

# **PIRAIA-study**

**(Prevalence of Influenza RelATed Invasive Aspergillosis)**

**A Multicenter Prospective Observational Cohort Study**

## **Study Protocol**

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## 1. STATEMENT OF COMPLIANCE

The signature below constitutes that this study will be comply with the requirements of Good Clinical Practice (ICH E6) and the Swedish Ethical Review Authority.

Principal Investigator

Signed:

Date:

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## 2. ABBREVIATIONS

ARDS	Acute Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
CI	Confidence Interval
CRF	Case Report Form
eCRF	electronic Case Report Form
CT	Computed Tomography
EORTC	European Organization for Research and Treatment of Cancer
GM	Galactomannan
GCP	Good Clinical Practice
ICU	Intensive Care Unit
IPA	Invasive Pulmonary Aspergillosis
MSG	Mycoses Study Group
PCR	Polymerase Chain Reaction
SAPS3	Simplified Acute Physiology Score
SOFA	Sequential Organ Failure Assessment

### 3. PROTOCOL SUMMARY

- Title:** Prevalence of Influenza Related Invasive Aspergillosis; A Multicenter Prospective Observational Cohort Study
- Overview:** A multicenter prospective observational study conducted at 12 intensive care units in Sweden. All patients >18 years of age with a PCR-verified influenza A or B diagnosed up to 7 days before admission to the ICU or during ICU care, will be included in the study. A new clinical routine will be implemented: During the ICU stay screening with Beta-D-glucan and Galactomannan in blood/serum will be performed twice weekly and a respiratory sample will be retrieved for fungal culture and microscopy once weekly. All results from collected samples will be available to the patient responsible physicians. Clinical and microbiological data will be collected, and the diagnosis of invasive aspergillosis will be made using predefined diagnostic criteria.
- Objectives:**
1. To determine the prevalence of influenza-related invasive pulmonary aspergillosis in Swedish intensive care units
  2. To assess the clinical impact of and risk factors for influenzarelated invasive pulmonary aspergillosis
- Population:**  $\geq 18$  years of age. Confirmed influenza A or B diagnosed <7 days before admission to the ICU or during ICU stay. At least 50 patients will be enrolled in the study.
- Number of sites:** 12
- Study duration:** 1 year
- Enrolment:** Until the end of April 2020 if  $\geq 50$  patients have been enrolled. Enrolment will continue until  $\geq 50$  patients have been recruited.

#### 4. KEY ROLES AND CONTACT INFORMATION

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## 5. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

### 5.1 Background

Bacterial pneumonia is a well-known complication of influenza infection and contributes to both increased morbidity and mortality. A less known complication is a fungal infection with aspergillus species. Aspergillus is omnipresent and is commonly found in soil, dust, and food. We regularly inhale Aspergillus spores, but our immune system effectively takes care of the fungus, why disease does not occur [2]. Invasive pulmonary aspergillosis (IPA) has typically only been considered in immunosuppressed patients such as in haematological malignancies, hematopoietic stem cell transplantation, organ transplantation, or prolonged neutropenia [3]. Isolation of Aspergillus in respiratory samples from patients without these risk factors has been interpreted as colonization without any clinical relevance.

In recent years, several reports of influenza-related IPA in patients lacking classical risk factors have been published [4,5]. A possible explanation could be a viral injury to the respiratory epithelium as well as local and systemic lymphopenia [6]. The prevalence of IPA in influenza patients admitted to the ICU has been reported to be upwards of 23% [4]. In a large retrospective cohort study from the Netherlands and Belgium, published in The Lancet in October 2018, the prevalence of IPA was 19% among the 432 influenza patients admitted to the ICU [10]. The mortality was significantly higher among patients diagnosed with IPA. Most patients with influenza admitted to ICU lack classical risk factors for IPA and have, therefore, not been routinely investigated for IPA.

Aspergillus infections are challenging to diagnose and require specific culture media or the use of indirect diagnostic methods such as detection of Galactomannan in serum or BAL and detection of Beta-D-Glucan in serum. However, microbiological tests cannot distinguish between colonization and infection, which is why internationally recognized criteria that combine host factors, radiological signs, and microbiological findings have been published [7]. The infection is graded being confirmed, probable, or possible IPA. The criteria were originally developed for immunosuppressed patients, but modified criteria that are useful in the ICU has been published [8].

Effective treatment for Aspergillus is available and must be started promptly in cases of suspected IPA. If left untreated, IPA is associated with significant mortality.

The reports of influenza-related IPA have led the Netherland national guidelines to recommend that all influenza patients admitted to the ICU should undergo bronchoscopy with BAL sampling for Aspergillus diagnostics [9]. In Sweden, 300-400 influenza patients are annually admitted to intensive care units. Swedish data on the prevalence of aspergillosis are lacking, and there is a risk that IPA remains both an unknown and underdiagnosed complication of influenza infection in Swedish intensive care units.

## **5.2 Scientific Rationale**

IPA is associated with significant mortality, and routine tests are often incapable of diagnosing the infection. The infection may, therefore, remain undetected without specific testing aimed to diagnose IPA. Reports of influenza-related IPA has emerged from several parts of the world. Still, the prevalence cannot be assumed to be equal in different settings as the transmission is dependent on exogenous exposure.

By accounts of the accumulated evidence, a new clinical routine will be introduced at study centers; Patients  $\geq 18$  years of age admitted to ICU with PCR-verified influenza A or B will be routinely tested to diagnose IPA early. The study will collect clinical and microbiological data of the patients subject to this new clinical routine. If the study provides evidence of IPA prevalence similar to the published reports, the clinical importance would be significant. New national guidelines regarding testing of influenza patients admitted to the ICU should be created with the potential to save both life and reduce suffering.

## **5.3 Potential Risks and Benefits**

IPA testing is introduced as a clinical routine and is not part of the study. The study is a prospective observational cohort study where clinical and microbiological data of patients subjected to the new clinical routine will be collected and assessed. Study participants admitted to any of study centers will receive the benefit of routine IPA testing and the possibility of early intervention if IPA is diagnosed.

## **6. STUDY OBJECTIVES**

### **6.1 Primary objective**

To determine the prevalence of influenza-related invasive pulmonary aspergillosis in Swedish intensive care units.

### **6.2 Secondary objective**

To assess the clinical impact and risk factors related to influenza-related invasive pulmonary aspergillosis. Patients with proven or probable IPA will be compared to patients that do not meet the criteria for proven and probable IPA. Baseline characteristics and clinical data such as SAPS3, SOFA score, need for invasive ventilation and need for continuous renal replacement therapy, will be assessed to find risk factors for IPA.



## **7. STUDY DESIGN**

### **7.1 Study overview**

The study is a Swedish, nationwide, multicenter, prospective observational cohort study to determine the prevalence of IPA among patients admitted to the ICU with confirmed influenza infection.

A new clinical routine regarding IPA-testing will be implemented at the participating centers, similar to the current guidelines in the Netherlands. All samples will be retrieved and analyzed using local methods and logistics. All test results will be available to the patient responsible physician, who is free to act on them as deemed appropriate.

The study is non-interventional, no measurements or sampling will be made mandatory by the protocol and data collection at these pre-specified time-points will only take place if decided acceptable by the patient responsible physician. All aspergillus testing is part of the new clinical routine, and no additional sampling will be performed on accounts of the study.

All patients  $\geq 18$  years of age with confirmed influenza A or B diagnosed up to 7 days before ICU admission, or during ICU care, will be included in the study. Patient consent was waived in the ethics approval from *the Swedish Ethical Review Authority* (Dnr 201900557).

Recruitment is planned to begin in December 2019. Recruitment of patients is expected to stop in April 2020 if  $\geq 50$  patients have been recruited, or after that when 50 patients have been recruited.

### **7.2 Study inclusion**

At least 50 patients will be enrolled in the study. The target sample size has been calculated using the expected prevalence of 15% IPA. The sample size is then sufficient to describe the prevalence with 10 % margin of error at the 95% confidence level. Approximately 100 influenza patients are admitted to ICUs of the participating centers every influenza season.

### **7.3 Inclusion criteria**

Patients must meet all the inclusion criteria to be eligible for the study:  $\geq 18$  years of age, PCR-verified influenza A or B diagnosed within 7 days of ICU admission, or during ICU stay.

#### **7.4 Exclusion Criteria**

Patients  $\leq 18$  years of age cannot be enrolled in the study as the evidence motivating the implemented routine IPA testing does not contain patients under 18 years of age.

#### **7.5 Study locations**

ICU (CIVA) at Karolinska Universitetssjukhuset Solna, Stockholm  
ICU at Karolinska Universitetssjukhuset Huddinge, Stockholm  
ICU at Danderyds sjukhus, Stockholm  
ICU (CIVA) at Akademiska sjukhuset, Uppsala  
ICU at Universitetssjukhuset, Linköping  
ICU, at Västmanlands sjukhus, Västerås  
ICU at Mälarsjukhuset, Eskilstuna  
ICU at Universitetssjukhuset, Örebro  
ICU at Länssjukhuset Ryhov, Jönköping  
ICU at Skånes universitetssjukhus, Malmö  
ICU at Skånes universitetssjukhus, Lund  
ICU at Helsingborgs lasarett

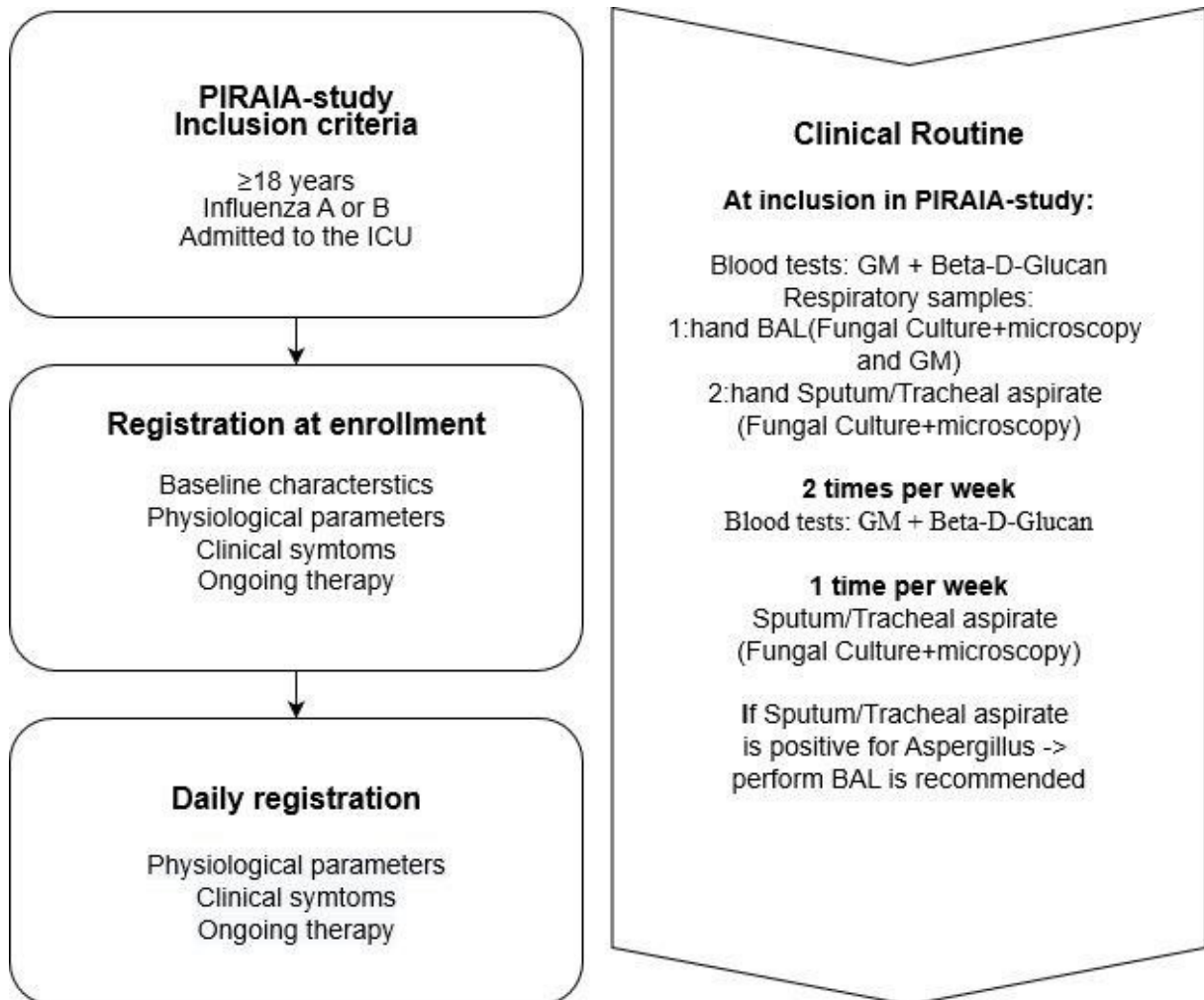
#### **7.6 Screening**

Influenza testing will be performed on the basis of clinical suspicion, and part of routine care.

#### **7.7 Informed consent**

This is a non-interventional study and the need for informed consent was waived in the approval from the Swedish Ethical Review Authority (DNR (Dnr 201900557))

## 8. WORKFLOW: OVERVIEW



## 9. DATA COLLECTION

All patient responsible physicians in the participating ICUs will be eligible to enrol patients in the study. All participating ICUs will receive both written and oral instructions about the study from the study site investigator. Data will be obtained from the participant's medical records and initially entered on a paper CRF. The empty CRF will be made available by sending prepared folders to the study sites. Additional CRFs will be made available for print from study website ([piraiastudy.se](http://piraiastudy.se)). Study site investigators will then enter the collected data into a web-based eCRF. The procedure will ensure continuous monitoring of the collected data.

### 9.1 Data to be collected Baseline

#### characteristics

Date of ICU admission

Age

Sex

SAPS3-score on arrival to the ICU SOFA-

score on arrival to the ICU Presence of:

Liver cirrhosis (defined as Child-Pugh-score  $\geq$ A)

Heart failure (defined as ejection fraction  $<$ 40%)

Diabetes mellitus (not diet treated)

Renal failure (defined as creatinine clearance  $<$ 30 mL/min/1.73m<sup>2</sup>)

Body Mass Index  $>$ 30 kg/m<sup>2</sup>

Haematological malignancy

Hematopoietic stem cell transplantation

Organ transplantation

Daily smoking in the last year

Solid tumour

Neutropenia (defined as  $<$ 0,5 x10<sup>9</sup> neutrophils)

Chronic obstructive pulmonary disease

New lung infiltrate

Treatment with corticosteroids in the last 28 days

Treatment with corticosteroids in a dose equal to or above 15 mg/day prednisone

#### At enrolment

Date of enrolment

Influenza A or B

Treatment with oseltamivir or zanamivir

SOFA-score the day of enrolment

Use of vasopressor

Use of continuous renal replacement therapy

Use of invasive ventilation

Use of non-invasive ventilation  
Body temperature above 38.0 degrees Celsius in the last 24 hours  
ARDS grade (defined by PaO<sub>2</sub>/FiO<sub>2</sub> 300-200 mmHg mild, 200-100 mmHg moderate, <100 mmHg severe) Treatment with corticosteroids  
Presence of haemoptysis  
Presence of pleural pain  
Ongoing treatment with antibiotics  
Ongoing treatment with antifungals

#### **Daily registration after enrolment**

SOFA-score  
Use of vasopressor  
Use of continuous renal replacement therapy  
Use of invasive ventilation  
Use of non-invasive ventilation  
Body temperature above 38.0 degrees Celsius in the last 24 hours ARDS grade (defined by PaO<sub>2</sub>/FiO<sub>2</sub> 300-200 mmHg = mild, 200-100 mmHg = moderate, <100 mmHg = severe)  
Treatment with corticosteroids  
Presence of haemoptysis  
Presence of pleural pain  
Ongoing treatment with antibiotics  
Ongoing treatment with antifungals

#### **At discharge from the ICU**

Date of discharge from the ICU  
Discharge to (home, ward, other ICU or death)  
Treatment with systemic antifungals during the care time in the ICU  
Microbiological findings: Positive Aspergillus culture or microscopy, GM in BAL or serum or Beta-D-Glucan.

#### **Medical records**

Medical records will be reviewed for information on microbiological findings and survival up to 90 days from ICU admission.

## **9.2 Confidentiality**

Study participants will be assigned a unique study identification number (e.g., LU01 - first patient in Lund). CRF will be pseudo-anonymized using the identification number. Each study site will receive a "code list" where the personal number of the study participant will be linked to the study identification number. Study site investigators are responsible for keeping the "code list" separated from the CRFs. The

study site investigators will continuously transfer the information to the eCRF to ensure the security of the data.

### **9.3 Access to data**

All CRFs will be archived for 15 years. De-identified data (individual data that underlie the results reported in the study) will be made available beginning 3 months and ending 5 years after the publication of the study.

## **10 QUALITY ASSURANCE AND QUALITY CONTROL**

The principal investigator will be responsible for organizing the study sites including education of study site investigators. Study folders will be prepared for the study investigators, including outlines of the data collection process, study protocol and CRF. After initiation of the study, the study site investigators will be responsible for all study-related procedures at their site.

Using the eCRF, data collection will be continuously and closely monitored. The principal investigator will issue queries about missing data, out of range values, or illogical data relations.

## **11 STUDY ENDPOINTS**

Enrolled patients will be categorized as proven or probable IPA at discharge from the ICU to answer the primary objective of evaluating the prevalence of influenza related invasive pulmonary aspergillosis. A modified version of the Asp-ICU criteria will be used, described below 11.1, 11.2.

All recorded baseline characteristics will be compared between patients diagnosed with IPA and those that were not. The following outcome parameters will also be assessed: SOFA-score, use of mechanical ventilation, use of non-invasive ventilation, use of vasopressor, use of renal replacement therapy, ARDS-grade using the Berlin definition, median number of ICU stay, ICU mortality, and 90-day mortality after ICU admission.

### **11.1 Proven invasive pulmonary aspergillosis**

Microscopic analysis on sterile material: histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or sterile biopsy in which hyphae are seen accompanied by evidence of associated tissue damage. Culture on sterile material: recovery of *Aspergillus* by culture of a specimen obtained by lung biopsy.

### **11.2 Probable invasive pulmonary aspergillosis (fulfil both clinical, radiological and mycological criteria)**

**Clinical criteria:** One of the following signs or symptoms must be present:

- Fever refractory to at least 3 days of appropriate antibiotic therapy.
- Recrudescence fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause
- Dyspnea
- Hemoptysis
- Pleural friction rubs or chest pain

**Radiological criteria:**

Any infiltrate on pulmonary imaging by portable chest x-ray or CT-scan of the lungs.

**Mycological criteria:** One of the following findings:

- Positive microscopy or culture of *Aspergillus* from BAL
- Galactomannan optical index on BAL >0,8
- Galactomannan optical index on serum  $\geq 0,5$

## **12 STATISTICAL ANALYSIS**

Prevalence of Proven/Probable IPA will be calculated at the 95% confidence level. Patients meeting the criteria for proven/probable IPA will be compared to the influenza positive patients who do not meet the criteria, in terms of underlying risk factors and outcome measures. Categorical variables will be assessed by Fischer exact test and  $X^2$  test, and continuous variables with t-test or Mann Whitney U test where appropriate. We will also perform multivariable analysis by binary logistic regression to detect independent risk factors for IPA. All analyses will be conducted using a threshold of 95% confidence level (P-value 0.05). ICU mortality and 90 days after admission to the ICU mortality will be compared using survival analysis.

## **13 FUNDING**

The study has received financial support from:

- Regionala forskningsrådet i Uppsala-Örebroregionen
- Centrum för klinisk forskning, Västerås

#### **14 INSURANCE**

Svenska patientförsäkringen

#### **15 PUBLICATION**

The study will be published in a peer-reviewed medical journal. All study site investigators will be awarded recognition as collaborator in the manuscript. To gain full authorship the site must include at least 10 participants and the investigator contribute according to the Vancouver definitions.



## 16 REFERENCES

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