicative for virus entry into the CNS, we will analyze if these are present in the CSF.
2. Histological lesions in historical brains from patients with paraneoplastic encephalitides, neuroceliac disease, acute demyelinating encephalomyelitis, multiple sclerosis, and thrombo-embolic events / stroke, will be extensively characterized and compared to COVID-19 patients for the distribution and the nature of cellular infiltrates, as well as the effects on blood vessels and surrounding myelin. Pathological findings will be correlated with MRI, if available. As a number of these conditions have a known or likely cause, this might help to shed light on the mechanisms involved in COVID-19.
3. The innate immunity in the brain is mediated largely by microglial cells that can both stimulate immune activity (pro-inflammatory), so-called M1 microglia, as well as dampen immunity (anti-inflammatory), M2 microglia[42]; though the majority of microglial cells are not exclusively M1 or M2, microglial cells can be skewed towards one of the types[43] steering immune activity. A simple IHC paradigm (4 markers) will be applied to characterize microglial cells.
4. In the adaptive immune system both cells and mediators play an important role. Many of these will be retrievable from CSF. As we have CSF samples of all COVID-19 patients we have brain tissue from, we will isolate immunoreactive cells and mediators, and apply both separately to organotypic brain slice cultures of mouse brains[44]. The changes this will induce in the organotypic slices are an indication for the effects of both cells and mediators from the CSF in COVID-19 neuropathology.

We expect to:

1. **Map virus distribution in the CNS in COVID-19 and correlate with neuroimaging;**
2. **Delineate immune mechanisms involved in CNS damage in COVID-19.**

WP 2.3. Systemic: Thrombotic / thrombo-embolic disease in COVID-19 5.1.2e

1. **What are the spectrum and underlying mechanisms of thromboembolic events in COVID-19?**

COVID-19 patients frequently develop significant vascular events[45]. While some of these appear to be thromboembolic in nature, there is also evidence for *in situ* arterial thrombosis in the context of endarteritis[18], as well as microvascular angiopathy[17]. These are likely to have differing etiologies. Unravelling the spectrum of these vascular phenomena in histological specimens will enable the development of avenues for prevention and treatment for these serious complications of COVID-19. We will do this by analyzing:

1. Histological composition of thrombi and local histological environment;
2. microCT imaging of FFPE blocks for vascular damage patterns 5.1.2e Univ. Southampton)
3. Presence of viral infection by immunohistochemistry / NGS / EM (WP 1.2);
4. Expression pattern by NanoString DSP / proteomics (WP 2.1);
5. Correlation with clinical and serological parameters. To this end, we will work in close collaboration with the ZonMW/Trombosestichting – funded top-down initiative no. 5.1.1c 5.1.2e EMC). This will allow for linkage of histopathological findings with:
   a. Basic scientific findings and circulating biomarkers (for which a similar virtual biobank is being created, incl. plasma proteomics; 5.1.2e EMC);
   b. Follow-up data from post-COVID thromboembolic event out-patient clinics 5.1.2e LUMC). To enable better delineation of venous thrombotic events in COVID-19 patients, and their relation to thromboembolic disease, specific guidance will be included in the national standard COVID-19

autopsy protocol (see below), to also include the acquisition of fresh thrombus material for ultrastructural studies (in collaboration with 5.1.2e (LUMC) / 5.1.2e (EMC)).

We expect to:

1. **Describe the spectrum of vascular involvement in COVID-19 patients;**
2. **Outline the mechanisms leading to thrombosis and thrombo-embolism COVID-19.**

WP 3. Sequelae of COVID-19.

WP 3.1. Short-medium term: secondary infectious complications of COVID-19 5.1.2e

1. **What is the histological prevalence of aspergillus infection in fatal COVID-19?**
2. **What is the predictive value of serological and microbiological test for the presence of aspergillus infection in tissue of COVID-19 patients?**
3. **What are risk factors for tissue infection by aspergillus in COVID-19?**
4. **What are the imaging findings of aspergillus superinfection in COVID-19 patients?**

We will closely collaborate with researchers involved in ZonMW application nr. 5.1.1c (main applicant 5.1.2e Radboud UMC) to:

1. Study autopsy material from the first SARS-Cov-2 infection wave, following a) correlation with microbiology data to identify Aspergillus positive patients and b) classification according to the AspICU algorithm and IAPA case definition[46,47]. For each case two (Aspergillus-negative) controls will be selected.
2. Use the CAPA-PLUS case registry to identify patients that underwent autopsy.

In this FFPE material, we will:

1. Use ancillary stains to demonstrate Aspergillus, and correlate this with findings on histopathology and expression profiling (WP 2.1) to characterize the immune response, and correlate with imaging findings.
2. Apply PCR, ISH, IHC and EM as needed to characterize the interaction between SARS-CoV-2 infection, the fungus and the host 5.1.2e

The results will be compared to COVID-19 cases without CAPA and correlated to Aspergillus diagnostic markers, specific risk factors and radiology.

We expect to:

1. **generate biomarkers of symptomatic aspergillus infection in COVID-19 and gain insight into underlying risk factors.**

WP 3.2. Long-term: chronic organ damage by COVID-19 (5.1.2e

1. **What are the long-term effects in lung tissue of COVID-19 survivors?**

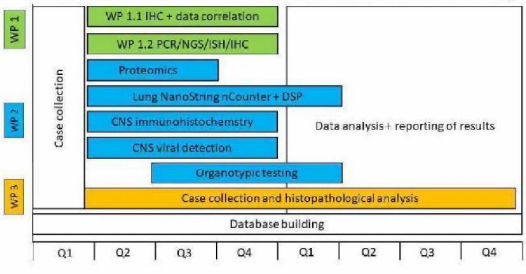
A significant proportion of patients show slow and prolonged recovery with severe limitations to daily functioning[48]. As yet, it is unknown which pathophysiological mechanisms are responsible for this chronicity of the disease after viral clearance and what the extent of long-term effects of COVID-19 will be on a tissue level, although it is well known that coronavirus infection can results in permanent lung damage, as shown in reports from patients SARS and MERS[32]. We therefore aim to answer this question by:

1. Registration, central review and further analysis of future histological specimens from patients in clinical follow-up cohorts (through close collaboration with post-COVID-19 thrombo-embolic (according to the published protocol of the NIV; 5.1.2e and pulmonary 5.1.2e LUMC) outpatient registries, and correlation with imaging findings (BIGR-NVvR imaging database 5.1.2e (NVvR))
2. Following patient consent, analysis tissue from prospective autopsies and relevant resection / (cryo)biopsy specimens through the infrastructure that is described in this project proposal. In these, we will primarily focus on evidence of (1) post-COVID pulmonary fibrosis and correlate this with CT (incl. air trapping seen in 90%)/imaging/cardiac ultrasound findings, as well as (2) CTEPH.

We expect to:

1. **Gain insight into the prevalence of post-COVID-19 pulmonary fibrosis and CTEPH;**
2. **Generate radiological biomarkers for the presence of structural complications of COVID-19;**
3. **Provide a standard national post-COVID-19 autopsy protocol.**

4. PLAN VAN AANPAK (ONDERBOUW KEUZES)



Time plan: As most tissues and all techniques are readily available at the start of the project, the proposal can start rapidly, yield first results within months and be finished within a 2 year time frame.

Feasibility: As the study of pathogenetic mechanisms in tissue is core in pathology research, this led to the early foundation of the Dutch COVID-19 Pathology Consortium (DCPC). Recently, the DCPC has indicated to NVVP members its intention to set up a virtual biobank, also to serve as a national resource

and international partner for tissue-based COVID-19 research. The DCPC biobank administrative hub will be housed at Erasmus MC and embedded in the ICT infrastructure of EraCORE, with registration in ResearchSuite/PaNaMa and the Erasmus MC central biobank. Medical-ethical approval for DCPC data registration, sharing and research use will be applied for though the Erasmus MC METC. Data and tissues for which there is a legal base for research use will be obtained locally by participating centers, and registered in the DCPC database. Considering the number of autopsies thus far, we expect to have tissue of 50-100 cases of COVID-19 available from current archives and in addition of non-COVID-19 lung tissues, which are already present in sufficient numbers in participating biobanks, with adequate clinical annotation. The biobank will also incorporate prospective material. The participants are experts in their field and their laboratories are ISO15189 certified.

5. RELEVANTIE

This proposal deals with the call-specific relevance criteria on virus, immunity, immune response and pathogenesis. It provides knowledge and models of the pathophysiology and pathogenesis of the virus in interaction with the human body for the various populations (gender, age, et cetera), which during the pandemic is vitally important[49] for supporting the medical action perspective and/or policy formulation, for example in relation to restrictive measures. This proposal brings together disciplines ranging from basic virology and non-human primate models, through pulmonary and cardiovascular specialists, to radiologists and pathologists, as well as a well-documented biobanks of tissues, imaging and clinical data from COVID-19 patients and non-COVID-19 controls. This has great potential for significant break-throughs, by accelerating the understanding of the pathogenesis, specifically in relation to known risk factors, and therefore hold real promise for **significant and rapid contributions to (primary, secondary and tertiary) prevention, biomarker development and the institution of mechanism-based treatment strategies – leading to a decrease in disease burden for patients and societies.**

The Netherlands are uniquely positioned to perform this type of multi-center tissue-based research due to the highly organized, networked, aligned and connected nature of its pathology (through PALGA/DNTP/NVVP working groups), and wider clinical research communities. By aggregating histological material in this highly organized way and aligning appropriate investigational tools for all Dutch COVID-19 patients of whom tissue is available, we answer current and future questions that might specifically refer to the national situation and can improve diagnosis and outcome for our population when infected with COVID-19. The assembly of available COVID-19 tissue with appropriate patient characterizations will generate a nationwide critical mass in scientific projects, foster collaborations, prevent duplication of research, and (continue to) ensure multi-disciplinary embedding. The intended structures and collaborations will remain operative for a possible 'second wave', and could also be used for other viral outbreaks, or even non-virus related tissue-based research.

6. PROJECTGROEPLEDEN EN HUN ROLLEN

1) 5.1.2e EMC, 5.1.2e WP 1.2
2) 5.1.2e EMC, 5.1.2e WP 2.1, WP 2.3, WP 3.2
3) 5.1.2e UMCG, WP 1.1
4) 2.1, WP 3.1, WP 3.2
5) 5.1.2e t, Radboud UMC, WP 2.2, WP 3.1, WP 3.2
6) 5.1.2e LUMC, WP 1.1, WP 2.3, WP 3.2
7) 5.1.2e 5.1.2e 5.1.2e EMC, WP 1.2, WP 2.1, WP 2.2
8) UMCG, WP 1.1
9) , AUMC, WP 2.2.

7. KENNISOVERDRACHT, IMPLEMENTATIE, BESTENDIGING

The DCPC has already contributed to national guidance on COVID-19 pathology diagnostics, FMS webinars, consultation for media communication by NVVP members, and served as a consultative resource for pathology and clinical colleagues in the Netherlands. The integrated nature of the proposed research will facilitate rapid dissemination of the findings of this research to the research and clinical community via similar channels. In addition, selected illustrative cases will be used to create an educational resource of annotated images, which will be made freely accessible. Findings will, whenever possible, be linked to data available from the nationwide CovidPredict project and feed into / be accessible through the Dutch COVID-19 Data Support Programme (Health-RI / BBMRI) and BigPicture (IMI). Data management will be according to FAIR principles using standard nomenclature (WHO / VODAN, and in collaboration with the data steward we will strive for alignment with COVID-19 standards which are currently being developed. This project came about through collaboration with and with support from pulmonary and intensive care physicians, and we will frequently meet with them to discuss the preliminary results of the ongoing project and the translation of the new knowledge of COVID-19 pathogenesis to improved treatment of hospitalized patients.

8. DEELNAME VAN DE STAKEHOLDER(S)/EINDDOELGROEPEN

The DCPC has endorsement by the NVVP for this initiative and will actively pursue participation by, and inform Dutch pathologists and trainee pathologists. We will seek close collaboration with patient advocacy groups representing at-risk populations, including Diabetesvereniging Nederland, Nederlandse Stichting Overgewicht, Harteraad and Longfonds. Interaction with pulmonary physicians (NVALT; post-COVID-19 registry), intensive care specialists (NVIC; MONITOR-IC), radiologists (NVvR; Biomedical Imaging Group Rotterdam (BIGR), national imaging biobank) and thrombosis and infection specialists ((NIV; post-thrombosis registry) is central to the success of the proposed research, and representatives from these specialties have been involved in this grant proposal and added to the project group.

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Reactie op opmerkingen in email met positief advies

positief advies projectidee, projectnr. 5.1.2e

- De commissie is van mening dat het projectidee erg inventariserend van aard is, en voorbijgaand aan wat er al aanwezig is. Zij mist aansluiting op bevindingen en hypothesen van basaal en klinisch onderzoek. De commissie verzoekt daarom aanvullende partners te overwegen.

De projectgroep heeft de organisatiestructuur van het in deze aanvraag beschreven beoogde onderzoek grondig herzien, en het onderzoek getracht zoveel mogelijk te laten aansluiten bij belangrijke klinische knelpunten. Hierdoor zijn 3 work-packages ontstaan, incl. hiertoe behorende sub-packages, die beter aansluiten bij de klinische praktijk van COVID-19, en de daarbij behorende kennislacunes. De work-packages richten zich achtereenvolgens op de initiële infectiefase en de daaraan ten grondslag liggende risicofactoren (WP1), de acute ernstige uitingen van COVID-19 in de longen, het centraal zenuwstelsel, en de circulatie, met ook specifieke aandacht voor stollingsproblematiek (WP2) en de chronische gevolgen van COVID-19 met betrekking tot secundaire infecties (in casu aspergillose), fibrose en pulmonale hypertensie (WP3). De projectgroepleden hebben allen tevens op basis van de tot dusverre bekende literatuur de onderbouwing van het voorgestelde onderzoek verstevigd, en de literatuurlijst is derhalve ook aanzienlijk uitgebreid. Wij zijn van mening dat de vraagstellingen in deze definitieve aanvraag aansluiten bij de meest prangende klinische problemen en reeds bestaande kennis omtrent COVID-19, en dat het voorgestelde onderzoek een goede kans biedt om aan het oplossen hiervan significant te kunnen gaan bijdragen. De eerste resultaten zouden al op korte termijn kunnen worden gegenereerd, omdat het materiaal en de technieken reeds grotendeels beschikbaar zijn. Bij het totstandkomen van deze definitieve aanvraag is tevens contact gezocht met klinische groepen die het COVID-19 onderzoek in Nederland organiseren, en zo is er contact geweest en wederzijds de wens tot verregaande samenwerking uitgesproken met vertegenwoordigers van de registries van de 5.1.2e, vasculair geneeskundigen 5.1.2e, en radiologen 5.1.2e. Ook is er aanvullende aansluiting op basaal onderzoek gezocht en gevonden (o.a. 5.1.2e WP 2.2).

- In het projectidee wordt samenwerking met een aantal partijen besproken. De commissie verzoekt deze samenwerkingen en organisatie daarvan verder uit te werken.

In het kader van het bovenstaande zijn afspraken gemaakt met bestaande klinische registries over samenwerking op het vlak van klinisch-pathologische correlaties, zoals beschreven in de aanvraag (zie tevens 5.1.2e, bijlage). Wij hebben voorts advies ingewonnen van de Patiëntenfederatie over het te volgen beleid t.a.v. het betrekken van patiëntenbelangen, en met diverse partijen is hiervoor contact gezocht (Diabetesvereniging Nederland, Nederlandse Stichting Overgewicht, Harteraad en Longfonds). Gezien de tijdsdruk is het helaas nog niet mogelijk gebleken hierbij tot concrete afspraken te komen, maar wij zullen deze contacten koesteren in het verloop van het project en hierdoor hopelijk alsnog tot een uitgewerkte samenwerking en betrokkenheid bij het onderzoek te komen.

Dutch COVID & Thrombosis Coalition

Betreft: projectaanvraag 88796, bottom-up ronde COVID-19 aandachtsgebied 1

Rotterdam 12 juni 2020

Geachte commissie,

Graag wil ik namens de Dutch COVID & Thrombosis Coalition onze steun uitspreken voor het project "Identifying pathogenesis of COVID-19 pathology in the Dutch population and unravelling differences in pathogenetic mechanisms in high- and low-risk groups", aanvraag 5.1.1c De onderzoeksvragen en projecten behorende bij dit project zijn complementair aan de vragen en projecten van het gehonoreerde project "Caging the dragon: translational approach to unravel and prevent COVID-19 associated thrombosis"(projectnummer 5.1.2e . Onderlinge samenwerking is al opgezet en functionerend.

Met vriendelijke groet,

5.1.2e
5.1.2e
5.1.2e
5.1.2e Erasmus MC
5.1.2e

Specification staff

1.a Staff costs (based on salary scale)

nr	Function / Name	NFU / VSNU member / other staff ruling	Function/Scale	Months	Gross salary - based on table / 1 FTE	Monthly Gross salary (for Other	% fte (for the project)	Salary costs	Gross salary, 40% increment (for Other ruling only)	Overhead % (for Other ruling only)	Total
1	to be specified	NFU	IA(rs) onderzoeker	24	5.1.1c		100%	5.1.1c	€ -		€ 145.377,00
2	to be specified	NFU	NWP-Ac	24			15%		€ -		€ 26.131,95
3	to be specified	NFU	Promovendus	18			100%		€ -		€ 82.004,00
4	to be specified				€ 0		100%	€ -	€ -		€ -
5	to be specified				€ 0		100%	€ -	€ -		€ -
6	to be specified				€ 0		100%	€ -	€ -		€ -
7	to be specified				€ 0		100%	€ -	€ -		€ -
8	to be specified				€ 0		100%	€ -	€ -		€ -
9	to be specified				€ 0		100%	€ -	€ -		€ -
10	to be specified				€ 0		100%	€ -	€ -		€ -
11	to be specified				€ 0		100%	€ -	€ -		€ -
12	to be specified				€ 0		100%	€ -	€ -		€ -
13	to be specified				€ 0		100%	€ -	€ -		€ -
14	to be specified				€ 0		100%	€ -	€ -		€ -
15	to be specified				€ 0		100%	€ -	€ -		€ -

1.b Staff costs (based on hourly rate)

The hourly rate should be acceptable, reasonable and fair

nr	Function	Activity / Actions	Hourly rate	number of hours	Total
1	to be specified		€ -		€ -
2	to be specified		€ -		€ -
3	to be specified		€ -		€ -
4	to be specified		€ -		€ -
5	to be specified		€ -		€ -
6	to be specified		€ -		€ -
7	to be specified		€ -		€ -
8	to be specified		€ -		€ -
9	to be specified		€ -		€ -
10	to be specified		€ -		€ -
11	to be specified		€ -		€ -
12	to be specified		€ -		€ -
13	to be specified		€ -		€ -
14	to be specified		€ -		€ -
15	to be specified		€ -		€ -

DAEB Format

Organisation	Type of organisation	Quoted costs	Quoted co-funding	Requested budget
Erasmus MC	Knowledge institution		-	
AUMC-VU	Knowledge institution		-	
UMCG	Knowledge institution	5.1.1c	-	5.1.1c
Radboud UMC	Knowledge institution		-	
LUMC	Knowledge institution		-	
		-	-	-
		-	-	-
		-	-	-
		-	-	-
		-	-	-
	Total	499.412,95	-	499.412,95

Staff costs

			Costs	Own contribution / 3rd party co-funding	Requested budget ZonMw
Function (will be filled based on "staff" sheet)	Remarks	Organisation (dropdown menu)			
to be specified	arts-onderzoeker	Erasmus MC	€ 5.1.1c	€ -	€ 5.1.1c
to be specified	radiologie fellow	Erasmus MC	€	€ -	€
to be specified	analist	UMCG	€	€ -	€
to be specified			€ -	€ -	€ -
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to be specified			€ -	€ -	€ -
			€ 253.512,95	€ -	€ 253.512,95

Material, equipment & consumer goods (itemised)

			Costs	Own contribution / 3rd party co-funding	Requested budget ZonMw
Discription	Remarks	Organisation (dropdown menu)			
NanoString nCounter analysis	80 cases, E.500 / case	Erasmus MC	€	€ -	€
NanoString DSP analysis	80 cases, 2 ROI each, E.600 / case	Erasmus MC	€	€ -	€
Antibodies and FISH probes WP 1.1		UMCG	€	€ -	€
microCT analysis	50 cases, E.400 / case	LUMC	€	€ -	€
Antibodies and detection kits WP 2.2	20-25 cases	AUMC-VU	€ 5.1.1c	€ -	€ 5.1.1c
ISH probes and RNA PCR	100 slides	AUMC-VU	€	€ -	€
Isolation and culture microglia and astrocytes		AUMC-VU	€	€ -	€
Histochemistry		Radboud UMC	€	€ -	€
Proteomics	80 cases, E.500 / case	Erasmus MC	€	€ -	€
			€ -	€ -	€

			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ 200.900,00	€ -	€ 200.900,00

Other costs (itemised)					
			Costs	Own contribution / 3rd party co-funding	Requested budget ZonMw
Description	cost sort (dropdown menu)	Organisation (dropdown menu)			
Implementation	Open Access publication	Erasmus MC	€ -	€ -	€ -
Implementation	Open Access publication	AUMC-VU	€ -	€ -	€ -
Implementation	Open Access publication	UMCG	€ -	€ -	€ -
Implementation	Open Access publication	Radboud UMC	€ -	€ -	€ -
Implementation	Open Access publication	LUMC	€ -	€ -	€ -
Database management, incl. alignment with clinical registries	Others, please specify in description	Erasmus MC	€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ -	€ -	€ -
			€ 45.000,00	€ -	€ 45.000,00

5.1.1c

5.1.1c

Total costs project	€ 499.412,95	
Own contribution / co-funding		€ -
TOTAL REQUESTED BUDGET ZONMW		€ 499.412,95

Additional explanation for budget

Approval financial responsible receiving organisation

Receiving organisation
Name:
Function:
E-mail adres:
Date:

Justification of the budget.

General

Only costs incurred during the project period are remunerated. Erasmus MC, Amsterdam MC, Radboudumc, UMCG and LUMC contribute to the project and receive part of the budget.

Staff costs

Erasmus MC is responsible for collection, administration, initial scoring an distribution of samples, as well as data integration from autopsy cases and clinical registries. This applies to all work packages, and these tasks will be assigned to a physician-researcher (preferably with (some) training in pathology) for the full period of the budget, with the intention of also giving the candidate a role in the generation of output from the data acquisition phase, ideally contributing towards a PhD thesis. In addition, WP2 and WP3 will require integration with imaging modalities (CT and MRI), and re-evaluation of any scans available. For this, dedicated research time of a radiologist is necessary, and this has been included in the proposed budget. Additional salary costs for the applicants and supervisors are covered by the institution. Staff costs are budgeted according to NFU salary tables. Total staff costs at Erasmus MC are € 171,508.95.

Groningen UMC will perform immunohistochemistry in WP 1.1, and correlate findings with available clinical, immune cell profiling and gene expression data. For this, technical assistance has been requested. Additional salary costs for the applicants and supervisors are covered by the institution. Staff costs are budgeted according to NFU salary tables. Total staff costs at Groningen UMC are € 82,004.00.

Material, equipment & consumer goods

Erasmus MC will perform pulmonary gene expression analysis with NanoString cCounter and DSP, as well proteomic methodologies, which generate data for WP 2.1, but also for other work packages, esp. WP 1.1 and WP 2.3. We expect to have pulmonary tissue available from approx. 80 cases. The costs of NS nCounter (RNA analysis, 770 genes, with COVID-19 spike-in) will be E5.1.1c per case, and for DSP (targeted protein analysis) E5.1.1c per 10 regions of interest (ROIs), with an expected 2 ROI per block, i.e. E5.1.1c per case. Proteomics will be performed on all available cases, with a cost of E5.1.1c per case. Equipment and analysis tools are covered by the institution. Viral presence detection (WP 1.2, WP 2.1, WP 2.3; by ISH, NGS, IHC) is covered by the Department of Virology. Total material, equipment & consumer goods costs at Erasmus MC are € 128,000.00.

Groningen UMC will perform immunohistochemistry and in situ hybridization in WP 1.1, estimated at € 20,000.00.

Radboudumc will perform additional histochemical stains for fungal infection (WP 3.1), estimated at E5.1.1c per case. Salary costs for technical assistance are covered by the institution. Total material, equipment & consumer goods costs at Radboudumc are € 6,000.00.

Amsterdam UMC will perform experiments for WP 2.2, including antibody staining, in situ hybridization, PCR, and organotypic cultures. Salary costs for technical assistance are covered by the institution. Total material, equipment & consumer goods costs at Amsterdam UMC are € 26,900.00.

LUMC (WP 2.3) will correlate findings of vascular pathology in lung tissue with microCT images, to be generated by the University of Southampton, costing E. 5.1.1c per case, for an expected 50 cases, i.e. total € 20,000.00.

Other costs

Central database set-up and maintenance, including alignment with clinical registries and slide scanning/hosting, is costed at E. 5.1.1c
As requested, we reserved at least 5% 5.1.1c Euro per institute) of the total costs of the project for communication and implementation, including lectures, exchange meetings, expert meetings, and (open access) publication.
We did not budget costs for open science and FAIR data, because all partners have experience with FAIR data collection and we have resources (data steward) available for this for the project.

Checklist

for Open science & FAIR data elements in the COVID-19 research programme

Version 1.0

This checklist is for the first 4 out of **8 requirements and recommendations** for the **activities for open science and FAIR** data. They relate to the preparation phase of a research project.
The checklists shows a number of options for open science and FAIR data. Please consult **Open science in COVID-19 research** for more information about what you can do, for recent updates on the guidance, new practices, and instructions.

Choose the options that suit your project best!
The purpose of the checklist is to fill in the options that you choose for your project. Discuss with your data steward (or other data expert) the options that suit your project best. If you have options that are not listed below, you may indicate this as well.

Please fill in the form and attach it as a PDF file to your grant application. This is mandatory.

Requirements & Recommendations	Applicants must report as follows
Who is the data steward who supports the open science and FAIR data planning in your project? Check the website ZonMw's webinars to inform and support data stewards.	☒ I involve a data steward: Name: 5.1.2e Institute: Erasmus MC E-mail: 5.1.2e @erasmusmc.nl Attended the webinar: ☒ Yes ☐ No ☐ I do not have a data steward yet.
Requirement 1: Alignment and reuse Please show the options for reusing data, biological materials, and/or other resources (from research or from practice) in your project. Check whether it is possible to use resources that are made in the context of COVID-19.	Name the existing resources that you plan to use: ☒ Data: VODAN ☐ Biological materials: ☒ Research software: Castor eCRF ☐ Other resources, i.e. Klik of tik om tekst in te voeren. ☐ No, I will not use existing resources, because Klik of tik om tekst in te voeren. Please mark the resources that you indicated above in **bold** if it is a COVID-19 related resource
Requirement 2: preregistration of all animal studies **(for all other studies, preregistration is strongly recommended)** You are required (for animal studies) and recommended (for all other studies) to preregister your research plan (including the protocols, methods, etc).	☐ I n case of preregistration: Provide the link or registration code: Klik of tik om tekst in te voeren. ☐ For animal studies, the code at the Preclinical Trial Register is: Klik of tik om tekst in te voeren. ☒ No, I do not preregister my research proposal.

Requirement 3: FAIR data within COVID-19 research community **Choose the options that suit your project best! (MAATWERK!)** Here you can show the COVID-19 specific standards, technology or infrastructure for FAIR data that you have selected to apply during your project. Once your application is granted, you can use these to fill in your data management plan (DMP) (= requirement 5). Read for more information: 3.Creating FAIR data, tailored to COVID-19	Name the COVID-19 specific FAIR data standards, technologies or infrastructure that are applicable in your study, and you plan to use: ☒ eCRF of the WHO (machine actionable) ☒ A COVID-19 related or other FAIR data point ☒ COVID-19 research platform for data sharing ☒ Data will be recorded in RDF format ☒ I plan to use the metadata scheme that will be developed for COVID-19 research (planned in summer 2020) ☐ Other COVID-19 related standards, etc: Klik of tik om tekst in te voeren. ☒ Collaboration with COVID-19 data collection(s), namely VODAN, glimdna ☐ A new standard, technology or infrastructure will be developed in the project with the COVID-19 research community. Comment on your choice(s) Klik of tik om tekst in te voeren. ☐ None of the above. Comment: Klik of tik om tekst in te voeren. ☐ I did not decide yet.
Requirement 4: Budget for FAIR data and Open Access Publications You need to plan a budget for open science and research data management during your research project. This budget should include data stewardship, and – if applicable - costs for additional services from data service providers (e.g. from Health-RI), or extra e-infrastructure.	**Explain how you budgeted for open science and FAIR data in your project:** ☐ I specified the costs in the budget form. ☒ I cannot specify the costs right now, and make a reservation of 5% maximum of my research budget for data stewardship. ☐ I did not budget the costs, because Klik of tik om tekst in te voeren. **When you fill in the budget form, you could consider the following aspects:** o Data stewardship o Data services providers (e.g., at Health-RI, other others) o Additional e-infrastructure, exceeding the regular institutional infrastructure. o Other open science and FAIR data related costs. o (Optional) Open access publication(s): ZonMw requires researchers within the covid-19 programme to make all publications resulting from scientific research, that is fully or partially subsidised by ZonMw, immediately (without embargo) open access available with an open license. You are allowed to include costs for full gold Open Access publications in the project budget up to a maximum amount of € 5000,- (specify with 'Open Access'). Immediate Open Access publishing via other routes is also permitted, but ZonMw does not provide financial resources for this. For the specific conditions we kindly refer to the programme texts.
