TriReg
Europe-wide Study of Trichosporonosis

A project of the European Confederation of Medical Mycology (ECMM)

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1. Study coordinators ........................................................................................................ 2
2. Introduction ...................................................................................................................... 3
3. Objectives ......................................................................................................................... 5
4. Study Period ..................................................................................................................... 6
5. Patient Population .......................................................................................................... 6
6. Case Report Form ............................................................................................................ 6
7. Epidemiological Survey .................................................................................................. 7
8. Data Analysis .................................................................................................................. 8
9. Specimen Collection and Laboratory-Based Research .................................................. 8
10. Budgetary Information ................................................................................................... 9
11. Ethical Considerations and Data Privacy Protection ...................................................... 9
12. Authorship Policy ......................................................................................................... 10
13. Contact Information ..................................................................................................... 10
14. References .................................................................................................................... 10
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2. Introduction

The incidence of invasive fungal infections is increasing in all parts of the world. The etiology of this ongoing epidemiological development is not completely understood. However, major contributing factors are the increasing number of transplantation procedures undertaken around the world (estimated at 500,000 per year), a widening of the indications for intensive chemotherapy, and the growing number of other clinical conditions requiring immunosuppressive treatment.

Therapeutic standards have been developed for the most frequent invasive fungal infections, i.e. candidiasis, aspergillosis and cryptococcosis. However, the so called “rare fungi” account for a significant number of invasive fungal infections. Thus, clinicians are facing infections due to a variety of different fungi without any reliable treatment recommendations. Today, therapeutic decision making is not evidence based.

Trichosporonosis is an example of these challenging “rare fungal infections”.

The genus *Trichosporon* belongs to the basidiomycetous yeast-like fungi. They are found in soil, composing wood and natural water reservoirs like rivers, lakes and the sea, as well as a wide variety of animals living predominantly in temperate and tropical areas [1]. They can also be part of the normal flora of the human skin and gastrointestinal tract [2, 3]. The genus encompasses about 50 species of which only some are considered clinically relevant. These include *T. domesticum*, *T. montevideense*, *T. cutaneum*, *T. dermatis*, *T. jiroveci*, *T. mucoides*, *T. asahii*, *T. asteroides*, *T. coremiiforme*, *T. faecal*, *T. inkin*, *T. japonicum*, *T. lactis*, *T. ovoides*, and *T. dohaense* [1].

The most common clinical presentation of infections with *Trichosporon* spp. is white piedra which causes white superficial lesions of hair. Tinea pedis and onychomycosis have been associated with *Trichosporon* spp. as well [1]. From Japan cases of allergic pneumonitis have been reported [4]. In recent years, *Trichosporon* spp. emerged as increasingly important causative agents of invasive disease in critically ill and immunocompromised patients. In a large review of 287 published cases of trichosporonosis almost two thirds of patients had an underlying haematological malignancy [5], but other conditions like solid tumors, organ transplantation, bone marrow transplantation, severe neutropenia, diabetes mellitus and HIV infection have also been reported. Medical interventions like prosthetic cardiac valves, central venous lines, peritoneal dialysis and concurrent antibiotic therapy or corticosteroid therapy have been identified as further risk factors [5-7]. Currently, there are no
guidelines available for the diagnosis and treatment of invasive trichosporonosis.

Limited epidemiological data on invasive trichosporonosis are available. In a small serious from the Slovak Republic, Trichosporon spp. were identified as the most common non-Candida species causing invasive yeast infections in cancer patients [8]. In a series from Italy between 1992 and 2000, 0.4% of patients with acute leukemia developed invasive trichosporonosis [5]. Data on risk factors are currently retrieved from case reports and case series and have never been investigated systematically.

The diagnosis of invasive trichosporonosis relies on cultural findings as well as the identification of fungal elements considered compatible with Trichosporon spp. in tissue specimens. As with all fungal infections, obtaining an appropriate specimen can be difficult and cultures remain negative in many cases. Non-culture based methods for diagnosing invasive trichosporonosis are highly desirable, but no standardized methods have been established.

(1-3)-β-D-Glucan has not been evaluated as a diagnostic marker for Trichosporon spp. systematically, but appears to be detectable in 50% of cases [9]. Trichosporon spp. are known to have glucuronoxylmannan as a cell wall constituent, which can be detected in serum. However, cross-reactions with Cryptococcus neoformans antigen have been observed [10]. Overall, no specific antigen-based diagnostic test for trichosporonosis is currently available.

PCR-based methods have been developed to diagnose trichosporonosis from a number of clinical specimen (including blood cultures, serum, and sputum), but no standardized procedure or commercial system is available. Furthermore, experimental PCR-based methods and can only be performed in specialised laboratories [1].

Phenotypic identification can be performed once cultural proof of yeasts has been obtained. However, this requires the presence of an experienced mycologist in the laboratory. Further identification may be carried out by commercial systems, but these rely on databases of previously identified reference strains. In case of a rare pathogen a matching reference may not always be available.

Despite a considerable number of published cases of clinically refractory trichosporonosis, few data exist on in vitro susceptibility testing of clinical isolates of Trichosporon spp. Currently, no standardized procedures for susceptibility testing are available and minimal inhibitory concentrations (MIC) were not correlated with clinical outcome in a systematic approach. Thus cutoff values / breakpoints for different antifungals remain unknown [11, 12].
With limited data on the susceptibility of *Trichosporon* spp. available and contradicting information from the published case reports on susceptibilities for single isolates, reliable treatment recommendations cannot be made. Therefore, systematic evaluation of cases of invasive trichosporonosis is urgently needed to improve clinical care of patients developing trichosporonosis.

### 3. Objectives

The objective of this study is to overcome the lack of knowledge on epidemiology, clinical course, biology, and pathomechanisms of trichosporonosis in order to develop an evidence-based diagnostic-therapeutic integrated approach. The specific objectives are:

#### 3.1. Epidemiology
- To describe the incidence of trichosporonosis in Europe
- To define risk groups

#### 3.2. Clinical course
- To describe the clinical pattern of disease
- To document diagnostic procedures performed for confirmation of diagnosis
- To describe first-line and salvage treatment regimens applied, their efficacy and outcomes

#### 3.3. Biology and pathomechanisms
- To collect isolates and set up a publicly accessible strain collection
- To characterize isolates by molecular typing
- To perform a proteome mapping of the most important species
- To determine in vitro susceptibility
- To sequence important clinical isolates

#### 3.4. ECMM Recommendations for diagnosis and treatment
- To establish a consensus guideline
- To develop potential screening procedures
- To identify treatment approaches for first-line and salvage situations
4. Study Period

Start date: December 1, 2012
End date: November 30, 2014

5. Patient Population

5.1. Inclusion criteria
- Cultural, histopathological, or DNA evidence of invasive fungal infection with *Trichosporon* spp.

5.2. Exclusion criteria
- Infection due to other fungal pathogen (co-infection is allowed)
- Colonisation or other non-invasive infection with *Trichosporon* spp. only

6. Case Report Form

The Case Report Form (CRF) will be created using the survey software EFS Survey™ (Globalpark). This software is used by many international research groups for epidemiological and sociological research projects. Data entry is carried out via an interactive macro created by the software that can be accessed via any internet browser. All documented data are automatically collected in a database. Regular data-backup, hierarchized management of rights and authentication protocols ensure the protection of data from unauthorized access and loss. All Good Epidemiological Practice (GEP) requirements are met by the software [13].

The CRF will be accessible through at least the following websites:

www.ecmm.eu
www.fungiscope.net

The study protocol, the full CRF as portable document file, and the ethics committee’s approval of the study will be available on these sites. Participants wishing to contribute cases will receive account-details for login. Account details have to be requested via E-mail. Full name, institution and E-mail address have to be supplied.

The following core data set will be collected:
1 Demographic data: age (decade), gender, ethnicity, country
2 Data of trichosporonosis: year of infection, species identification, co-infections with other fungi, clinical characteristics upon diagnosis
3 Data of concomitant diseases: diagnosis, year of diagnosis, current status and treatment
4 Potential risk factors for developing fungal infection: immunosuppressive therapy, chemotherapy, biologicals, use of corticosteroids, radiotherapy, prosthetic material, diabetes mellitus, renal failure and dialysis, trauma and major surgery, HIV/AIDS, total parenteral nutrition, antibiotic therapy, neutropenia, mucositis, and other risk factors
5 Antifungal prophylaxis if given: drug, route, dose, duration prior to diagnosis of trichosporonosis
6 Diagnostic measures and findings
7 Antifungal treatment: drug, route, dose, duration, side effects and treatment outcome
8 Overall and attributable survival at week 12 and 6 months from diagnosis of trichosporonosis
9 Cause of death

7. Epidemiological Survey

All participating institutions will be asked annually to complete an additional epidemiological survey questionnaire.

The following data will be collected:

1 Catchment area of the institution
2 Specialty of the reporting department
3 Level of care provided
4 Annual number of invasive yeast infections
5 Annual number of invasive fungal infections
6 Annual number of SOT and allo HSCT
7 Annual number of cases with acute leukemia
8. Data Analysis

Data will be analysed using descriptive statistical methods using SPSS™ software by IBM.

9. Specimen Collection and Laboratory-Based Research

If *Trichosporon* spp is identified in isolates that were sent to the cooperating laboratory, for second opinion formal identification will be carried out based on culture and molecular biology results. The availability of these tests will be communicated to all ECMM-members to encourage formal identification of suspected cases of trichosporonosis. The result will only be communicated to the provider of the specimen, but they will be encouraged to document the clinical data along with the microbiological results in the case report form. The following laboratory-based research will be conducted:

1. Strain identification by micro- and macro-morphology, culture and molecular tools
2. In vitro susceptibility testing according to EUCAST and E-test

The laboratory based part aims to overcome the lack of knowledge about the biology and the pathogenic mechanisms of these emerging fungi, a step essential to develop new therapeutic approaches, using an integrated approach. By the simultaneous sequencing of several isolates of this species complex, our innovative sequencing strategy will permit the development of comparative genomics and will feed, associated with the proteome mapping, into the identification of molecular mechanisms determining virulence and antifungal resistance. This new scientific knowledge will be particularly helpful to develop molecular tools essential to perform rapid and early detection and thus diagnosis.

In order to elucidate the differences observed in antifungal susceptibility profiles of isolates (some clinical isolates being susceptible, while others exhibit resistance independently of previous antifungal treatment), alternative mechanisms in the resistance process will be investigated. Single nucleotide polymorphism of various genes (identified by *in silico* approach or the orthologues of which are known to be involved in antifungal resistance) will be studied by cost-efficient high throughput real-time PCR assays in large collections of phenotypically characterized isolates in order to assess the relationship between genetic polymorphisms and phenotype.
10. Budgetary Information

For evaluable patient documentations filled in by the participating center a compensation of € 100 each will be paid. If the documentation workload is too high, centers are encouraged to ask the study office for personnel to be sent to the site. For isolates or tissue made available to the central laboratory an additional compensation of € 50 will be paid.

11. Ethical Considerations and Data Privacy Protection

In the current study 2 aspects of the study have to be considered separately:

1. Documentation of clinical data
2. Work with isolates of *Trichosporon* spp.

Regarding aspect 1. Only data created during standard medical care will be documented in the CRF. There is no interventional aspect to this study. Therefore, there are neither associated risks nor benefits for the patient when participating in the study. The digital documentation of the clinical data will take place in an anonymized fashion. No identifiable data, e.g. name or date of birth will be entered into the database. Instead of the exact age of the patient we will only collect information on the decade of birth of the patient as a further measure to ensure anonymity. There will also be no pseudonyms which would make a retrospective re-identification of the patient possible. Clinical data collected refers to common conditions and treatment modalities in medical care, such that no re-identification of the individual case on the basis of these data will be possible. Queries after completion of documentation by the reporting institution are not planned. Under these circumstances, we consider an informed consent of the patient not necessary. Regular data backup, hierarchized management of rights and authentication protocols ensure the protection of data from unauthorized access and loss. Contributors can only view the cases submitted by themselves. All study procedures are liable to Good Epidemiological Practice (GEP) requirements German and European legislation [13]. All clinical data fall under medical confidentiality. All data and results will be stored for at least 10 years after publication of results.

Regarding aspect 2, to ensure anonymity, the results of microbiological examinations will only be communicated to the treating physician and entered.
into the database along with the clinical data in one session by the treating physician. The microbiological examination of isolates of *Trichosporon* spp. does not require informed consent of the patient, as there is no patient material involved in the microbiological examinations.

12. Authorship Policy

Authorship will be restricted to those centers contributing clinical/microbiological data or translational work. For each contributing center, there will be authorship positions available. This will extend to a maximum of two: one clinician, and one microbiologist/medical mycologist, if applicable.

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