Posaconazole for the Prevention of COVID-19 Associated Pulmonary Aspergillosis in Critically-Ill Patients: A European Multicenter Case-Control Study

(NCT05065658)

The study titled *"Posaconazole for the Prevention of COVID-19 Associated Pulmonary Aspergillosis in Critically-Ill Patients: A European Multicenter Case-Control Study" (*NCT05065658) was conducted as a sub-study within the protocol of a larger prospective, multicenter trial including amendments of the local ethic committees. The overarching trial, titled *"Pulmonary Aspergillosis in Critically Ill COVID-19 Patients in Intensive Care Units – A Multinational Explorative Trial"* provided the framework for exploring key outcomes related to pulmonary aspergillosis in critically ill COVID-19 patients. The sub-study leveraged data collected within the parent study to specifically assess the role of posaconazole in preventing COVID-19-associated pulmonary aspergillosis. This design ensured consistency and robust collaboration across the two investigations.

The study protocol of the multicenter, prospective trial *“"Pulmonary Aspergillosis in Critically Ill COVID-19 Patients in Intensive Care Units – A Multinational Explorative Trial"* can be found below.

Pulmonary Aspergillosis in Critically Ill COVID19 Patients in Intensive Care Units – a multinational explorative trial

Running title:

Aspergillosis in COVID19 on ICU

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Abstract

In December 2019, a novel coronavirus named SARS-CoV-2 spread throughout the world and is causing a global pandemic. In contrast to many other ordinary respiratory viruses, a significant proportion of patients diagnosed with SARS-CoV-2 infections need to be hospitalized or even admitted to intensive care units (ICUs). We know that patients with severe influenza infection are at a higher risk of developing invasive pulmonary aspergillosis (IPA). First reports of IPA have also emerged among SARS-CoV-2 infected patients admitted to the ICU. We hypothesize that patients with severe SARS-CoV-2 infection requiring ICU-level care are also at a higher risk of developing IPA.

The main objective of this explorative trial is to screen patients retrospectively based on routinely performed investigations for the presence of IPA. In addition, remainders of routinely performed bronchoalveolar lavage fluid (BALF) samples and endotracheal aspirates will be collected and stored for retrospective biomarker testing. Left-over blood samples (plasma, serum, and urine) will also be collected and stored for the measurement of fungal biomarkers, soluble urokinase plasminogen activator receptor and interleukins 6 and 8, as well as urine biomarkers for IPA including Triacetylfusarine C (Innsbruck Medical University). In addition, the respiratory tract microbiome will be analyzed in a subset of patients (University of Manchester).

No procedures or interventions beyond those done as a part of routine clinical care will be performed. All tests will be performed in samples left over from clinical routine. Data will be collected in an anonymized fashion in an electronical case report form.

Background

Infections with human coronavirus are common, especially among the cold weather season. They are responsible for approximately 10 – 15% of common colds in the winter time (1). However, within the last 20 years there have been three relevant outbreaks with highly pathogenic coronavirus leading to a large number of infected patients and a significant number of deaths. The outbreak of the severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) in 2002 infected more than 8000 people in 28 countries, with a case fatality rate of 9.8% (2).
In 2012 another outbreak with a highly pathogenic coronavirus started on the Arabian Peninsula. The middle east respiratory syndrome coronavirus (MERS-CoV) has been infecting over 2400 people since 2012, with a case fatality rate of approximately 35% (3). However, none of these two highly pathogenic coronavirus infections caused a global pandemic.

In December 2019, an outbreak with a novel coronavirus started in China. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has now caused a pandemic and has been infecting at least 2.8 million people in 185 countries (up to 27-APR-2020, source: WHO). As up to 27-APR-2020 more than 195 000 people around the world died due to coronavirus disease 2019 (COVID19). Although the vast majority of COVID19 cases are mild to moderate (approximately 80%), approximately 15% of patients diagnosed with COVID19 require hospitalization and supplementary oxygen, and 5% of patients develop critical disease with acute respiratory distress syndrome (ARDS) and require ICU admission and mechanical ventilation (4).

ARDS may also be a consequence of infections with other respiratory virus, like influenza. Recently, it was shown that patients with influenza-associated ARDS are at a higher risk for developing pulmonary mold infections, primarily from IPA. In large multicenter trial the prevalence of pulmonary aspergillosis among patients with influenza associated ARDS was 19% (5). As patients with influenza associated aspergillosis have a mortality rate of over 50% (5), mold active antifungal prophylaxis is a standard of care in many centers for these patients. From Wuhan, epidemiological studies indicate that invasive fungal infections may occur in between 4-5% of COVID-19 episodes requiring ICU admission (6,7). Given that in Wuhan galactomannan testing is rarely available and fungal diagnostics are sparse, this is likely an underestimate of the real burden of IPA in patients with COVID-19 requiring ICU admission.

Diagnosis of IPA is based on patients risk factors (e.g. severe influenza), imaging studies (chest X-ray, chest computed tomography) and microbiological investigations, like culture from bronchoalveolar lavage fluid (BALF), galactomannan (GM), aspergillus lateral-flow device (LFD), galactomannan lateral-flow assay (LFA), Triacetylfusarine-C (TAF-c) or aspergillus PCR (8).

The aims of this study are to determine the prevalence of IPA in COVID19 patients in the ICU and to determine the diagnostic performance of BALF fungal biomarkers for diagnosis of IPA.

The study consists of three different sub-studies:

Base module “A”: Epidemiological study (ICU patients only)

Add on Aim “B”: Biomarker study (ICU patients only)

Add on Aim “C”: Microbiota study (ICU patients and patients on general wards)

All participating centers must participate in Base module “A” plus at least one of the two add on modules (B or C or both).

Objectives

Aims “A”

Primary: Provide necessary data to interpret laboratory findings for Module B and/or C

Secondary: Determine the prevalence of IPA in patients with critical COVID19 in intensive care units

Aims “B”

The objectives of the biomarker (“B”) sub study are as following:

1.) Determine diagnostic performance of BALF-GM for IPA in COVID19

2.) Determine diagnostic performance of BALF-LFD for IPA in COVID19

3.) Determine diagnostic performance of BALF-LFA for IPA in COVID19

4.) Determine diagnostic performance of BALF Aspergillus PCR in COVID19

5.) Determine performance of urine biomarkers for IPA in COVID19

6.) Determine radiological findings on chest imaging in COVID19 patients with and without IPA

7.) Outcome of COVID19 patients on ICU with and without IPA

8.) Length of ICU stay in COVID19 patients with and without IPA

9.) Prognostic potential of plasma and BALF soluble urokinase plasminogen activator receptor (suPAR), IL-6, and IL-8 in COVID19 patients with and without IPA (single measurements and kinetics)

Aim “C”

The primary objective of the microbiota sub study is to determine diagnostic performance of mycobiome tests in COVID-19 patients.

Study population

There is no pre-defined upper limit of patients that may be included in this study by the participating centers. The total number of patients will depend on the course of the COVID19 pandemic within the next months.

Inclusion criteria

Inclusion criteria required for all sub studies

1.) Patient has PCR confirmed SARS-CoV-2 infection

Additional inclusion criteria for biomarker study "B"

1.) Bronchoscopy or endotracheal aspiration is performed during routine clinical work (indicated by the treating physician only – no intervention for study purpose only)

2.) Chest imaging (e.g. CT chest scan or chest x-ray) available +/- 7 days of bronchoscopy/endotracheal aspiration

Additional inclusion criteria for microbiota study "C"

1.) Bronchoscopy or endotracheal aspiration is performed during routine clinical work (indicated by the treating physician only – no intervention for study purpose only) in ICU patients **OR** patients are willing to give sputum samples if they are on general wards after signing an informed consent

2.) Chest imaging (e.g. CT chest scan or chest x-ray) available +/- 7 days of bronchoscopy/endotracheal aspiration

Exclusion criteria

1.) <18 years of age

Material, Methods and Study Period

Specimen for Biomarker Testing (Aim "B")

Respiratory specimen from patients fulfilling the inclusion criteria (BALF or endotracheal aspirate), obtained at ICU admission and any available follow up samples obtained during the ICU stay will be collected, aliquoted (up to three aliquots each at least 1ml specimen) and stored at -80°C. In case remaining samples volume is less than 1ml, addition of sterile NaCl 0.9% is necessary to obtain at least one aliquot containing at least 1 ml of respiratory specimen. Highly viscid samples may be diluted with NaCl 0.9% to enable further work up. Liquifying agents, such as Suptasol® (=dithiothreitol) must not be used as they significantly affect results of biomarker testing, such as galactomannan. If available at the participating site, respiratory samples should be cultured on sabouraud-dextrose-agar and sent to the Innsbruck Medical University (Prof. Lass-Floerl) for further analysis.

Left-over blood samples (plasma and serum) from routinely performed blood drawings at the time of the respiratory sample and during the course of ICU admission (at the day of ICU admission, followed by 1 – 3 times per week, depending on the local screening strategies), will be stored at -80°C for retrospective suPAR, IL-6, and IL-8 testing.

Urine samples will be collected 1 – 3 times a week (on same day as blood samples). Total samples volume from each visit should be 10 ml of urine. Samples should be divided in aliquots, with a total volume of 10 ml for each visite and stored at -80°C without any pretreatment.

After the recruiting phase of the study the following parameters will be tested from the obtained specimens and samples:

1.) Galactomannan ELISA from BALF or endotracheal aspiration and serum

2.) 1,3-ß-D-Glucan from serum

3.) Aspergillus lateral-flow device from BALF or endotracheal aspiration and serum

4.) Galactomannan lateral-flow assay from BALF or endotracheal aspiration and serum

5.) Aspergillus PCR from BALF or endotracheal aspiration

6.) suPAR, IL-6, and IL-8 from BALF and plasma samples

7.) GM, triacetylfusarinine C from urine samples

In case any of these parameters are performed during routine clinical work, they will not be determined a second time from stored samples. All laboratory tests performed for study purposes will be done anonymized. Only left-over samples obtained during routine clinical work

will be collected for this study. No intervention to the patients will be performed for study purposes only.

Specimen for Mycobiome Study (Aim "C")

In a subset of patients, lower-respiratory tract samples (BAL and endotracheal aspiration) or sputum samples from patients on ICU and sputum samples from COVID-19 patients on general wards will be collected and processed according to the attachment "Respiratory sample collection for sub-study C".

Respiratory samples will be collected and inactivated either by adding an inactivating agent (containing >0.3% TnBP and 0.1% Triton X100 or Tween 80) or by heat inactivation by heating the samples to 65°C for 15 minutes. A sample volume of at least 0.5 ml is required, but a sample volume of >1mL would be optimal. Samples will then be stored at the participating center and sent to Prof. Paul Bowyer from the University of Manchester in batches on dry ice.

Study Period

Inclusion of patients may start as soon as possible in the participating centers. The end of the study period is currently open. It will highly depend on the course of the pandemic within the next months. Patients may be included in the study until 31st-DEC-2020.

Case Report Form

For data acquisition and storage FungiScope® Registries will be used. The anonymized electronic case report form will be accessible through www.clinicalsurveys.net. ClinicalSurveys.net employs a customized version of Questback's internationally acclaimed EFS Survey® and EFS Leadership® technology to provide the user with an easy-to-use online documentation system. Following data will be obtained in the CRF:

1.) Date of SARS-CoV-2 diagnosis (=date of positive PCR)

2.) Duration of ICU stay

3.) Results from routinely performed microbiological investigations (BALF culture, biomarkers, etc.)

4.) Routine laboratory biomarkers (Galactomannan, BDG, etc.)

5.) Chest imaging results +/- 7 days of bronchoscopy/endotracheal aspiration

6.) Host factors and genetic and other risk factors for IPA

7.) Antifungal treatment

8.) Outcome data

9.) Cause of death (if applicable)

The FungiScope® Registries are in accordance with all applicable laws and regulations including the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP) the ethical principles that have their origins in the Declaration of Helsinki (current official version: Fortaleza, 2013), and applicable privacy laws (Regulation (EU) 2016/679). All Good Epidemiological Practice (GEP) requirements are met by the registry. Users can only view and modify their own contributions. All data transmissions are encrypted via TLS 1.2 with an AES 256 GCM bit key and ECDHE RSA key exchange; certificate is provided by COMODO RSA Domain Validation Server. Data are only documented anonymously; no directly identifying data other than the investigator names and email addresses are stored on Questback servers. Administration of the eCRF is limited to selected and named administrators at the University Hospital Cologne. Any data manipulation by users and administrators is logged in an audit trail allowing complete data reconstruction. The platform has been extensively used in hundreds of surveys and studies and has received approval by the responsible data protection officers at the University Hospital Cologne.

Criteria for defining aspergillosis

Invasive aspergillosis will be classified according to the scheme published by Blot et al. (9) with some modifications:

1.) COVID19 requiring ICU admission was added as host factor

2.) Tracheal aspiration was equaled to BALF in terms of microbiological testings'

3.) Serum and BALF GM was added as entry criterion

The modified diagnostic criteria are displayed in table 1.

Table 1 Modified diagnostic criteria for invasive aspergillosis

Proven invasive aspergillosis
- Idem to the EORTC/MSG criteria
Putative invasive aspergillosis (all four criteria must be met)
1.) *Aspergillus* recovery from BAL or endotracheal aspirate or positive galactomannan [either serum (>0.5 ODI) of respiratory sample (>1 ODI)]
2.) Compatible signs and symptoms - Fever refractory to at least 3 d of appropriate antibiotic therapy - Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause - Pleuritic chest pain - Pleuritic rub - Dyspnea - Hemoptysis - Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support
3.) Abnormal medical imaging by portable chest X-ray or CT scan of the lungs
4.) Either 4a or 4b 4.a) Host risk factors (one of the following conditions) - Patient with COVID19 requiring ICU admission - Neutropenia (absolute neutrophil count <500/mm3) preceding or at the time of ICU admission - Underlying hematological or oncological malignancy treated with cytotoxic agents - Glucocorticoid treatment (prednisone equivalent, >20 mg/d) - Congenital or acquired immunodeficiency 4.b) Semiquantitative *Aspergillus*-positive culture of BAL fluid (+ or ++), without bacterial growth together with a positive cytological smear showing branching hyphae
When ≥1 criterion necessary for a diagnosis of putative IPA is not met, the case is classified as *Aspergillus* colonization.
Abbreviations: BAL = bronchoalveolar lavage; CT = computed tomography; EORTC/MSG = European Organization for the Research and Treatment of Cancer/Mycosis Study Group; ICU = intensive care unit.

References

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ECMM *Aspergillus* in COVID-19

European Confederation of Medical Mycology ECMM

The Focus of this study is to evaluate Diagnostics, Biomarkers and Mycobiome findings in COVID19 patients with and without Invasive Aspergillosis

The study consists of a base module and two different sub-studies:

Base module “A”: Epidemiological study (ICU patients only)

Add on Aim “B”: Biomarker study (ICU patients only)

Add on Aim “C”: Microbiota study (ICU patients and patients on general wards)

All participating centers must participate in Base module “A” **plus** at least one of the two add on modules (B or C or both).

Base module "A": Epidemiological study (ICU patients only)

Aims: **Primary:** Provide necessary data to interpret laboratory findings for Module B and/or C
Secondary: Determine the prevalence of IPA in patients with critical COVID19 in intensive care units

Inclusion criteria: Patient has PCR confirmed SARS-CoV-2 infection and admitted to ICU

Required tasks for this sub-study:

- Enter you cases in the case report form (www.clinicalsurveys.net).
    - Go to www.clinicalsurveys.net → go to survey list → select the study "ECMM *Aspergillus* in COVID-19" → enter your case
    - For getting access to www.clinicalsurveys.net please contact jon.salmanton-garcia@uk-koeln.de
    - In case you need assistance regarding documentation or problems with the questionnaire please contact Jon Salmanton-Garcia (jon.salmanton-garcia@uk-koeln.de)

Note:
We encourage you to enter the cases in a timely manner, as you need to label the samples collected for Aim "B" and/or Aim "C" for storage. The labeling need to be addressed in the eCRF.

Aim "B": Biomarker study (ICU patients only)

Aim: Determine diagnostic and prognostic performance of different biomarkers in blood, respiratory samples and urine

Inclusion criteria: See Aim "A" plus routinely performed blood, urine and respiratory tract specimen sampling

Required tasks for this sub-study:

- Collecting left-over blood samples, urine and respiratory tract samples from routine clinical work-up
- Sampling schedule:
    - **Blood** (left-over samples only): Plasma and serum at the day of ICU admission, followed by 1 - 3 times per week. Blood samples need to be aliquoted in up to 3 aliquots each containing at least 500 µL.
    - **Urine**: Sampling at the day of ICU admission, followed by 1 - 3 times per week (on the days of blood sampling). Samples should be divided in aliquots, with a total volume of 10 mL for each visit.
    - **Respiratory tract samples**: BALF or tracheal aspirate whenever collected during clinical routine. Divide in up to 3 aliquots, each containing at least 1 mL. In case remaining samples volume is less than 1 ml, addition of sterile NaCl 0.9% is necessary to obtain at least one aliquot containing at least 1 mL of respiratory specimen. Highly viscid samples may be diluted with NaCl 0.9% to enable further work up.

Note:
All samples should be stored at -80°C without any pretreatment at the participating center. For low volume respiratory tract samples or highly viscous samples please see above. Don't use liquefying agents such as Sputasol® for this part of the study. For labeling of the samples, see the attachment "*Sample labeling*".

Aim "C": Microbiota study (ICU patients and patients on general wards)

Aim: Determine diagnostic performance of mycobiome tests in COVID-19 patients

Inclusion criteria: See Aim "A" plus routinely performed BAL or tracheal aspiration in ICU patients OR patients are willing to give sputum samples if they are on general wards after signing an informed consent

Required tasks for this sub-study:

- Collecting BAL or tracheal aspiration in ICU patients and/or sputum samples (once) from patients on general wards

Respiratory sample processing for sub-study "C":

Respiratory samples will be collected and by heating the samples to 80°C for 40 minutes. A sample volume of at least 0.5 ml is required, but a sample volume of >1mL would be optimal. Samples will then be stored at the participating center and sent to Prof. Paul Bowyer from the University of Manchester in batches on dry ice.

- Also see attachment "COVID-19 Sample Inactivation v5.1""

Sample labeling for biomarker study "B"

All samples collected within this study are supposed to be stored at -80°C at the participating centers and sent to the different labs in batches. We will introduce you where and when to send the samples as required.

Label samples as shown here:

"*CenterID_PatientID_Specimen_Day*"

CenterID: every participating center will receive a two digit ID that you will receive on the first page of the questionnaire on clinicalsurveys.com

PatientID: patients should be coded by consecutive two digit numbers, starting with 01 for the first included patient at each site

Specimen: should be coded as following:

- Bronchoalveolar lavage fluid = BAL
- Tracheal Aspirate = TA
- Sputum = SP
- Plasma = PL
- Serum = SE
- Urine = UR

Day: indicates the day after the first sample for this study was drawn, with day 0 being the first day were an study sample was drawn and stored (regardless of the specimen).

Examples:

"01_05_BAL_6" = "Graz/AUT_Patient 5 from Graz_Bronchoalveolar lavage fluid_sampled on day 6 after first sample (e.g. urine) was drawn.

"01_05_PL_0" = "Graz/AUT_Patient 5 from Graz_Plasma_sampled on day 0 after first sample (e.g. urine) was drawn – in this case Plasma was the first sample drawn for the study.