Diploma Thesis

Retrospective analysis of plasma levels and efficacy of antifungal prophylaxis with different posaconazole formulations

submitted by

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Graz, 10. January 2018 *David Lenczuk eh*

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Zusammenfassung

Einleitung

Posakonazol ist ein Breitbandantimykotikum, welches Patienten und Patientinnen einnehmen, die ein hohes Risiko haben invasive Pilzinfektionen zu erleiden, wie beispielsweise Patienten und Patientinnen die eine Induktionstherapie aufgrund einer hämatologischen Erkrankung erhalten oder solche die aufgrund von einer HSCT immunsuppressive Medikamente benötigen.

Methoden

Diese retrospektive Studie wurde in Graz durchgeführt und Patient und Patientinnen, die im Zeitraum von Juni 2015 bis Dezember 2016 Posakonazol als Pilzprophylaxe erhalten haben wurden eingeschlossen. Patientendaten, mikrobiologische Ergebnisse und Labordaten wurden via Medocs und Fieberkurven gesammelt. Die Messungen wurden während den ambulanten Routineuntersuchungen oder während eines stationären Aufenthaltes gemessen. Analysiert wurde ob während der gesamten Einnahmezeit die Tablette gegenüber der oralen Suspension häufiger einen Spiegel von min. 0,7 µg/ml erreichte. Eine mögliche Durchbruchsinfektion wurde anhand der EORTC/MSG Kriterien diagnostiziert.

Ergebnisse

446 Messungen von 61 Patienten und Patientinnen, wovon 48 die Tablette und 13 die orale Suspension einnahmen, wurden analysiert und aufgeteilt in mindestens eine Messung (DRT 91,1% vs. OS 51,6%, p-value 0,001), mindestens zwei Messungen (DRT 91,6% vs. OS 56,0%, p-value 0,004) bzw. mindestens drei Messungen (DRT 90,7% vs. OS 62,1%, p-value 0,073). Der Vergleich von Periode Tag 1-6 und Tag 7-14 zeigte signifikante höhere Spiegel in der Tablettengruppe - (DRT mean 1,53 µg/ml vs. OS mean 0,67 µg/ml, p-value 0,009). Keine bekannten Faktoren beeinflussten den Spiegel der Tablette, ausgenommen Patienten und Patientinnen mit GvHD hatten niedrigere Spiegel - DRT 22/86,7% vs. OS 13/97,4%, p-value 0,026. Eine Durchbruchsinfektion, Orbita Abszess durch Candida glabrata, wurde diagnostiziert welches einer Rate von 1,6% entspricht.

Diskussion

Die Tablette hat während der gesamten Einnahmedauer suffizientere Spiegel erreicht als die orale Suspension. Spiegelmessungen sind in Zukunft womöglich nur mehr bei Patienten und Patientinnen mit GvHD nötig.

Abstract

Background

Posaconazole is a broad spectrum antifungal drug used as a prophylactic agent in patients receiving induction chemotherapy for hematological malignancies and in those for whom immunosuppressive drugs are needed to prevent GvHD after HSCT. These patients have a high risk of invasive fungal disease.

Methods

This retrospective study conducted from June 2015 until December 2016 in Graz, analyzed 97 patients of which 61 were enrolled, 48 were on delayed-release tablet and 13 on oral suspension formulation. Their medical data, microbiological data, laboratory data, and medications were extracted from patient's charts via Medocs. Posaconazole plasma concentrations were obtained during outpatient treatment or hospitalization. This thesis assesses the superiority of DRT during the whole period of intake compared to OS, cutoff 0.7 µg/ml. Breakthrough infections were defined as "proven", "probable" and "possible" according to the EORTC/MSG.

Results

The 446 measurements of 61 patients (1 to 30 PPCs per patient) were analyzed and divided in min. one PPC (DRT 91.1% vs OS 51.6%, p-value 0.001), min. two PPCs (DRT 91.6% vs OS 56.0%, p-value 0.004) and min. three PPCs (DRT 90.7% vs OS 62.1%, p-value 0.073). Comparison of the periods day 1-6 and day 7-14 showed significant higher PPCs in the DRT group (mean 1.53 µg/ml) than in OS group (mean 0.67 µg/ml) – p-value 0.009. Only one breakthrough infection was diagnosed as Candida glabrata orbital abscess, 1.6%. No decrease in PPC was seen in patients suffering from diarrhea or taking concomitant PPI, only patients diagnosed with GvHD had lower plasma levels DRT 22/86.7% vs. OS 13/97.4%, p-value 0.026.

Conclusion

The posaconazole delayed-release tablet is superior compared to the oral suspension in reaching sufficient plasma concentrations during the period of intake. Therapeutic drug monitoring may be needed for selected patients only.

Table of contents

List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
ALL	Acute lymphatic leukemia
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
AP	Alkaline phosphatase
APL	Acute promyelocytic leukemia
AST	Aspartate aminotransferase
BAL	Bronchoalveolar lavage
BMI	Body mass index
CD	Cluster of differentiation
CLL	Chronic lymphatic leukemia
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CNS	Central nervous system
CSF	Cerebral spine fluid
CT	Computer Tomography
CV	Coefficient of variances
DLBCL	Diffuse large B-cell lymphoma
DNA	Deoxyribonucleic acid
DRT	Delayed-release tablet
EBV	Ebstein-Barr-Virus
ECG	Electrocardiogram
EMA	European Medicine Agency
EORTC	European Organization of Research and Treatment of Cancer
FDA	Food and Drug Administration
GGT	Gamma-glutamyltransferase
GvHD	Graft-vs-Host-Disease
HHV	Human Herpes Virus
HIV	Human Immunodeficiency Virus
HLA	Histocompatibility leukocyte antigen
HPMCA	Hydroxypropyl Methylcellulose Acetate Succinate
HSCT	Hematopoietic stem cell transplantation

HSV	Herpes simplex virus
HTLV	Human T-cell leukemia Virus
i.v.	Intravenous
IFD	Invasive fungal disease
IFI	Invasive fungal infection
IFICG	Invasive Fungal Infection Cooperative Group
MDS	Myelodysplastic Syndrome
mEq	Milliequivalent
MGUS	Monoclonal gammopathy of undetermined significance
MPN	Myeloproliferative Neoplasms
MSG	Mycoses Study Group
NHL	Non-Hodgkin lymphoma
NIAID	National Institute of Allergy and Infectious Diseases
NOS	Not otherwise specified
OS	Oral suspension
PCZ	Posaconazole
PPC	Posaconazole plasma concentration
PPI	Proton pump inhibitor
SBECD	Sulfobutylether β-cyclodextrin
spp	Species
TDM	Therapeutic drug monitoring
UDP	Uridine diphosphonate
VOD	Veno-occlusive disease
VZV	Varicella-zoster virus

List of figures

List of tables

1 Introduction

Patients receiving chemotherapy for hematologic malignancies or receiving immunosuppressive drugs after human stem cell transplantation for preventing Graft-vs-host-disease have a compromised immune system. Under these circumstances, they are at high risk for invasive fungal disease.(1,2) Especially aspergillosis, candidiasis and zygomycosis are the most common infections.(1–4) Therefore it is crucial to have an adequate prophylaxis for these patients to reduce the possibility of infections, according to the increased morbidity and mortality.(5)
Drugs used for prevention are mostly azole as fluconazole, itraconazole, and posaconazole. Different studies have shown the superiority of posaconazole against the other agents.(6,7)

Posaconazole was only available as an oral suspension until June 2014. Now it is obtainable as pH-sensitive tablets and intravenous suspensions. Due to the significant interindividual and intraindividual variability and drug-drug interactions of the oral suspension, it is standard practice to ensure the adequate plasma level of posaconazole by therapeutic drug monitoring during administration and therefore to improve the clinical outcome.(8–10) Several factors affect the absorption of the drug negatively. Such as gastric acid pH, gastric motility, diarrhea, vomiting and concomitant food intake.(8,10–16) The new available posaconazole tablet provides a formulation that ensures a better intestinal uptake and therefore a higher and more consistent plasma level in prevention and treatment of invasive fungal diseases.
Because of the potential harm of having an inadequate plasma level and lack of evidence, therapeutic drug monitoring is established with the posaconazole tablet.(17)

Previously studies only measured plasma concentration in the first two weeks. The present thesis analyzes the therapeutic plasma levels during administration of posaconazole oral suspension and posaconazole tablets during long time use and at least 1 to 30 measurements.
The next few chapters provide a short overview of hematologic malignancies, invasive fungal disease and, posaconazole formulations followed by methods and study results.

1.1 Hematological malignancies

Hematological neoplasia is a disorder of the bone marrow and the lymphatic system characterized by infiltrating the bone marrow, blood and other tissues by clonal, abnormal differentiated cells. There are three different cells produced: erythrocytes, thrombocytes, and lymphocytes. The stem cells are the common basis of all cells, which can differentiate to specialized cells with specialized functions. While dividing into different cells, there are a lot of DNA spots which can be damaged or falsely replaced by other bases. Usually, there is a repair system taking care of these mistakes, so there is no further problem in the differentiation of cells. Unfortunately, some errors cannot be replaced or repaired because of defective repair genes.
There are lots of different factors influencing this system such as endogenous factors (chromosomal aberration, trisomy 21, etc.), exogenous factors (radiation, pesticides, chemotherapy, benzoyl, immunosuppression) and infections such as EBV, HIV, Helicobacter pylori.

As the hematological disorders primarily affect the blood system, changes in blood cells are their major clinical presentation. Lots of diseases are infiltrating the bone marrow and therefore are suppressing the hematopoiesis. The result is a decrease in blood cells. Symptoms of weakness, fatigue, and poor concentration are present in anemia. Severe infections are due to inefficient immune cells, and spontaneous bleeding occurs due to small numbers of thrombocytes. Clonal cells are infiltrating lymphatic tissue which leads to hepatosplenomegaly, swelling of lymph nodes or mediastinal swelling causing dyspnea, dysphagia and dry cough.
The diagnosis can be made by collecting blood samples, bone marrow biopsy or excising abnormal lymph nodes.
These disorders are systemic diseases, and therefore the therapy includes cytotoxic chemotherapy and hematopoietic stem cell transplantation.

1.1.1 Epidemiological facts and etiology(18–20)

TYPE	INCIDENCE	AGE	ETIOLOGY
LYMPHOMA			
• HODGKIN'S LYMPHOMA	3	30a, 60a	EBV, HIV, immunosuppression, chemicals
• NON-HODGKIN'S LYMPHOMA	10-12	mostly elder people	immunodeficiency, EBV, immunosuppressive drugs, AIDS, HHV 8, H. pylori, HTLV, pesticides
MYELOMA	5	70a	unknown, associated with MGUS, chemicals, radiation
LEUKEMIA			
• ALL	1.5	80% <20a	radiation, chemicals, genetic disorders, HTLV
• CLL	4	90% >50a	unknown, children of CLL are at higher risk
• AML	3.7	65 a	radiation, chemicals, genetic disorder, HTLV, a transformation from other hematological disorders
• CML	2	60	radiation, chemicals
MDS	0,4*	70a	90% unknown, 10% radiation, chemotherapy, chemicals
MPN	0.8-2.1	>60a	unknown, radiation, chemicals

*>70a incidence rate 20-50 per 100 000/year

Table 1: Epidemiological facts and etiology of common hematological disorders

1.1.2 Classification

In 2016, the WHO updated the classification of hematological malignancies. The following tables give an overview of existing diseases.

Lymphoma			
Hodgkin's lymphoma		*Non-Hodgkin's lymphoma*	
Classical Hodgkin lymphoma	**Nodular lymphocyte predominant Hodgkin lymphoma**	**B-cell NHL**	**T-cell NHL**
Nodular sclerosis		Chronic lymphatic leukemia	T-cell prolymphocytic leukemia
Lymphocyte-rich		Hairy cell leukemia	Extranodal NK-/T-cell lymphoma, nasal type
Mixed cellularity		Diffuse large B-cell lymphoma	Angioimmunoblastic T-cell lymphoma
Lymphocyte-depleted		Burkitt lymphoma	peripheral T-cell lymphoma, NOS
		High-grade B-cell lymphoma	Mycosis fungoides
		Follicular lymphoma	Sézary syndrome
		Mantle cell lymphoma	Primary cutaneous CD30+ T-cell lymphoproliferative disorders

Table 2: Classification of lymphoma

Myelodysplastic syndromes (MDS)	**Myeloproliferative neoplasms (MPN)**
MDS with single lineage dysplasia	Chronic myeloid leukemia
MDS with ring sideroblasts	Chronic neutrophilic leukemia
MDS with multilineage dysplasia	Polycythemia vera
MDS with excess blasts	Primary myelofibrosis
MDS with isolated del(5q)	Essential thrombocythemia
MDS, unclassifiable	MPN, unclassifiable

Table 3: Classification of myelodysplastic syndromes and myeloproliferative neoplasms

Acute leukemia*		
Acute lymphocytic leukemia		Acute myeloid leukemia
B-Lineage ALL	*T-Lineage ALL*	M0 Undifferentiated acute myeloblastic leukemia
B-Precursor ALL • Pro-B • c-(common) • Pre-B	"Early" T	M1 Acute myeloblastic leukemia with minimal maturation
Mature B	Thymic	M2 Acute myeloblastic leukemia with maturation
	"Mature" T	M3 Acute promyelocytic leukemia (APL)
		M4 Acute myelomonocytic leukemia
		M4 eos Acute myelomonocytic leukemia with eosinophilia
		M5 Acute monocytic leukemia
		M6 Acute erythroid leukemia
		M7 Acute megakaryoblastic leukemia

Table 4: Classification of acute leukemia

*ALL classified by GMALL Studies and AML classified by FAB-Classification because of use in clinical practice

1.2 Hematopoietic stem cell transplantation

So far, the term bone marrow transplantation was used because hematopoietic stem cells have been obtained from the donor's bone marrow. Since blood and umbilical cord blood are used as a source, the term hematopoietic stem cell transplantation is preferred.

There are two different strategies for the use of HSCT:(21)

- Replacement of a deficient but not neoplastic hematopoietic system with healthy stem cells
- Cure for a neoplastic disorder with high-dose myelosuppressive chemotherapy which requires a stem cell transplantation afterwards

The annual number of performed HSCT has increased the last decades due to the broad availability of stem cell donors.

Hematopoietic stem cells have an enormous ability to regenerate and to find the way to the bone marrow after intravenous application. It is possible for them to be cryopreserved and can be stored for a long time. Only a few cells are needed to replace the whole hematopoietic system of a patient like erythrocytes, granulocytes, thrombocytes, B- and T-lymphocytes as well as the macrophage system.

There are three different types of donating stem cells:

- autogeneic transplantation - be one's own donor
- – allogeneic transplantation - have someone else donating the cells – related or unrelated
- syngeneic transplantation - one identical twin is donating

The best situation is if there is an identical twin available for transplantation as there is no possibility of developing Graft-vs-Host-Disease and of course the HLA-match is perfect. Unfortunately, in less than 1% such a donor is present. (21)
In case of an allogeneic transplantation, donor and recipient are not genetically identical, and the risk of immune cells attacking the host is possible which can lead to a GvHD. The high number of potential donors related or unrelated make this option very interesting for clinical use. Large registers are existing which all have

information about the important HLA-A, -B, -C and –DR genes needed to be compared for HSCT. The likelihood to match with a sibling is 1:4, therefore most donors are in the patient's family. The risk of rejection when having stem cells from a relative is only 1-3%, whereas with stem cells from unrelated persons the risk for severe or life-threatening GvHD is as high as 15%.(21,22) Because of genetic polymorphism, the possibility of finding an unrelated HLA-match is very low – about 1:10.000 but since there are these large donor registers including more than 20 million possibilities, 70% of all patients in need for transplantation succeed in finding a match.(21)

It is possible to have the patient's stem cells extracted, cryopreserved and reinjected after myeloablative conditioning. The advantage of an autogenic transplantation in not having external lymphocyte attacking the patient's body reduces the risk of GvHD to zero. But because of the lack of foreign lymphocytes, the Graft-vs-Tumor-Effect is not present either, and the risk of contamination with tumor cells makes the risk of relapse very high. Different "purging" technics do exist to reduce the number of tumor cells from transplants, but several studies could not show a difference in relapse or survival compared to allogeneic transplantation. So, in clinical practice, the autogenic version is less common.

Before transplantation, the donor receives a chemotherapy plus radiation as conditioning therapy to eradicate bone marrow. Different regimes are existing which differ in dose and substance applied. Some diseases do not need high dose chemotherapy to achieve remission due to their compromised immune system. Also, patients with lots of comorbidities benefit from low to intermediate dose chemotherapy.(21,22) This regime provides another advantage due to not having all immune cells damaged. The effect of Graft-vs-Leukemia/Tumor is more present in reduced doses strategies. The option of having donor lymphocytes infused to regain remission in relapse supports the benefit of immunological anti-tumor activity. As conditioning chemotherapy is associated with higher mortality, low and intermediate chemotherapy is preferred in elderly patients.
A small number of CD34+ hematopoietic stem cells is circulating in our blood, and after administration of granulocyte colony-stimulating factor, this number increases rapidly after four to five days. Now, these cells are obtained by a leukapheresis

procedure and are injected into the recipient's blood system which is usually done by a central venous catheter. These stem cells are quickly moving into the bone marrow – called engraftment. Now the hematopoietic system should recover soon.
The time after transplantation is characterized by problems due to chemo-radiotherapy and an insufficient hematopoiesis. Adverse effects from chemotherapy drugs range from mucositis, hemorrhagic cystitis, non-exclusive disease to growth retardation and infertility.

Hematopoietic problems are based on reduced blood cells leading to anemia (weakness, fatigue, and poor concentration), thrombocytopenia (spontaneous bleedings) and leukocytopenia (opportunistic infections).
Another common complication of HSCT is GvHD. The donor's T-lymphocytes are attacking the recipient's cells. There are two types differentiated by time of onset. The acute GvHD is occurring within 90 days with symptoms of diarrhea, maculopapular exanthema, and increased liver enzymes and the chronic GvHD, which normally starts after three months to two years. It presents itself with symptoms such as an autoimmune disorder like exanthema, Sicca-Syndrome, arthritis, bronchiolitis obliterans and destruction of bile ducts leading to cholestasis. The treatment includes immunosuppressive drugs as glucocorticoids, Cyclosporine A, Tacrolimus and a prophylaxis of opportunistic infections.(21)

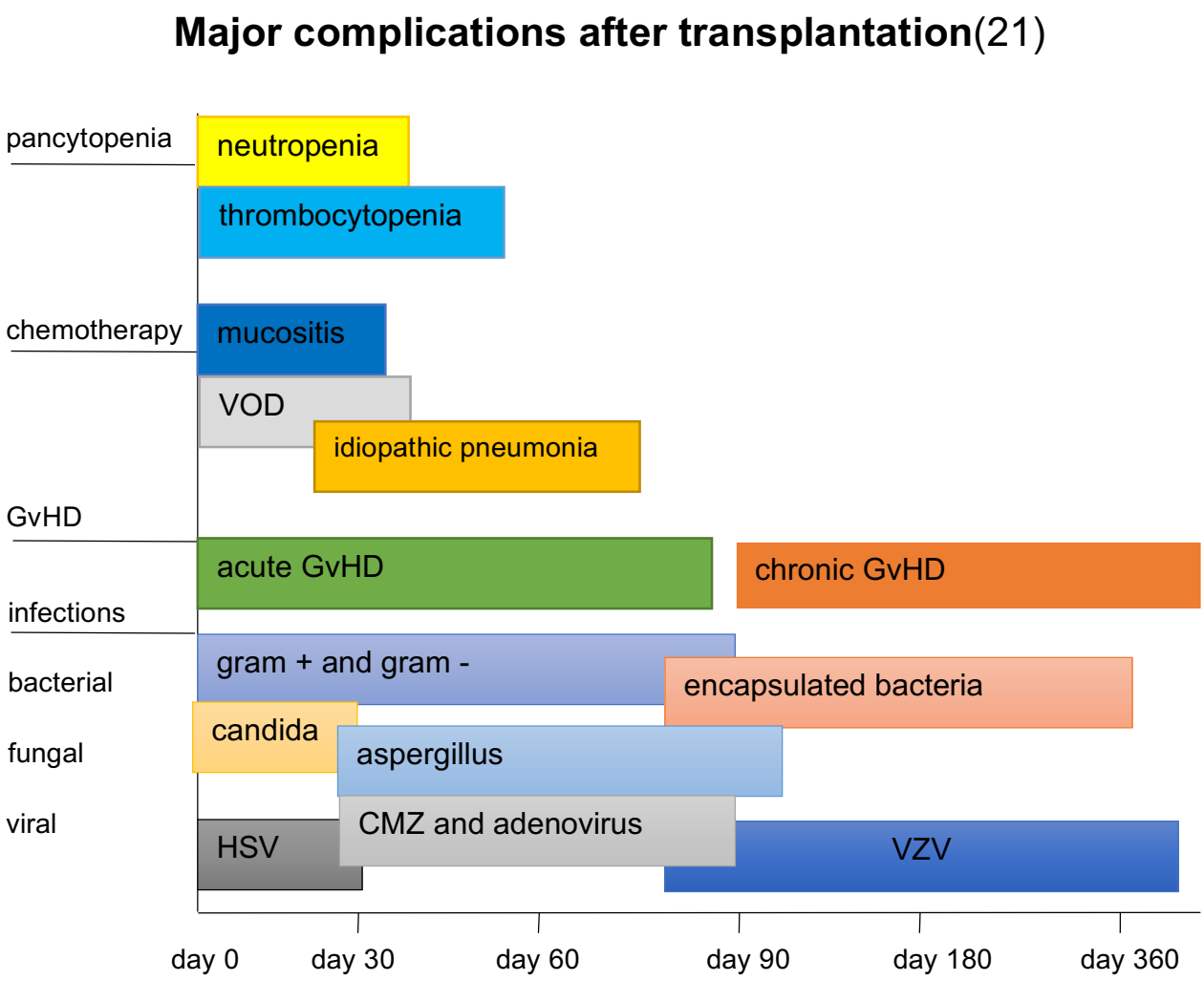


Figure 1: Major complications after HSCT

1.3 Invasive fungal diseases

Several different fungi are enabled to cause invasive fungal infections. They can be split up into three major groups: **yeasts** such as *Candida spp, Cryptococcus spp* and relatively rare ones like *Saccharomyces spp, Trichosporon spp, Malassezia spp, Geotrichum candidum, Hansenula anmola, Rhodotorula spp* and *Pichia spp*; **molds** such as *Aspergillus spp, Fusarium spp, Scedosporium prolificans, Mucor, Rhizopus* and *Rhizomucor Absidia* and **dimorphic fungi** including *Histoplasma capsulatum, Coccidoides immitis, Blastomyces dermatitidis, Paracoccidoides spp, Sporothrix spp* and *Penicillium marneffi*.(2)

Many fungi can be present as commensally in humans which is called fungal colonization, where a balance between virulent factors and the immune system prevents invasive infection. Primary, colonization is made by *Candida spp, Cryptococcus spp, Aspergillus spp, Mucor,* and *Rhizopus*. In healthy individuals, the immune system controls their growth. In contrast to immunocompromised patients due to immunosuppressive drugs or hematologic disorders where no or insufficient immune response leads to life-threatening infections.(1,2) The last decades the incidence for IFD has increased worldwide as more frequent immunosuppression is present due to AIDS, diabetes, a wide availability of HSCT with more success rates, a higher use of immunosuppressive drugs and an increased possibility to diagnose hematological diseases which require chemotherapy.(1)

Invasive fungal infections have been described as the presence of fungal elements either as mold or yeast in deep tissue biopsy or needle aspirates that is confirmed on culture and histopathological examinations. Classification is made by the Invasive Fungal Infections Cooperative group (IFICG) of European Organization for Research and Treatment for Cancer (EORTC) and Mycology Study group (MSG) of National Institute of Allergy and Infectious Disease (NIAID).(23) In clinical practice, it is often difficult to make the diagnosis of an IFD because of uncertain diagnostic tools which only suggest but do not proof a fungal infection, especially in periods of neutropenia where an immune response is minimized. Inter-practitioner variability made it hard to compare cases in clinical research. Therefore, in 2002 the EORTC/MSG developed three definitions of IFD “proven”, “probable” and “possible” in immunocompromised patients to ease comparison of clinical and epidemiological research.(23) These definitions were reviewed by the EORTC/MSG in 2008 to

improve uncertainness. Also, the term “invasive fungal infection (IFI)” was revised to “invasive fungal disease (IFD)” to reflect more accurately that we are dealing with diseases caused by fungal infections. De Pauw et al. pointed out that the following definitions are only used in a research context and are not suitable for clinical practice since in some cases fungal infections are present but cannot be diagnosed with several tools and therapy is still needed.

1.3.1 Proven invasive fungal diseases

Proof of an IFD can only be made by extracting fungal elements from diseased tissue of the patient, except for disseminated cryptococcosis the presence of capsular antigen in cerebral spinal fluid (CSF) is considered as proven IFD. Endemic dimorphic fungal are several listed with different approaches in diagnostic. Molds and yeasts can be confirmed by microscopic analysis and culture from sterile materials, blood or in the case of suspect cryptococcosis antigen in CSF.(24)

1.3.2 Probable invasive fungal diseases

The diagnosis of a probable IFD can only be made if there is at least one host factor, one clinical feature and one mycological evidence present.

Criteria for probable IFD except for endemic mycoses*

Host factors:

- Recent neutropenia related to onset of fungal disease
- Receipt of allogeneic stem cell transplant
- Prolonged use of corticosteroids – min. 0.3 mg/kg/d, > 3 weeks
- Treatment with T-cell immunosuppressant, specific monoclonal antibodies or nucleoside analogs
- Inherited severe immunodeficiency

Clinical criteria:

- Lower respiratory tract fungal disease shown with CT
- Tracheobronchitis
- Sinonasal infection
- CNS infection
- Disseminated candidiasis

Mycological criteria:

- Direct tests non-sterile (cytology, direct microscopy or culture)
  - Mold in sputum, BAL, bronchial brush or sinus aspirate samples
- Indirect tests
  - Aspergillosis
    - Galactomannan antigen detected in plasma, serum, BAL or CSF
  - Invasive fungal disease other than *cryptococcosis* and *zygomycoses*
    - Beta-D-glucan detected in serum

*this table only provides an overview, for further information see the original paper(24)

Table 5: Criteria for probable IFD

1.3.3 Possible invasive fungal diseases

The category possible IFD is similar to probable IFD except that there is no sufficient evidence of mycological criteria present.

1.3.4 Endemic mycosis

For endemic dimorphic fungi, separate diagnostic criteria are existing because there is no category “possible IFD” due to the non-specific host factors and clinical factors.

Diagnosis and criteria for endemic mycoses

Proven endemic mycosis:

- In a host with an illness consistent with an endemic mycosis, 1 of the following:
  - Recovery in culture from a specimen obtained from the affected site or blood
  - Histopathologic or direct microscopic demonstration of appropriate morphologic forms with a truly distinctive appearance
  - characteristic of dimorphic fungi
  - For coccidioidomycosis, demonstration of coccidioidal antibody in CSF, or a 2-dilution rise
  - For paracoccidioidomycosis, demonstration in 2 consecutive serum samples of a precipitin band to paracoccidioidin concurrently in the setting of an ongoing infectious disease process

Probable endemic mycosis:

- Presence of a host factor plus a clinical picture consistent with endemic mycosis and mycological evidence, such as a positive Histoplasma antigen

*this table only provides an overview, for further information see the original paper(24)

Table 6: Diagnosis and criteria for endemic mycoses

1.3.4.1 Limitations of the definition

These categories refer only to immunocompromised patients and are not suggestive in critical ill patients. The failure to receive the criteria for IFD does not mean that there is no IFD. It only presents the state where not enough evidence is present to support the diagnosis.(24)

1.4 Posaconazole

Posaconazole is a broad-spectrum triazole antifungal agent, second generation which has shown to be highly active against fungi such as *Candida spp*, *Aspergillus spp, Cryptococcus spp, Fusarium spp* and in therapy of mycetoma, chromoblastomycosis and coccidioidomycosis.(9,25–32) It is approved and established for prophylaxis and treatment of IFD.(6,7,9,33) It is as effective as previously used drugs like itraconazole or fluconazole in prophylaxis but has fewer side effects.(6,7) Nowadays, the three different forms – oral suspension formulation, delayed-release oral tablet formulation (DRT) and intravenous formulation are all approved by the FDA (2005, 2013, 2014) and EMA (2005, 2014,2014).

Labeled EMA indications for the use of Posaconazole(32,34)
Fungal infections in adults: • Invasive aspergillosis in patients with disease that is refractory to amphotericin B or itraconazole or in patients who are intolerant of these medicinal products • Fusariosis in patients with disease that is refractory to amphotericin B or itraconazole or in patients who are intolerant of these medicinal products • Chromoblastomycosis and mycetoma in patients with disease that is refractory to amphotericin B or itraconazole or in patients who are intolerant of these medicinal products

• Coccidioidomycosis in patients with disease that is refractory to amphotericin B, fluconazole or itraconazole or in patients who are intolerant of these medicinal products • Oropharyngeal candidiasis as first-line therapy in patients who have severe disease or are immunocompromised, in whom response to topical therapy is expected to be poor • Refractoriness is defined as progression of infection or failure to improve after a minimum of 7 days of prior therapeutic doses of effective antifungal therapy
Prophylaxis of IFDs in the following patients: • Patients receiving remission-induction chemotherapy for acute myelogenous leukemia or myelodysplastic syndromes expected to result in prolonged neutropenia and who are at high risk of developing IFDs • Hematopoietic stem cell transplant recipients who are undergoing high-dose immunosuppressive therapy for GvHD and who are at high risk of developing IFDs

Table 7: Labeled EMA indications for the use of Posaconazole

1.4.1 Pharmacological facts

The drug name of posaconazole is Noxafil and is invented and patented by Merck & Co, Inc., Whitehouse Station, NJ, USA. As a class member of azole, it inhibits the enzyme 14α-demethylase (CYP51), which is responsible for the conversion of lanosterol to ergosterol. Thereby the inhibition of the biosynthesis of the fungi's cell membrane component ergosterol causes an accumulation of methylated sterol precursors leading to cell death.(9,32) It is highly protein bound (>98%), mostly bound to albumin depending on the administrated dose. Several circumstances can enlarge the unbound fraction – e.g., hyperbilirubinemia or hypoalbuminemia, which leads to an increased metabolism.(35)

Posaconazole has an average half-life period ($t_{1/2}$) of 35h (range from 20h to 66h) and is therefore eliminated slowly. After administration of ^{14}C-marked posaconazole, the predominant excreted portion was documented in feces (77% of radiolabeled), primarily as a parent drug (66% of radiolabeled dose). Due to a small amount of

elimination through urine as glucuronide conjugates, renal clearance is of minor importance (14% of radiolabeled dose).(32,34) When taking posaconazole continuously, steady state is reached after 7-10 days.(34)

1.4.2 Cutoff level and therapeutic drug monitoring

For prophylactic treatment, a plasma concentration of posaconazole greater than 0.7 µg/ml is recommended. Despite the fact, that some authors prefer a lower threshold level of 0.5 µg/ml, 0.7 µg/ml is generally accepted. In therapeutic use recommended plasma levels are normally higher than 1.0 µg/ml which is achieved by higher daily doses.(9,12,14,16,31,36–38) Especially using the oral suspension formulation therapeutic drug monitoring by plasma concentration measurement is recommended.(8,10,17) Suggestions for the delayed-release tablet are discussed in chapter 4.

1.4.3 Adverse effects

In general, posaconazole has shown an excellent safety profile and a good tolerability through healthy volunteers and sick patients.(32) Reported adverse events are normally mild, such as a headache, fatigue and dry mouth. Clinical relevant side effects are primarily gastrointestinal distress, like vomiting, diarrhea, abdominal pain and decreased appetite.(38,39) Neutropenia, elevated liver enzymes and prolonged QT-time have also been described.(6,7,32) Since neutropenia is a more concerning situation blood cell count should be regularly analyzed. QT-time prolongation is mentioned in very few studies with azoles, but regarding the high morbidity caused, ECG-monitoring is needed.(32,37) Hepatotoxicity is defined as an increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP) three times the upper limit of normal or patient's baseline, or bilirubin greater than one and half times the upper limit of normal or patient's baseline. Increased liver enzymes have been mentioned in many studies, but after discontinuing therapy with posaconazole, normal levels have been reached within 11 days.(6,7,39,40) More serious adverse events are seen in patient with higher plasma concentrations, indicating a dose-related toxicity, but in contrast, only a few patients (about 3%) exceeded concentrations beyond 3.750 µg/ml in prophylactic treatment.(12,14,38,39)

The intravenous formulation is known to cause several injection site adverse reactions such as thrombophlebitis, which requires an infusion through a central venous catheter for at least 90 min.(17,32)

Common adverse events
Headache
Fatigue
Dry mouth
Diarrhea
Vomiting
Abdominal pain
Decreased appetite
Neutropenia
Elevated liver enzymes
QT-prolongation

Table 8: Common adverse events

In general, posaconazole has an excellent safety profile with mild and easy to recognize changes.

1.4.4 Drug-drug interactions

The antifungal agent's azoles are likely to inhibit several CYP P450 enzymes and the p-glycoprotein transporter which results in higher concentrations of numerous drugs metabolized by these enzymes mentioned in the following table. However, posaconazole is a less potent inhibitor than other azoles. Especially the inhibition of the CYP3A4 is important in a patient receiving antifungal prophylaxis due to immunosuppression in GvHD. Reduction in the metabolism of these agents leads to higher plasma concentrations with potential toxicity. Administrating posaconazole and drugs known to be metabolized by CYP3A4 demands therapeutic drug monitoring and clinical evaluation to adjust doses of these drugs. The importance of phase 2 metabolisms has been demonstrated in studies. Co-administration of UDP glucuronidation inducers such as rifabutin or phenytoin resulted in decreased plasma concentration.(41,42)

Reduction in plasma concentration is more common in the use of the oral suspension formulation because of interactions with gastrointestinal drugs, which are described in the following table.

Agents of potential interaction with posaconazole(32)

Immunosuppressive drugs:

- Sirolimus
- Cyclosporine
- Tacrolimus
- Corticosteroids

HIV drugs:

- Efavirenz
- Lopinavir
- Ritonavir
- Fosamprenavir

Cardiovascular drugs:

- Amiodarone
- Statins
- Calcium channel blockers
- Digoxin

Oncological drugs:

- Taxanes
- Vina alkaloids
- Irinotecan
- Antinausea agents (5HT3 antagonists)

Table 9: Agents of potential interaction with posaconazole

1.4.5 Oral suspension formulation

Since October 2005 posaconazole is available as an oral suspension in Europe, taken three times a day as prophylaxis each 200mg/5ml and for treatment of salvage IFD four times a day each 200mg/5ml. It is necessary to have a concomitant intake of high-fat food to provide optimal drug exposure. There is a significant variability in the absorption of posaconazole with or without food. The influence may be due to delayed gastric emptying and increased gastric secretion in the presence of food.(13) A study conducted in Graz has shown, that personal on-site patient education regarding the intake of posaconazole (e.g., with fatty/acid food or the prevention of nausea/vomiting) increased absorption and therefore the plasma concentration.(15)

Figure 2: Noxafil® Oral Suspension

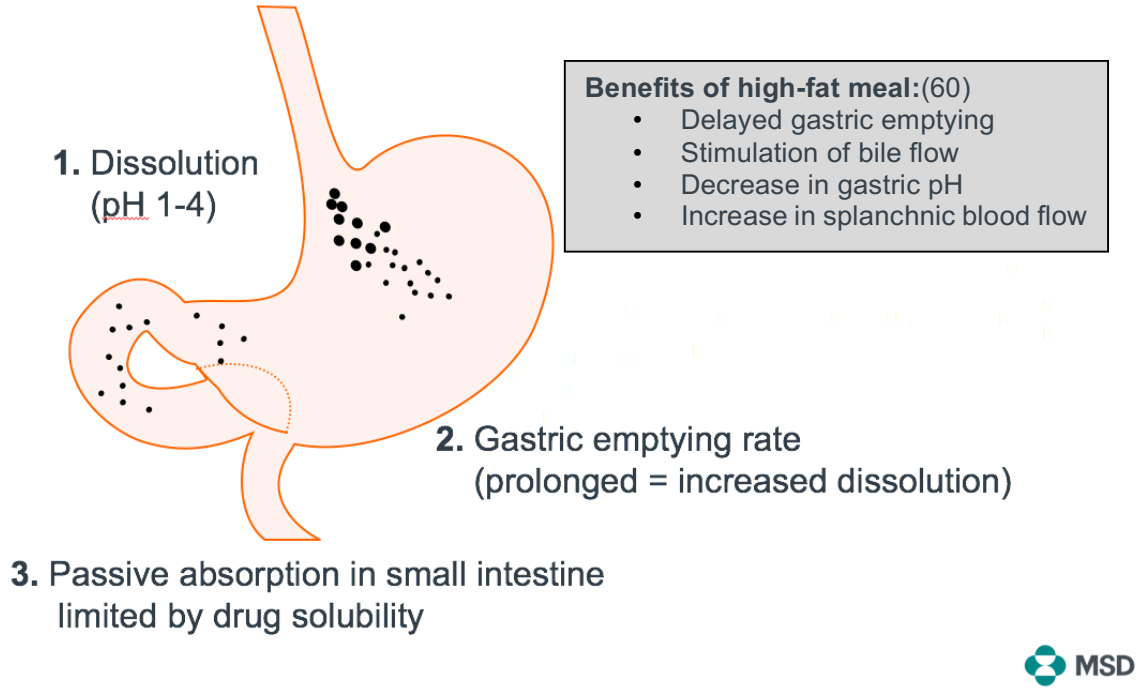


Figure 3: Gastrointestinal passage of Oral Suspension Formulation - MSD

Several drugs, influencing the gastrointestinal environment also lead to an increased variability of bioavailability. Medications such as proton pump inhibitors (PPI) are likely to decrease the blood concentration as well as metoclopramide which alters gastric pH and motility, and therefore increases gastric clearance.(43) Recipients of hematologic stem cell transplantation who developed GvHD need

antifungal prophylaxis. Due to the effect on the mucosa and the gastrointestinal tract, GvHD complications such as mucositis, infections, vomiting and toxic diarrhea result in reduced absorption of posaconazole suspension formulation.(10)
For this reason, it is recommended to perform therapeutic drug monitoring in patients receiving this formulation, but no study has shown an improved outcome depending on TDM.(8,10,17)

1.4.6 Delayed-release tablet formulation

Based on the fact that oral suspension formulation's plasma concentration is depending on several factors and patients suffering from gastrointestinal problems are often not able to swallow large volumes of medication and therefore do not achieve adequate plasma levels, the need for a better absorbed and easier to use preparation is present.
In April 2014, the EMA approved the gastro-resistant tablet for clinical use, and since June 2015 it is available at the General Hospital in Graz.

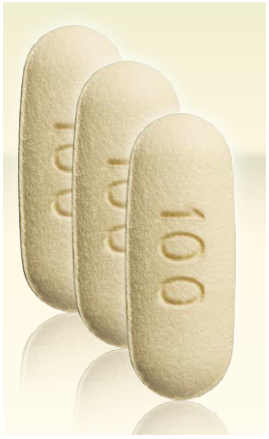


Figure 4: Delayed-release Tablet

For prophylactic use, on day one posaconazole is dosed 300mg (three tablets) twice as a loading dose and afterwards 300mg (three tablets) daily. In case of therapeutic application, the dose is raised to 400mg (four tablets) twice on day one as a loading dose and the following days 400mg (four tablets) once.
Noxafil 100 mg tablets are packaged in a blister in cartons of 24 (2x12) of 96 (8x12) tablets which are yellow-coated, capsule-shaped, length of 17.5 mm debossed with "100" on one side.(44)

According to the enormous variability of absorption of posaconazole oral suspension formulation, the tablet is needed to be released explicitly in the intestine to ensure a higher bioavailability. The gastro-resistant tablet consists of a pH-sensitive polymer guaranteeing that the drug reaches the elevated pH environment (pH 6.8) of the small intestine to be released over 30 min in order to maximize the absorption.

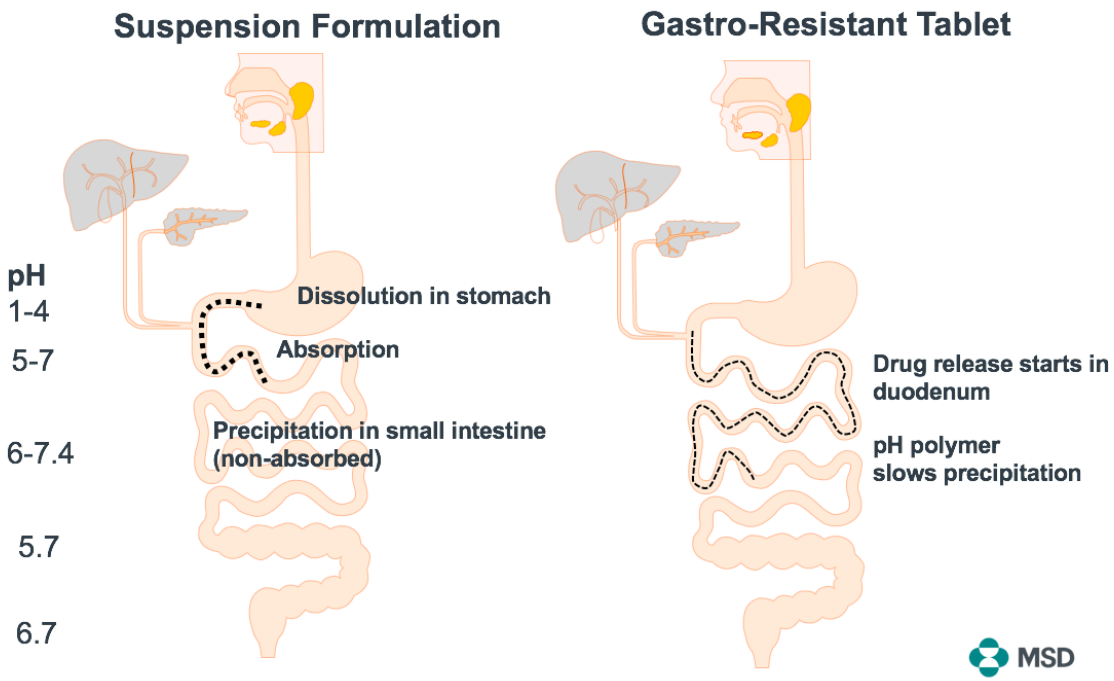


Figure 5: Absorption of Suspension Formulation vs. Gastro-Resistant Tablet - MSD

This goal is achieved by mixing the solid dispersion of posaconazole with the pH-sensitive polymer hypromellose acetate succinate (HPMCAS) at a ratio of 1:3 (weight-to-weight) via a hot melt extrusion process.(44) This is a new strategy to produce formulations with enhanced bioavailability and solubility for drugs which are poorly soluble. Due to drug-polymer combination and termed or solid solutions, the crystallization of the drug is decreased by maintaining this amorphous state. Therefore, improving the solubility of posaconazole in the elevated pH environment of the intestine results in an enhanced absorption.(9)

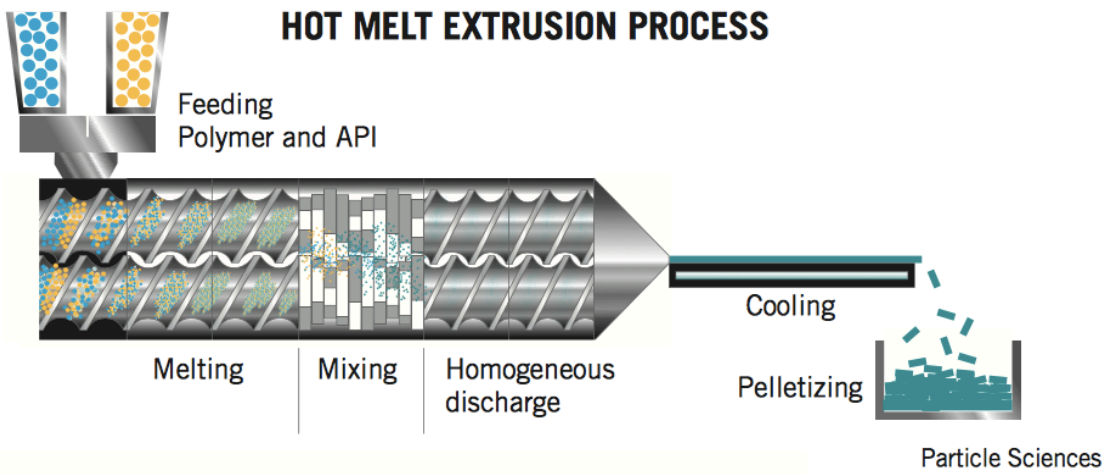


Figure 6: Hot melt extrusion process

Phase one and phase three studies showed high rates of healthy volunteers achieving plasma concentration above the threshold level of 0.5 µg/ml (>95%).(38,39) In hematological patients, several studies presented a significantly higher plasma concentration in patients receiving the delayed-release tablet of posaconazole.(9,11,12,16)

Concomitant administration of medications such as proton pump inhibitors (PPI), or metoclopramide, altering gastric motility or gastric pH is not likely to influence the absorption and is therefore considered as safe.(31,32,45,46) Comparred to the oral suspension formulation, there is no need of application together with food.(47)

In patients suffering from GvHD, the presence of mucositis, diarrhea or other gastrointestinal integrity did not have an impact on achieving threshold levels.(9,16) Concerning to diarrhea, different studies have shown different effects on the plasma level measured.(10) Only one study suggested a significantly lower concentration in patients weighing ≥90kg or in patients with a body mass index ≥30.(48) The findings regarding body weight are of less importance in clinical practice because hematologic patients are normally underweighted.

Steady state has been reached consistently after seven days.(11) Adverse effects are quite similar to the oral suspension formulation, and there is a need to monitor hepatic enzymes, QTc-time, blood cells and plasma levels of several concomitant used drugs mentioned above.

These circumstances underline the superiority of the new gastric resistant tablet and recommend its use in clinical practice.

Therapeutic drug monitoring is suggested for a delayed-release tablet in patients with GvHD and treatment of IFD.(8,10,17) Due to a lack of data, more randomized studies are needed to support this recommendation better. However, compared to other diagnostic procedures, imaging with CT or biomarker for IFD, TDM is relatively cheap, and it can be easily cost-effective concerning the expensive antifungal treatment.

1.4.7 Intravenous formulation

Patients who receive posaconazole as prophylaxis are often affected by their primary medication/chemotherapy which influences the ability to swallow or causes diarrhea and are not able to take oral medications. Also, intubated critically ill patients are unlikely to get oral drugs. In this situation, posaconazole intravenous formulation was approved by EMA in September 2014. Each bottle contains 300mg of posaconazole mixed with the solubilizer sulfobutyl ether-beta-cyclodextrin (SBECD) in 16.7ml stored at 2-8°C. For utilization the concentration is mixed with either 5% dextrose in distilled water, 0.9% or 0.45% sodium chloride, 5% dextrose and 0.45% or 0.9% sodium chloride or 5% dextrose and 20 mEq KCl, maximum end-volume between 150ml and 283ml. Due to a high rate of infusion site reactions when injected peripherally, administration of the intravenous posaconazole formulation is recommended through central venous catheter over 90 min.(49)

Figure 7: Noxafil® Intravenous formulation

The mean plasma concentration is normally beyond the prophylactic threshold level (≥0.7 µg/ml) as well as the therapeutic threshold level (≥1.0 µg/ml). It has a similar safety profile compared to other posaconazole formulations and the effect on the liver and the renal function is minimal.(11,38,39)

1.5 The clinical problem

Having such difficulties to get sufficient plasma concentration of posaconazole by the oral suspension formulation, the development of the delayed-release tablet seems to be the achievement which guarantees adequate trough levels in prophylaxis and treatment of IFD. In healthy volunteers, the blood concentration was beyond the defined threshold level during 28 days.(38,39) Several studies have shown the superiority of posaconazole tablet regarding absorption over the oral suspension in patients receiving posaconazole. Plasma levels were measured on day seven or in one study measured between day five and day fourteen.(12,14,16,36,37) Therefore there is no doubt that the tablet is more efficient than the oral suspension within the first two weeks. Due to the long duration of intake of immunosuppressive agents as prophylaxis of GvHD in patients who received

HSCT (min. 90d) or as concomitant administration during therapy of GvHD, two weeks do not reflect the whole period of posaconazole tablet intake. Additional studies are needed to assess the role of therapeutic drug monitoring for the delayed-release tablet.

This retrospective thesis compares the sufficiency of plasma levels during extended period intake of two groups (DRT vs. OS). Therapeutic drug monitoring during prophylactic intake is analyzed which may be reduced to only one measurement on day seven as proof of steady state or in patients in danger to have reduced plasma levels.

2 Materials and Methods

2.1 Study population

97 patients at the Medical University Hospital of Graz were eligible for this retrospective study, all were receiving posaconazole in different formulations between June 2015 and December 2016. These patients with hematological disorders have been recruited from various wards.

The following inclusion criteria were applied:

- ≥ 18a and ≤ 80a
- receiving posaconazole oral suspension or delayed-release tablet for prophylaxis

One case represented one patient during hospitalization or outpatient treatment. Those patients receiving long-term antifungal prophylaxis were counted as one single case regardless of the number of times they were readmitted. If the formulation has changed, irrespectively to the reason why the patient was excluded.

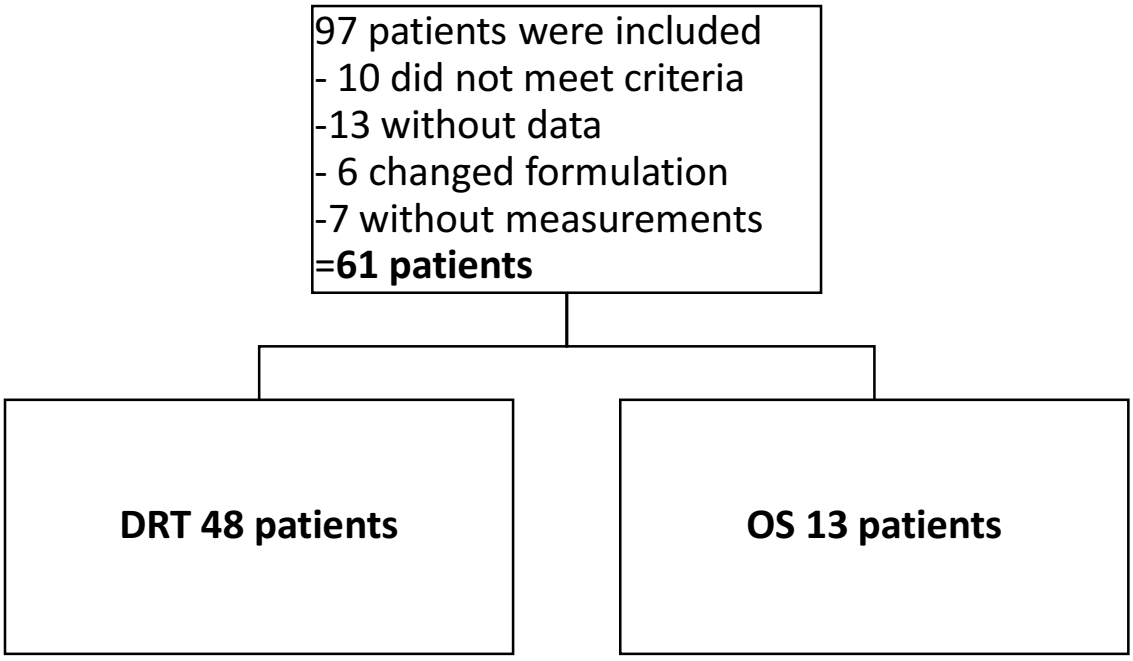


Figure 8: Included patients

Within the 97 patients, ten did not meet the inclusion criteria, thirteen patients had no medical charts at the Medical University Hospital of Graz (only measurements of posaconazole plasma concentration have been made), six patients changed the formulation during their period of prophylaxis, and seven patients had no documented measurements during their intake. In the end, 48 patients received the delayed-release tablet and thirteen patients the oral suspension formulation. The patient characteristics are shown in the following table.

Patient characteristics

			DRT (N=48)	OS (N=13)	P value*
Sex – no.(%)					
	m		24(50)	6(46)	0.001
	w		24(50)	7(54)	0.002
Age		mean	55	49	0.260
BMI		mean	25.18	23.71	0.200
Measurements		mean	7	8	0.550
Primary underlying disease – no.(%)					
	AML		31(65)	3(23)	<0.001
	ALL		1(2)	4(31)	0.180
	CLL		0(0)	1(8)	
	Myelofibrosis		3(6)	0(0)	
	DLBCL		3(6)	1(8)	0.317
	Aplastic anemia		2(4)	0(0)	
	others		8(17)	4(31)	0.248
Duration of intake		mean	92	124	0.280
HSCT – no.(%)					
	allogenic		34(71)	8(62)	0.039
GvHD – no.(%)					
	Yes		25(52)	5(38)	<0.001
PPI – no.(%)					
	Yes		34(71)	8(62)	<0.001
Diarrhea – no.(%)					
	Yes		12(25)	1(8)	0.002

Table 10: Patient characteristics

*P values for continuous variables were calculated with Student's t-test; P values for categorical variables were calculated with Fisher's exact test.

2.2 Study design

The retrospective study was conducted from the 1st of June 2015 to the 31st of December 2016 at the Division of Infectious Diseases and the Division of Hematology at the Medical University Hospital of Graz, Internal Medicine. Patients receiving posaconazole oral suspension or delayed-release tablet for prophylaxis due to hematological disorders have been included. At the Medical University Hospital of Graz, PCZ prophylaxis is mostly described in patients with prolonged neutropenia due to (re-)induction chemotherapy or in patients with GvHD treatment.

The patient's characteristics such as concomitant medication, laboratory assessment, the dosage of PCZ, intake time, microbiological data, clinical data on the outcome of therapy and adverse events have been extracted from chart reviews and openMedocs. Each patient's medical record has been reviewed individually using an excel data collecting temple. Posaconazole plasma levels were obtained in the morning prior to scheduled PCZ intake during routine outpatient treatment, normally every 7-10 days or during hospitalization. A cutoff point of ≥0.7 µg/ml was determined according to previous study recommendations.(17,36,37) Levels above the target were defined as satisfactory and those below the target as low.

Trough posaconazole plasma concentrations were measured by an in-house laboratory employing CE-IVD-marked Chromsystems PCZ Reagent Kit (Chromsystems GmbH, Munich, Germany) based on high-performance liquid chromatography, analyzed by an UltiMate 3000 chromatography device (Dionex, Sunnyvale, CA, USA) and by Triple Quadrupole-TSQ-System (Thermo Fisher, Palo Alto, CA, USA) with Electrospray-Ionization-(ESI) source. The intraday coefficients of variation (CVs) was <6.0% and the interday CVs was <10.0% with a detection limit of 0,05µg/ml. At Medical University Hospital of Graz, posaconazole plasma levels of <0.2 µg/ml (issued as 0.2 µg/ml) are not further differentiated.

Measurements between day 1-6 and day 7-14 are compared to each other. Satisfactory PPCs were analyzed all together in the DRT and in the OS group.
According to previous studies with max. of three measured levels, patients were analyzed three times - min. one plasma concentration obtained, min. two obtained and min. three measurements during their period of intake.(12,14,16,36,37) These

mean values are showing the percentage of satisfactory PPCs referring to the intake period of each patient.

Hospitalization due to neutropenic fever and the need of changing antifungal medication has also been recorded. Severe adverse events like QT prolongation, neutropenia and hepatotoxicity have been observed by ECG or laboratory assessment of blood cell count and liver enzymes such as alanine aminotransferase (AST), aspartate aminotransferase (ALT) and alkaline phosphatase (AP). Mild events like a headache, abdominal pain, nausea or vomiting were asked during outpatient visits or daily assessment.

Acute GvHD was classified according to Glucksberg et al.(50), and chronic GvHD according to NIH – consensus criteria 2005 (51). Two groups of GvHD grading were made: group 1 includes acute GvHD grading 1-2 and chronic GvHD mild and moderate, group 2 contains acute GvHD grading 3-4 and chronic GvHD severe.

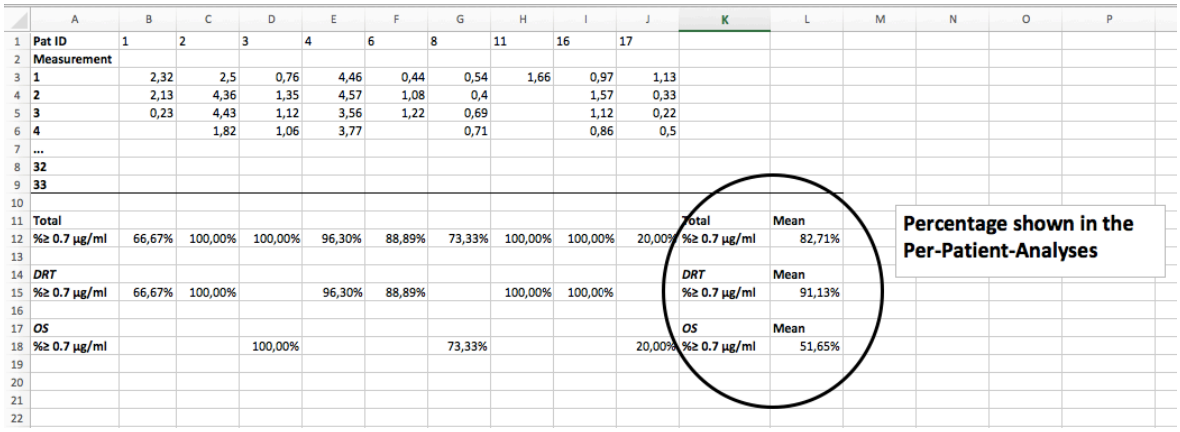
Break through infections have been defined by detection of antifungal presents as “proven”, “probable” or “possible” according to the European Organization of Research and Treatment of Cancer, Mycoses Study Group.(24)

The study adhered to the Declaration of Helsinki (1996) and Good Clinical Practice and the study protocol was approved by the local ethics committee of Medical University of Graz (Graz, Austria).

2.3 Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows v.23.0.0.0 (IBM Corp., Armonk, NY) and Microsoft Excel for IOS v.15.32 (Microsoft Corp., Redmond, WA). Continuous data are present as mean or median and interquartile range, categorical variables as numbers and percentage. The analyzation of OS and DRT group was performed three times relating to minimum measurements taken – one, two and three. Pre-steady-state PPCs in patients receiving DRT or OS and obtained from day 1 to 6 were compared between the two formulations and to steady-state - day 7-14.

During intake time, the percentage of satisfactory posaconazole plasma concentrations is calculated for each patient separate and issued as a mean value for each group DRT or OS, as shown in figure 9. For alternating factors like diarrhea, GvHD, and concomitant PPI intake the same procedure was used.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	**Pat ID**	**1**	**2**	**3**	**4**	**6**	**8**	**11**	**16**	**17**						
2	**Measurement**															
3	**1**	2,32	2,5	0,76	4,46	0,44	0,54	1,66	0,97	1,13						
4	**2**	2,13	4,36	1,35	4,57	1,08	0,4		1,57	0,33						
5	**3**	0,23	4,43	1,12	3,56	1,22	0,69		1,12	0,22						
6	**4**		1,82	1,06	3,77		0,71		0,86	0,5						
7	**...**															
8	**32**															
9	**33**															
10																
11	**Total**										**Total**	**Mean**				
12	**%≥ 0.7 µg/ml**	66,67%	100,00%	100,00%	96,30%	88,89%	73,33%	100,00%	100,00%	20,00%	**%≥ 0.7 µg/ml**	82,71%				
13																
14	***DRT***										***DRT***	**Mean**				
15	**%≥ 0.7 µg/ml**	66,67%	100,00%		96,30%	88,89%		100,00%	100,00%		**%≥ 0.7 µg/ml**	91,13%				
16																
17	***OS***										***OS***	**Mean**				
18	**%≥ 0.7 µg/ml**			100,00%			73,33%			20,00%	**%≥ 0.7 µg/ml**	51,65%				
19																
20																
21																
22																

Percentage shown in the Per-Patient-Analyses

Figure 9: Per-Patient-Analyses

Analyses of continuous data were made by Student's t-Test and categorical data by Mann-Whitney-U test. A p-value of <0,05 was considered as statistically significant.

3 Results

3.1 Per-sample analyses

Overall 515 posaconazole plasma concentrations were obtained in 97 patients with hematological disorders, of which 36 patients (69 plasma levels) were excluded due to several reasons mentioned above. Leaving 446 included measurements: 382/446 (85.65 %) achieved target level of 0.7 µg/ml, DRT 297/325 (91.38 %) vs. OS 85/121 (70.25 %) – p-value <0.001.

The average concentration was 2.0 µg/ml, with a minimum of 0.2 µg/ml and a maximum of 7.6 µg/ml, interquartile range [IQR] 0.95-2.71 µg/ml.

3.2 Per-patient analyses

In contrast to previous studies which analyzed only one to three posaconazole plasma concentrations each obtained on the first 14 days, this analyzation was divided into three parts with a different minimum of measurements. The median duration of PCZ prophylaxis was 92 days (min. 8, max. 341, interquartile range [IQR]

38-121) in patients receiving DRT and 124 days (min. 24, max. 294, IQR 41-211) in patients receiving OS.

3.2.1 Patients with minimum of one measurement

61 patients with 446 posaconazole plasma concentration were enrolled in this analyzation. 48 Patients receiving delayed-release tablet (no. of PPCs mean 7.06, median 4, min. 1 and max. 30, interquartile range [IQR] 2-8) and 13 patients receiving oral suspension formulation (no. of PPCs mean 8.23, median 7, min. 1, max. 17, interquartile range [IQR] 3-15). During intake time 91.1% in the DRT group and 51.6% in the OS group of mean-PPCs were satisfactory (p-value 0.001).

	DRT (N=48)	**OS (N=13)**	**p-value**
% ≥ 0.7 µg/ml	91.1%	51.6%	0.001
DRT (N=48) no./% PPCs			
	pos. vs. neg.		*p-value*
GVHD	25/88.3% vs. 23/94.2%		0.007
PPI	34/91.1% vs. 14/91.2%		0.910
Diarrhea	12/95.1% vs. 36/89.8%		0.717
OS (N=13) no./% PPCs			
	pos. vs. neg.		*p-value*
GVHD	5/60.3% vs. 8/46.3%		0.622
PPI	8/57.6% vs. 5/42.2%		0.524
Diarrhea	1/73.3% vs. 12/49.8%		0.833

Table 11: Results min. one measurement

In the DRT group, there was a statistically significant difference between patients suffering from GvHD (25/88.3% vs. 23/94.2%, p-value 0.007) in reaching PPCs above the threshold level of 0.7 µg/ml. Analyzing GvHD in subgroups of grading, no statistical significance was seen – group 1 92.5% (10 patients of which 5 had gastrointestinal GvHD) vs. group 2 85.5 % (15 patients of which 11 had gastrointestinal GvHD) – p-value 0.196. In patients with concomitant PPI intake (34/91.1% vs. 14/91.2%, p-value 0.910) or diarrhea (12/95.1% vs. 36/89.8%, p-value 0.717) no difference has been detected.

Neither GvHD, nor concomitant PPI intake or diarrhea do have an alternating effect on PPC in patients using the oral suspension formulation. No difference in satisfactory PPCs percentage has been found with GvHD (5/60.3% vs. 8/46.3%, p-

value 0.622), PPI (8/57.6% vs. 5/42.2%, p-value 0.524) or diarrhea (1/73.3% vs. 12/49.8%, p-value 0.833) in this analysis.

Among those receiving DRT insufficient PPCs were observed in 29% (14/48) of patients, while 69% (9/13) of those receiving OS had at least one insufficient PPC (p=0.008). 34 of 48 patients (71%) receiving DRT always had sufficient PPCs, while 13 of 48 patients (27%) had at least one insufficient PPC (one additional patient had only a single PPC which was below the target). In patients receiving OS 4 of 13 (31%) always had sufficient PPCs, 6 of 13 patients (46%) had at least one insufficient PPCs, and 3 (23%) patients never reached a PPC of 0.7mg/L (one, two, and four measured PPCs respectively).

Four patients occurred not to have PPCs reaching the threshold level of 0.7 µg/ml during the whole period of measuring. Two patients only had one measured plasma level, one patient hat two and the fourth patient had four obtained levels. Number 30, 52, and 89 have been on oral suspension formulation which is suggested to be the reason for unsatisfactory plasma concentrations. Although patient 56 took the delayed-release tablet, measured plasma levels did not exceed 0.7 µg/ml, maybe because he was suffering from HIV-associated lymphoma.

Patient ID	**30**	**52**	**56**	**89**
PCZ	OS	OS	DRT	OS
PPC	0.37	0.44	0.41	0.32
	0.34			0.20
				0.46
				0.20
no. of PPCs	2	1	1	4

Table 12: Patients below cutoff during hole intake period

3.2.2 Patients with minimum of two measurements

In this subject, only patients are analyzed having a minimum of two measured posaconazole plasma concentrations during the period of intake. 51 patients were enrolled, 39 received the delayed-release tablet (no. of PPCs mean 8.55, median 7, min. 2, max 30, interquartile range [IQR] 3-10) and 12 received the oral suspension formulation (no. of PPCs mean 8.46, median 6, min. 2, max. 17, interquartile range [IQR] 5-15). During the period of posaconazole intake, the percentage of satisfactory PPC was 91.6% in the DRT group and 56.0% in the OS group, p-value 0.004.

	DRT (N=39)	**OS (N=12)**	**p-value**
% ≥ 0.7 µg/ml	91.6%	56.0%	0.004
DRT (N=39) no./% PPCs			
	pos. vs. neg.		*p-value*
GVHD	24/87.8% vs. 15/97.8%		0.028
PPI	30/93.2% vs. 9/86.3%		0.366
Diarrhea	11/94.7% vs. 28/90.5%		0.842
OS (N=12) no./% PPCs			
	pos. vs. neg.		*p-value*
GVHD	5/60.3% vs. 7/52.9%		0.876
PPI	8/57.6% vs. 4/52.7%		1.000
Diarrhea	1/73.3% vs. 11/54.4%		1.000

Table 13: Results min. two measurements

On closer inspection of the different groups, only in the DRT group a statistical significance was reached in patients suffering from GvHD (24/87.8% vs. 15/97.8%, p-value 0.028). Analyzing GvHD in subgroups of grading, no statistical significance was seen – group 1 92.5% (10 patients of which 5 had gastrointestinal GvHD) vs. group 2 84.5 % (14 patients of which 10 had gastrointestinal GvHD) – p-value 0.138. During concomitant PPI intake (30/93.2% vs. 9/86.3%, p-value 0.366) and during periods of diarrhea (11/94.7% vs. 28/90.5%, p-value 0.842) no difference was detected.

Similar results in the OS group, GVHD (5/60.3% vs. 7/52.9%, p-value 0.876), PPI (8/57.6% vs. 4/52.7%, p-value 1.000) and diarrhea (1/73.3% vs. 11/54.4%, p-value 1.000). Despite the effects appeared in previous studies, no statistical significance in PPC alternating with oral suspension formulation was found.

3.2.3 Patients with minimum of three measurements

The last analyzation shows similar outcomes. Now, only patients with three or more obtained posaconazole plasma concentrations are enrolled, 35 in the DRT group (no. of PCC, mean 9.20, median 7, min. 3, max. 30, interquartile range [IQR] 4-11) and 10 patients in the OS group (no. of PCC, mean 10.20, median 9, min 3, max 17, interquartile range [IQR] 6-15). Comparing the percentage of satisfactory PPC (90.7% vs. 62.1%, p-value 0,073) no statistical significance is shown. This could be due to the reduced number of patients in the OS group, taking to the high difference in total percentage into account.

	DRT (N=35)	**OS (N=10)**	**p-value**
% ≥ 0.7 µg/ml	90.7%	62.1%	0.073
DRT (N=35) no./% PPCs			
	pos. vs. neg.		*p-value*
GVHD	22/86.7% vs. 13/97.4%		0.026
PPI	26/92.2% vs. 9/86.3%		0.469
Diarrhea	10/94.1% vs. 25/89.3%		0.900
OS (N=10) no./% PPCs			
	pos. vs. neg.		*p-value*
GVHD	5/60.3% vs. 5/64.0%		0.841
PPI	7/58.7% vs. 3/70.3%		0.667
Diarrhea	1/73.3% vs. 9/60.9%		1.000

Table 14: Results min. three measurements

The remaining subject showed similar results. Patients suffering from GvHD in the DRT group reached statistically significance (22/86.7% vs. 13/97.4%, p-value 0.026), PPI (26/92.2% vs. 9/86.3%, p-value 0.469) and diarrhea (10/94.1% vs. 25/89.3%, p-value) did not. Analyzing GvHD in subgroups of grading, no statistical significance was found – group 1 92.5% (10 patients of which 5 had gastrointestinal GvHD) vs. group 2 81.9 % (12 patients of which 9 had gastrointestinal GvHD) – p-value 0.059.

During oral suspension intake, again no significance was found. Neither in GvHD (5/60.3% vs. 5/64.0%, p-value 0.841), PPI (7/58.7% vs. 3/70.3%, p-value 0.667) nor in diarrhea (1/73.3% vs. 9/60.9%, p-value 1.000).

3.3 Posaconazole plasma levels on different days

As in previous studies, posaconazole plasma concentrations were compared on three separate days:

- Pre-steady-state – day 3±1
- Steady-state – day 7±1
- Late-steady-state – day 14±1

Because of the small number of measurements, the PPCs were combined between the periods of day 1 to day 6 and day 7 to day 14.

When PPCs were measured during clinical routine, blood samples were not explicitly collected on these defined periods, and the available plasma levels are obtained coincidentally. Therefore, it is likely to not have measurements in both periods in all patients.

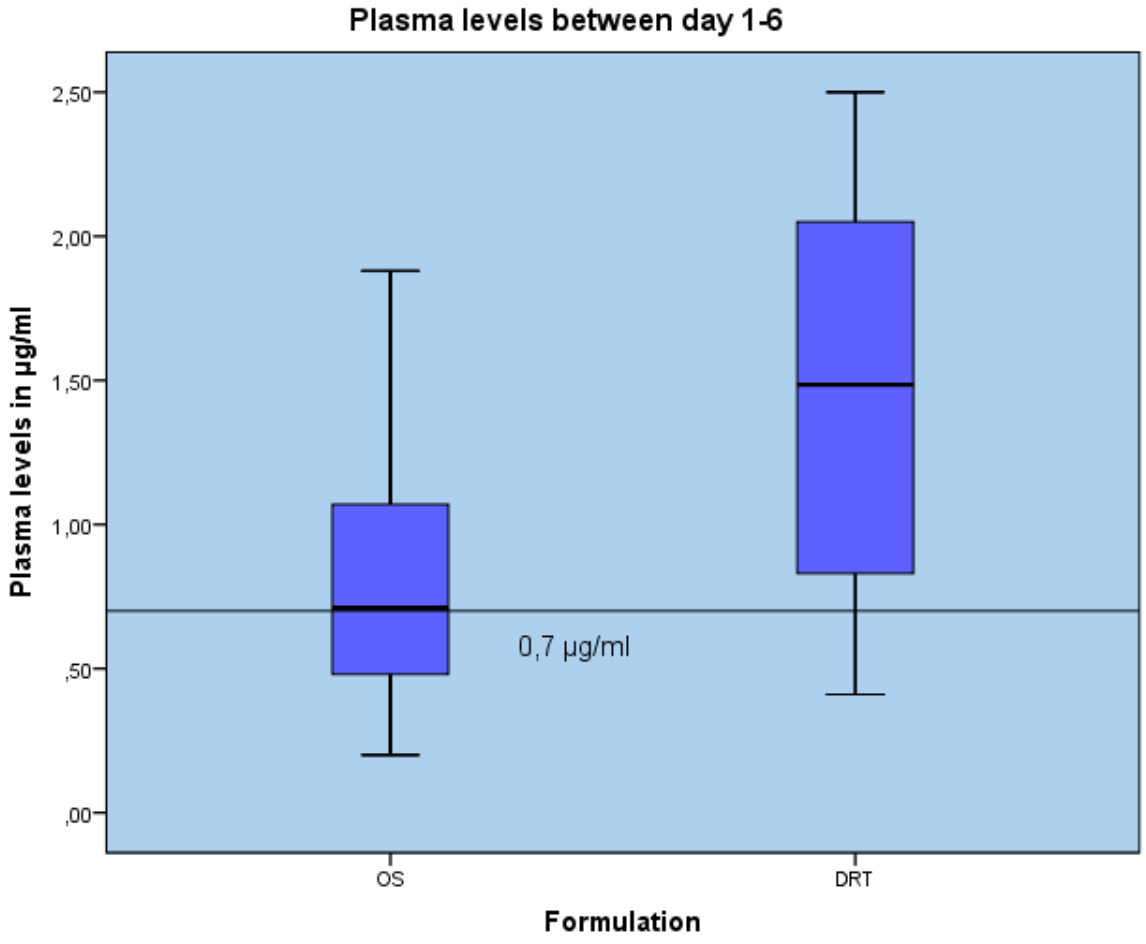


Figure 10: Boxplot plasma levels days 1-6

On the days 1-6, 11 PPCs in the OS group and 22 PPCs in the DRT group were obtained. The blood concentration was significantly higher in the posaconazole tablet formulation group (mean 1.47 µg/ml, SD 0.64, min. 0.41 µg/ml, max. 2.50 µg/ml, interquartile range [IQR] 0.86-1.97 µg/ml) compared to the OS group (mean 0.82 µg/ml, SD 0.49, min. 0.20 µg/ml, max. 1.88 µg/ml, interquartile range [IQR] 0.48-1.07 µg/ml) – p-value 0.008, Mann-Whitney-U test.

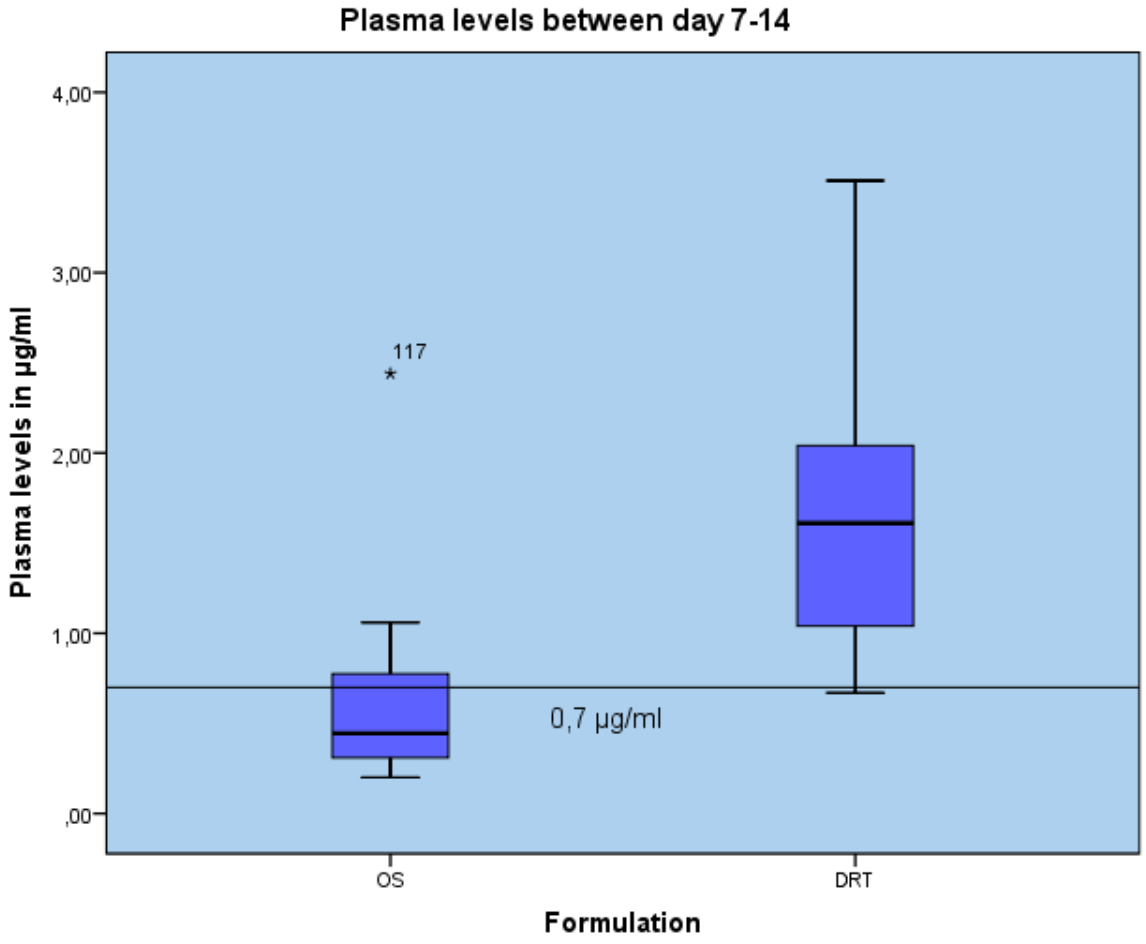


Figure 11: Boxplot plasma levels days 7-14

On days 7-14, 12 PPCs were obtained in the OS group and 28 in the DRT group. Like in the measurement on days 1-6, plasma levels are significantly higher in patients receiving posaconazole tablet formulation and overall the threshold level was reached in almost all measurements. DRT group (mean 1.63 µg/ml, SD 0.68, min. 0.67 µg/ml, max. 3.51 µg/ml, interquartile range [IQR] 1.05-2.04 µg/ml) compared to the OS group (mean 0.66 µg/ml, SD 0.62, min. 0.20 µg/ml, max. 2.4

µg/ml, interquartile range [IQR] 0.31-0.74 µg/ml) – p-value <0.001, Mann-Whitney-U test.

Comparison of days 1-6 to days 7-14 shows an early hit of the threshold level in both groups but no further increase of posaconazole plasma concentrations – OS (mean: 0.82 µg/ml to 0.66 µg/ml, p-value 0.144) or DRT (mean: 1.47 µg/ml to 1.63 µg/ml - p-value 0.398).

3.4 Adverse events

Only two patients complained about side effects, like loss of appetite, vomiting, and blurred vision. One patient receiving the delayed-release tablet reporting these symptoms, had to discontinue posaconazole intake due to increased liver enzymes [bilirubin 2.776 mg/dl – normal range 0.10-1.20 mg/dl, alanine aminotransferase (AST) 66 U/L – normal range 0-35 U/L, gamma-glutamyltransferase (GGT) 1046 U/L – normal range 0-55 U/L, alkaline phosphatase (AP) 145 U/L – normal range 40-130 U/L] indicating hepatotoxicity after one month of intake.
The second patient, receiving the oral suspension formulation got switched to the delayed-release tablet after two months of intake because of nausea and loss of appetite, but no hepatotoxicity has been seen.

The Medical University Hospital of Graz's clinical practice in patients with neutropenic fever (>38.5°C) includes Chest-X-Ray or CT-Thorax as well as blood cultures taken peripherally or centrally if a central venous catheter is applied. Antimicrobiological drugs are primarily changed to intravenous formulations. Having started with cefepime as a first-line antibiotic drug, the regime changes after persistence of fever for 48-72h to meropenem and linezolid. If the temperature does not decrease, Chest-X-Ray or CT-Thorax are repeated, and fungal detection tests are made. Changes in antifungal or antibiotic agents are made accordingly to the pathogen found, or as an empiric approach. The choice of drug is also dependent on the drugs used as prophylaxis and liver or kidney impairment.

Despite high percentages of satisfactory posaconazole plasma concentrations, 33 (27 DRT, 6 OS) of 61 enrolled patients (54.1%) had to be admitted to hospital due to neutropenic fever or increase of temperature of unknown source. It was

necessary to switch antifungal prophylaxis in 19 (16 DRT, 3 OS) patients (57.6%) assuming fungi resistant to posaconazole are causing these circumstances. Alternatively used agents that are used are Mycamine® (micafungin), Vfend® (voriconazole), Cancidas® (caspofungin) and Ambisome® (amphotericin B).

3.5 Breakthrough infection

Only one patient was diagnosed with proven C. glabrata orbital abscess due to pansinusitis during posaconazole intake, defined as break through infection – incidence1/61, 1.6%, which is as in the range documented by previous studies (0-3% incidence of breakthrough infection in posaconazole prophylaxis).(6,7,12,16,36,52,53)

In the following, a summary of this case is provided.

3.5.1 Orbital abscess

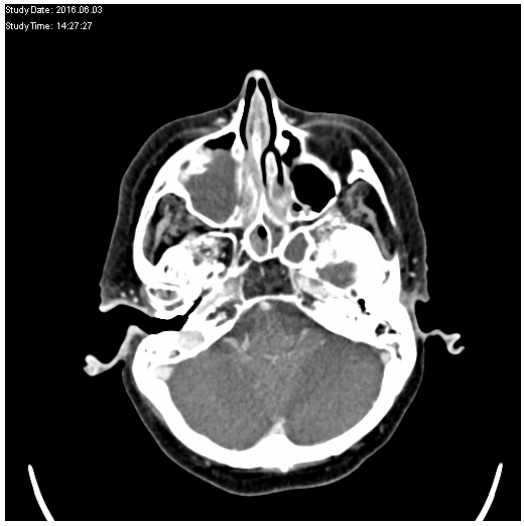


Figure 12: CT-Head 3.6.2016

The patient was admitted to the Medical University Hospital of Graz on 30.5.2016 due to fever and weakness. He was suffering from MDS which transformed into AML and therefore received an allogeneic stem cell transplantation on 22.1.2016. Further diagnoses were chronic obstructive lung disease COPD, a colonization of 4MRGN Klebsiella pneumoniae and acute GvHD treated with Urbason®. He already took Noxafil DRT (300mg/d) as antifungal prophylaxis and further a trip with Optinem® (meropenem) was started. A head CT taken on 3.6.2016 showed pansinusitis which lead the physician to extend the antibiotic spectrum with Rokiprim® (Cotrimoxazole) and Doxycyclin®.

Despite the broad antimicrobial spectrum, the patient's neurological status got worse and an MRT taken on 9.6.2016 showed signs of meningoencephalitis and right orbital abscess. The last obtained PPC on 30.5.2016 was 3.0 µg/ml.
The patient was taken to the ICU. On the same day, polypectomy and removal of the abscess were made. Noxafil® got switched to Ambisome® (Amphotericin B).

The bacteriological culture obtained from the abscess presented Candida glabrata. Empiric change of antifungal therapy should be effective against this yeast.

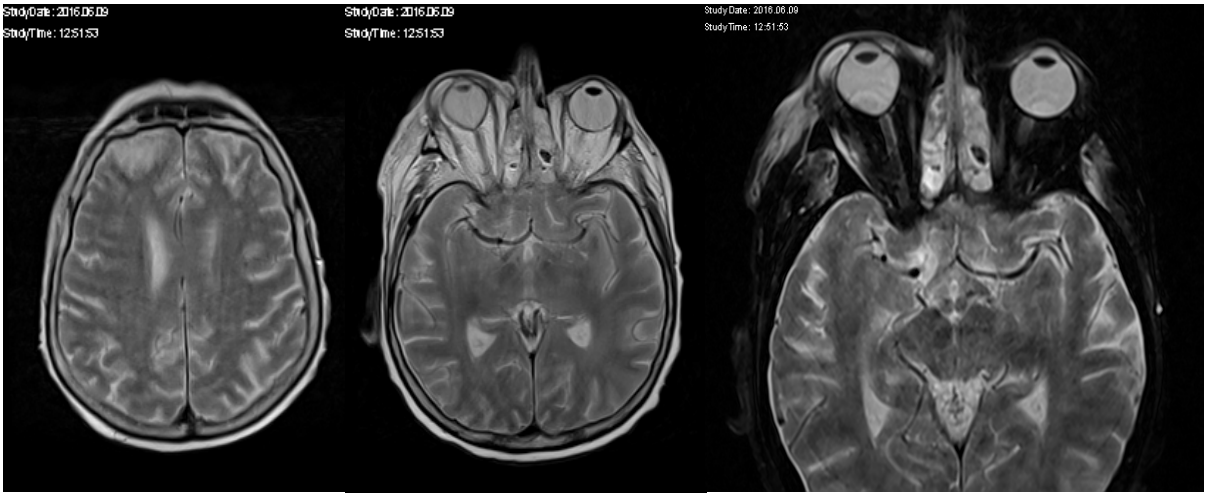


Figure 13: MRT-Head 9.6.2016

An MRT taken on 15.6.2015 showed complete removal of the orbital abscess but worsening of the meningoencephalitis and a subtotal ischemic stroke in the region of the pons.

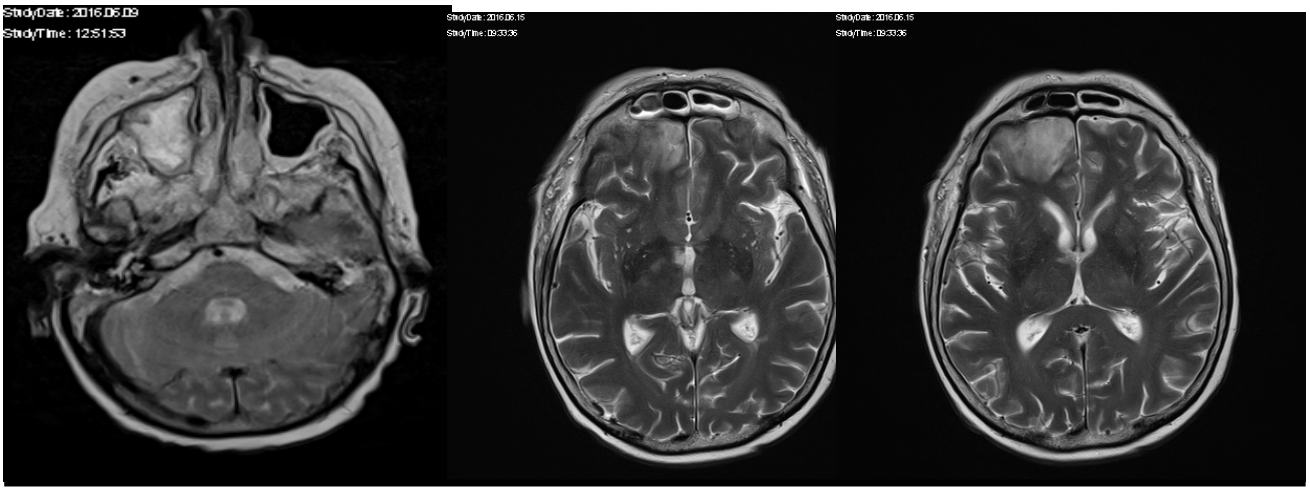


Figure 14: MRT-Head 15.6.2015

Due to the poor prognosis, best supportive care was initiated, and unfortunately, the patient died on 19.6.2016.

4 Discussion

Several studies have shown the superiority of tablet formulation compared to oral suspension formulation in patients with hematological disease preventing invasive fungal infections during neutropenia or concomitant intake of immunosuppressive drugs. These findings are based on a few measurements taken in early intake periods and do not represent the whole time frame of prophylaxis. Therapeutic drug monitoring was usually performed even though no evidence for the delayed-release tablet was existing, and these recommendations are only based on findings from the oral suspension formulation. Only two studies had more patients receiving DRT (64 and 86) and evaluated posaconazole plasma concentrations, but measurements were only made on day 7.(16,37) Despite the fact, that posaconazole is used in prophylaxis of GvHD and at least taken for 90 days, PPCs after day 14 have not been evaluated yet.

In October 2017 Tallman et al. presented a poster at IDWeek 2017, San Diego, Ca. including 547 patients, this is so far the largest trial comparing PCZ OS and PCZ DRT. The findings of satisfactory PPCs show similarities to this thesis – DRT 90.6% and OS 60.5% - p-value <0,001. The rate of break through infections is the same: 1.6%. In contrast, therapeutic drug monitoring and the need to change antifungal agents are not described on this poster, and the mean duration of intake was only 25 days.

Therapeutic drug monitoring was performed in clinical practice, and satisfactory plasma levels were seen in about 90% of patients during their period of intake. The findings of this study are identical to clinical experience, no matter if the minimum was set to one, two or three measurements (91.1%, 91.6%, 90.7%). Therefore, it showed a high percentage of satisfactory posaconazole plasma concentrations over the whole period of prophylaxis, also compared to the oral suspension formulation.

Several factors are known to influence the bioavailability of OS like diarrhea, GvHD, concomitant intake of PPIs or metoclopramide or simultaneous high-fat food administration, but none reached statistical significance in this study. As these findings are evidence-based, the different results may be due to the small number (13) of patients on oral suspension formulation.

In patients who were taking DRT, only GvHD was observed to influence posaconazole plasma concentrations negatively but not diarrhea or PPI.
Comparing days 1-6 and days 7-14 of intake, these results are similar to previous studies. DRT reached the threshold level of 0.7 µg/ml within six days with a significant difference to OS. After two weeks, plasma concentrations did not increase on average (1.53 µg/ml, 1.68 µg/ml, and 1.71 µg/ml) suggesting a pre-steady state measurement is sufficiently informative for steady state which also has been shown by Prattes et al.(54)

Adverse events were seen in only two patients, one receiving DRT suffering from hepatotoxicity and the other receiving OS suffering from appetite loss and visual disturbance. After termination of posaconazole intake, these symptoms improved and elevated liver enzymes normalized within ten days. The associated adverse effects on posaconazole intake can be monitored and treated efficiently.

Break through infections have been found in about 0-3% in previous studies and these findings are the same as in this thesis as only one patient has been diagnosed with proven Candida glabrata abscess due to pansinusitis (1.6%). Two patients were found to have colonization of C. glabrata, which does not meet the criteria for IFD (EORTC/MSC) and therefore do not need antifungal therapy.(55) The delayed-release tablet is highly effective in preventing invasive fungal infection according to the small number of break through infections. As a colonization is the primary source of infections with Candida and patients with neutropenia or patients receiving immunosuppressive agents are at high risk for IFD and treatment with antifungal drugs is indicated when colonization is detected.(56–59) C. albicans accounts only for half of the isolates identified and C. glabrata has become a more important pathogen in the northern Europe, the United States, and Canada. Immediately identification of it leads to adequate treatment with effective antifungal drugs.(4)

Therapeutic drug monitoring is yet established in clinical practice and well recommended in the oral suspension formulation. The lack of evidence in patients receiving posaconazole DRT leads to suggestions for this formulation based on studies concerning OS. Since plasma concentrations are only influenced by diarrhea or GvHD, adverse events can easily be monitored by ECG or by laboratory

assessment. Therapeutic drug monitoring may be recommended in selected patients suffering from these conditions. Further studies are needed to precisely assess the role of TDM in DRT and assess the financial benefit according to the reduced costs of not performing TDM and the costs of invasive fungal infections probably caused by missed posaconazole plasma levels below the threshold level.

4.1 Strengths and limitations

This study is strengthened by the high number of posaconazole plasma concentration measurements obtained during the whole period of intake and the high number of patients receiving DRT compared to previous studies. Sufficient trough levels were additionally analyzed for each patient which represents the intake time frame better.

It is limited by the range of measurements (1-30) between different patients, and if plasma levels were obtained on the same day, they would be more comparable. This can only be achieved by having regular time intervals between the measurements and therefore requires a prospective study design.

4.2 Conclusion and perspectives

This thesis retrospectively analyzed the sufficiency of posaconazole plasma concentrations during prophylaxis intake of posaconazole delayed-release tablets compared to oral suspension formulations in patients with hematological disorders. The present analysis showed a superiority of DRT to OS in achieving sufficient plasma levels. These findings indicate that therapeutic drug monitoring may not be necessary for all patients and can be reduced to selected cases. However, further studies are needed to evaluate the role of therapeutic drug monitoring.

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