**RESEARCH PROTOCOL**

**Invasive pulmonary aspergillosis complicating COVID-19 infection in critically ill patients: a retro- and prospective, multicentre study**

**PROTOCOL TITLE** Invasive pulmonary aspergillosis complicating COVID-19 infection in critically ill patients: a retro- and prospective, multicentre study

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| **Project leader** | Dr. J.A. Schouten, MD, PhD  Dept of Intensive Care  Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, the Netherlands  0031-629129083  Jeroen.Schouten@radboudumc.nl |
| **Coordinating Investigator** | Drs. N.A.F. Janssen, MD  Dept of Internal Medicine / Infectious Diseases  Radboud University Medical Center  Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, the Netherlands  0031-24-3619086  Nico.Janssen@radboudumc.nl |
| **Principal investigator(s)** |  |
| **Radboud University Medical Center, Nijmegen** | Dr. F.L. van de Veerdonk, MD, PhD  Dept of Internal Medicine / Infectious Diseases  Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, the Netherlands  0031-24-3619825  Frank.vandeVeerdonk@radboudumc.nl  Prof. dr. P.E. Verweij, MD, PhD  Dept of Medical Microbiology  Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, the Netherlands  0031-24-3614356  Paul.Verweij@radboudumc.nl  Dr. R.J.M. Brüggemann, PharmD, PhD  Dept of Clinical Pharmacy  Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, the Netherlands  0031-24-3617744  Roger.Bruggemann@radboudumc.nl  Dr. J.A. Schouten, MD, PhD  Dept of Intensive Care Medicine  Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, the Netherlands  0031-24-3617273  Jeroen.Schouten@radboudumc.nl |
| **Sponsor** | Radboud University Medical Center  Geert Grooteplein-Zuid 10  6525 GA Nijmegen, the Netherlands |
| **Subsidising party** | N/A |
| **Independent expert (s)** | N/A |
| **Laboratory sites** | Dept of Medical Microbiology  Geert Grooteplein-Zuid 10, 6525 GA, Nijmegen, the Netherlands  Dept of Medical Microbiology  Geert Grooteplein-Zuid 10, 6525 GA, Nijmegen, the Netherlands |
| **Pharmacy** | N/A |

**PROTOCOL SIGNATURE SHEET**

|  |  |  |
| --- | --- | --- |
| **Name** | **Signature** | **Date** |
| **Head of Department:**  Prof. dr. H. van der Hoeven MD, PhD  Intensivist  Radboudumc Nijmegen |  |  |
| **Project leader:**  Dr. J.A. Schouten MD, PhD  Intensivist  Radboudumc Nijmegen |  |  |

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**LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS**

|  |  |
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| **BAL**  **CAPA**  **CMO**  **CO** | **Broncho-Alveolar Lavage**  **COVID-19-Associated Pulmonary Aspergillosis**  **Committee on Research Involving Human Subjects**  **Coordinating Investigator** |
| **COVID-19** | **Coronavirus Disease 2019** |
| **EORTC/MSGERC** | **European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium** |
| **GCP**  **GM**  **GMI** | **Good Clinical Practice**  **GalactoMannan**  **GalactoMannan Index** |
| **IAA**  **ICH** | **Influenza-Associated Aspergillosis**  **International Conference on Harmonization** |
| **ICU** | **Intensive Care Unit** |
| **METC**  **PCR**  **PI** | **Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**  **Polymerase Chain Reaction**  **Principal Investigator** |
| **SARS-CoV-2** | **Severe Acute Respiratory Syndrome Coronavirus 2** |
| **Sponsor** | **The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical**  **company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.** |
| **WHO** | **World Health Organization** |
| **WMO** | **Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen** |

**SUMMARY**

**Rationale:** Invasive pulmonary aspergillosis can complicate severe influenza infections in patients admitted to the intensive care unit (ICU) in 16 to 28% and is associated with a high mortality of 44.4 to 61%. With the current coronavirus disease 2019 (COVID-19) pandemic, which can lead to severe viral pneumonitis, secondary infection with *Aspergillus* has been described to occur as well. After the instigation of dexamethasone as mainstay therapy in COVID-19 patients who require oxygen on admission in hospitals, the incidence of superposing infections like invasive pulmonary aspergillosis is likely to increase In ICU patients

**Objective**: Main objective: to assess the incidence of COVID-19-associated pulmonary aspergillosis (CAPA) in patients admitted to the ICU with COVID-19. Further, to compare mortality rates between patients with COVID-19 without and with CAPA. To evaluate which diagnostic tests are performed in Dutch ICUs to diagnose invasive pulmonary aspergillosis. To evaluate empirical and targeted therapy in all patients with CAPA in the ICU. To analyze Aspergillus isolates cultured from patients regarding resistance phenotype and genotype and additional genotyping markers.

**Study design:** A retro- and prospective, observational, multicentre study of patients in who(m) a broncho-alveolar lavage (BAL) was performed in the second COVID19 wave

**Study population:** All patients admitted to the ICU with COVID-19 and who underwent a BAL during ICU stay in the second COVID wave

**Main study parameters/endpoints:** Incidence of CAPA at the time of ICU discharge or death. Mortality rates compared between patients with COVID-19 with and without CAPA. Observational data on disease manifestation (a.o. incidence of tracheitis in CAPA patients) diagnostic procedures used in serum (galactomannan (GM)) and respiratory samples (*Aspergillus* culture / PCR or galactomannan (GM) detection and treatment provided (which empirical treatment, treatment duration).

**Nature and extent of the burden and risks associated with participation, benefit and group relatedness:** The risk of participation is non-existent. Participants will not be subjected to any intervention; only clinical data will be collected from their medical files.

# INTRODUCTION AND RATIONALE

Invasive pulmonary aspergillosis has long been regarded as an opportunistic infection in patients with a severely compromised immune system, such as recipients of allogeneic stem cell transplants, recipients of solid organ transplants, those using immunosuppressive medication and those with a severe primary immune deficiency. Invasive pulmonary aspergillosis can notably complicate severe influenza infections in patients admitted to the intensive care unit (ICU) in 16 to 28% and is associated with a high mortality of 44.4 to 61%.

There are increasing reports of COVID-19 patients in the ICU developing secondary invasive fungal infections. A variable frequency of COVID-19 associated pulmonary aspergillosis (CAPA) of 4% - 34% has been reported, primarily in European countries (1). The variability may be due to many factors including diagnostic approach, cohort size and CAPA case definition. A consensus CAPA case definition will be published shortly that may overcome variation due to case definitions. (2) Studies from the Netherlands are limited, but one cohort from Amphia, Breda, reported 6 cases in 31 (19%) COVID-19 patients in the ICU. (3) An unpublished case registry involving hospitals from Belgium and the Netherlands included 520 COVID-19 patients in the ICU (CAPA PLUS study). CAPA was diagnosed in 41 patients (7.9%) according to the new consensus definitions and even in 15.1% (41/271) if only patients were considered who underwent at least one diagnostic test required to diagnose CAPA according to this new classification. The ICU mortality in CAPA patients was 51.3% compared to 33.3% in COVID-19 patients without CAPA and 23.1% in those unclassifiable (p=0.001). Excess mortality was also reported in other cohort studies (4,5) and is similar to that observed in influenza associated pulmonary aspergillosis (IAPA; 51% versus 28%). (6) These data were obtained during the first corona wave. Based on interactions with other hospitals we have the impression that the number of CAPA cases are higher during the second wave compared with the first wave.

Invasive *Aspergillus* tracheobronchitis is a highly lethal manifestation in IAPA patients, with a mortality rate of 90%.(7) In our experience, cases of invasive *Aspergillus* tracheobronchitis are observed in COVID-19 patients, but the frequency may be lower than found in influenza patients (which is up to 30%). At present, the frequency, optimal management and mortality of *Aspergillus* tracheobronchitis in CAPA remains unclear.

Azole resistance has been reported in three CAPA-patients (including one patient from the Netherlands) (8) but the resistance frequency remains unclear. According to the Dutch SWAB invasive mycoses guideline, CAPA patients receive combination antifungal therapy.

Therefore, we propose to perform a retro- and prospective, observational, multicentre study to assess the incidence of CAPA in critically ill COVID-19 patients, in the second wave of COVID-19.

# OBJECTIVES

Primary Objective:

# To investigate the frequency of CAPA during the second wave of COVID-19 in ICUs in the Netherlands. The frequency data for CAPA will be compared to the cohort collected during the first wave (CAPA PLUS study).

Secondary Objectives:

1. To collect data regarding CAPA diagnosis, disease manifestation (e.g. tracheitis), host factors, pathogen ID and susceptibility profile, management and outcome.
2. To analyze *Aspergillus* isolates cultured from patients regarding resistance phenotype and genotype and additional genotyping markers.

**3. STUDY DESIGN**

We aim to perform a partially retrospective and partially prospective, observational, multicentre study. We aim to collect proven and probable cases according to the consensus guideline(2). This data collection will be done prospectively where still applicable but will involve a retrospective review of all cases in the ‘second wave’. The denominator will be determined through local ICU admission and SARS-CoV-2 diagnosis data. These data will be retrieved from NICE (National Intensive Care Evaluation) data and/or hospital /ICU admission data from participating hospitals

As the characteristics and epidemiology of the current pandemic are rapidly changing, it will be challenging to predict the number of patients eligible to participate in this study and when they will present to clinical care. Therefore, we propose to perform an open-ended study without a predefined number of patients to be included, nor a predefined time period during which patients will be included.

**4. STUDY POPULATION**

## 4.1 Population (base)

All patients with confirmed (proven by polymerase chain reaction, or PCR) COVID-19 admitted to the ICU, regardless of reason of admission to ICU, age, gender or ethnicity.

## 4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

# Confirmed (proven by PCR) COVID-19.

# Admission to the ICU

# Performance of a BAL

## 4.3 Exclusion criteria

Not applicable.

# 5. METHODS

## 5.1 Study parameters/endpoints

### 5.1.1 Main study parameter/endpoint

Number of patients with CAPA within the total number of patients with (confirmed) COVID-19 admitted to the ICU.

### 5.1.2 Secondary study parameters/endpoints

### Mortality rate among total number of patients with critical (confirmed) COVID-19 admitted to the ICU

### Mortality rate among patients with CAPA

### Demographic and/or clinical risk factors for the development of CAPA in COVID-19 (see “5.1.3. Other study parameters”)

* Differences in length of stay in ICU between patients with COVID-19 who develop CAPA and those who do not
* In case of development of CAPA, the time to CAPA diagnosis

### 5.1.3 Other study parameters

For a detailed description of all the demographic and clinical parameters being assessed, please refer to the copy of the electronic case report form (CRF) used in this study provided.

In summary, data will be collected regarding:

* Demographic characteristics
* Medical history
* Use of immunosuppressive medication
* EORTC/MSGERC risk factors for invasive pulmonary aspergillosis
* Clinical measures of diseases severity at ICU admission (i.e., the Acute physiology and chronic health evaluation II (APACHE II) score and quick sequential organ failure assessment (qSOFA) scores)
* Treatment data during ICU admission, including treatment for COVID-19 and antifungal treatment
* Details regarding the diagnosis and treatment of CAPA
* Presence confirmed CAPA tracheo bronchitis

**5.2 Participating investigators / medical centres**

Attempts will be made to include as many participants as possible in this study. Therefore, active attempts will be made to include as many medical centres in the Netherlands. The study is supported by the NVIC, which will assist in involving hospital ICUs in the Netherlands through their national research initiative called RCCnet.

## 5.3 Withdrawal of individual subjects

Centers can leave the study at any time for any reason if they wish to do so without any consequences.

**6. STATISTICAL ANALYSIS**

In general, all subjects who are included in this study will be included in the statistical evaluation. Descriptive statistics will be calculated using Excel 2010 software (Microsoft Corporation) and SPSS software version 25.0 (SPSS Inc.).

Appropriate statistical tools will be used to test for significance with respect to the primary and secondary endpoints. In all instances, a p-value < 0.05 will be considered statistically significant.

## 6.1 Primary study parameter

CAPA incidence at ICU discharge will be reported as a percentage/proportion. To correct for confounders, a multivariable method will be used, correcting for several baseline characteristics.

## 6.2 Secondary study parameters

Secondary study parameters will be reported and differences in these parameters between groups will be statistically assessed as follows:

* Mortality at ICU discharge: reported as a percentage/proportion; Statistical assessment by means of the Chi squared test or the Fisher’s exact test, as appropriate
* Length of hospital stay: reported as a mean ± SD or median and IQR, as appropriate; Statistical assessment by means of student’s t test or Mann-Whitney U test, as appropriate
* Choice of diagnostic tests to establish the diagnosis of CAPA will be reported as percentage/proportion
* Choice of empirical and targeted therapy and duration of this therapy will be reported as percentage/proportion and mean ± SD or median and IQR, as appropriate, respectively
* In case of development of CAPA, the time to CAPA diagnosis: reported as a mean ± SD or median and IQR, as appropriate; Statistical assessment by means of student’s t test or Mann-Whitney U test, as appropriate

## 6.3 Other study parameters

Other study parameters, as described in “5.1.3. Other study parameters” of all subjects will be included in the study report.

Descriptive statistics will be performed on these subject characteristics. Depending on the variable being assessed, statistical analysis by means of the Chi squared test, the Fisher’s exact test, student’s t test or Mann-Whitney U test will be performed, as appropriate.

# 7. ETHICAL CONSIDERATIONS

## 7.1 Regulation statement

The study will be conducted in compliance with the principles of the Declaration of Helsinki (version 10, 19th October 2013) and the principles of Good Clinical Practice (GCP). Due to its observation nature, the Medical Research Involving Human Subjects Act (WMO) is not applicable to this study.

Before the start of the study, this protocol and other related documents will be submitted for review to the Committee on Research Involving Human Subjects (CMO).

Any subsequent protocol amendments will be submitted to the CMO for approval. No substantial amendment that requires review by the CMO will be implemented until the CMO grants a favourable opinion for the study.

The coordinating investigator (CI) is responsible for ensuring that the study is performed in accordance with the protocol, current ICH guidelines on GCP, and applicable regulatory and country-specific requirements. GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial participants are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical trial data are credible.

Investigators based in other countries than the Netherlands are required and expected to conform to local legislation and regulation regarding medical research involving human subjects prior to participation in this study.

## 7.2 Recruitment and consent

Due to the observational nature of this study, participants will not be subjected to any procedures, nor will the care they receive be influenced by enrolment in this trial. Therefore, the WMO is not applicable to this study. Because of this and to ensure efficient progress of this study, written informed consent from participants is not required before enrolment. However, an opt-out system will be employed, where patients’ medical data will not be used in this study only when the participant, their relatives and/or their legal representatives object to this use of their (encoded) medical data. Naturally, attempts to actively inform patients, their relatives and/or legal representatives and acquiring their (oral) informed consent will be attempted, as far as reasonably possible.

Data shall be provided by the local investigator involved in the clinical care of the participant in encoded form in electronic CRFs, using Castor Electronic Data Capture (EDC) as a data management system, ensuring that the data collected are not directly traceable to the individual participant.

# 8. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

## 8.1 Handling and storage of data and documents

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented. These documents should be classified into 2 separate categories: (1) investigator’s study file, and (2) subject clinical source documents.

The investigator’s study file will contain the protocol/amendments, CRF and query forms, METC and governmental approval with correspondence and other appropriate documents and correspondence.

Subject clinical source documents would, for example, include subject hospital/clinical records, physician’s and nurse’s notes, appointment book, original laboratory reports, ECG, radiological study, pathology and special assessment reports, consultant letters, screening and enrolment log, etc..

All clinical study documents must be retained by the investigator for at least 15 years. The investigator must be notified prior to destroying any clinical study records.

For each subject enrolled, a CRF must be completed and approved by the principal investigator or co-/sub-investigator within a reasonable time period after data collection. If a subject withdraws from the study, the reason must be noted on the CRF.

The sponsor should ensure that the investigator has control of and continuous access to the CRF data reported to the sponsor. The sponsor should not have exclusive control of those data. When a copy is used to replace an original document (e.g. source documents, CRF), the copy should fulfil the requirements for certified copies. The investigator/institution should have control of all essential documents and records generated by the investigator/institution before, during and after the trial.

The sponsor is responsible for archiving trial specific documentation (such as, but not limited to, the protocol, potential amendments, copy of (e)CRF burned on CD, final report and database) for at least twenty years. Site-specific trial documents will be archived locally on site according to local practice and guidelines. Archived data may be held on electronic record, provided that a back-up exists and that hard copies can be obtained, if required. Destruction of essential documents will require authorisation from the sponsor.

The investigator must assure that subjects’ anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only a subject identification code should be recorded on any form submitted to the sponsor and ethics boards. The investigator must keep a screening log showing codes for all subjects screened and for all subjects enrolled in the study.

This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from the investigator. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

The medical record and other source documents are only accessible by the medical staff of the clinical research centre and the investigator. CRFs do not contain identifiable information and will be coded with identification codes only. The identification key will be accessible to the medical of the clinical research centre and the local investigator only. Information and study files that are necessary for the evaluation of the research are stored anonymously and the identification key will not be accessible by unauthorized parties.

The data management system that will be used for the handling and storage of data in this study is Castor Electronic Data Capture (EDC), in accordance with the standard procedures employed by the Clinical Research Center Nijmegen (CRCN).

## 8.2 Monitoring and Quality Assurance

Not applicable.

## 8.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

## 8.4 Annual progress report

Not applicable.

## 8.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient’s last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.  
  
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

## 8.6 Public disclosure and publication policy

Publications will be coordinated by the investigator of sponsor. Authorship of publications will be determined in accordance with the requirements published by the International Committee of Medical Journal Editors and in accordance with the requirements of the respective medical journal.

For multicentre trials, it is anticipated that the results of the overall trial shall be published in a multicentre publication, involving the data of all clinical sites participating in the trial.

Participating sites are not allowed to publish any data or results from the trial prior to the multicentre publication, provided however that participating sites are allowed to publish the results generated at the participating site if a multicentre paper has not been written after 12 months from trial database lock.

Any publication by participating sites will be submitted to the sponsor for review at least 30 days prior to submission or disclosure. The sponsor shall have the right to delay the projected publication for a period of up to 3 months from the date of first submission to the sponsor in order to enable the sponsor to take steps to protect its intellectual property rights and know-how.

# 

# REFERENCES

1. Marr KA, et al. Aspergillosis complicating severe coronavirus disease. Emerg Infect Dis. 2020;27(1).
2. Koehler P, et al. Defining and managing COVID-19 associated pulmonary aspergillosis: The 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. Lancet Infect Dis 2020; (in press).
3. van Arkel ALE, et al. COVID-19-associated pulmonary aspergillosis. Am J Respir Crit Care Med. 2020;202:132-5.
4. White PL, et al. A national strategy to diagnose COVID-19 associated invasive fungal disease in the ICU. Clin Infect Dis. 2020 Aug 29:ciaa1298.
5. Bartoletti M, et al. Epidemiology of invasive pulmonary aspergillosis among COVID-19 intubated patients: a prospective study. Clin Infect Dis. 2020 Jul 28:ciaa1065.
6. Schauwvlieghe AFAD, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. Lancet Respir Med 2018;6:782-92.
7. Nyga R, et al. Invasive tracheobronchial aspergillosis in critically ill patients with severe influenza. A clinical trial. Am J Respir Crit Care Med. 2020;202:708-16.
8. Meijer EFJ, et al. Azole-resistant COVID-19-associated pulmonary aspergillosis in an immunocompetent host: A case report. J Fungi 2020;6:79.