

Diploma Thesis

**Invasive fungal infections in patients with hematological malignancies receiving ibrutinib therapy (INFINITY)**

submitted by

**Egger Matthias**

for the academic degree of

**Doctor of medicine (Dr.med.univ.)**

at the

**Medical university of Graz**

**Department of Internal Medicine, Section of Infectious Diseases and Tropical  
Medicine**

under the supervision of

Priv.Doiz. Dr.med.univ. Dr.scient.med Juergen Prattes

Dr.med.univ. Christoph Zurl

Graz, am 30.11.2021

## **Declaration**

I declare that I have written this diploma thesis independently, that I have not used other than the sources/resources cited, and that I have explicitly marked all material which has been quoted either literally or by content from the sources used.

Graz, am 30.11.2021

Egger Matthias, eh.

## Acknowledgments

To begin with, I want to thank my supervisor Juergen Prattes, M.D, who constantly supported me on several occasions throughout recent years, giving me an understanding and introduction to the practice of research.

I would particularly like to thank my family for their unconditional aid and encouragement at any time throughout my whole study period. Together with awesome friends, who accompany me for many years now, I can look back on a blessed chapter of my life.

## Contents

1.	List of Abbreviations.....	6
2.1	List of Tables.....	7
2.2	List of Figures .....	7
3.	Abstract .....	8
3.1	Background.....	8
3.2	Methods .....	8
3.3	Results .....	8
3.4	Conclusion.....	8
4.	Zusammenfassung.....	10
4.1	Hintergrund.....	10
4.2	Methoden.....	10
4.3	Ergebnisse .....	10
4.4	Konklusion .....	11
5.	Introduction .....	12
5.1	Invasive fungal infections in patients with hematologic malignancies.....	12
5.1.1.	Invasive Aspergillosis.....	13
5.1.2.	Invasive Candidiasis.....	14
5.1.3.	Others.....	16
5.2.	Risk factors for IFIs in patients with hematologic malignancies .....	17
5.3.	Bruton’s Tyrosine Kinase.....	18
5.4	Ibrutinib.....	18
5.4.1	Invasive fungal infections in patients receiving ibrutinib therapy.....	19
5.4.1.1	<i>Current Data</i> .....	19
5.4.1.2	Potential mechanisms how ibrutinib enhances susceptibility to IFIs .....	20
5.5.	Diagnostic criteria for IFI.....	21
5.6	Importance of local epidemiology .....	24
6.	Material and Methods.....	25

6.1. Aim of the study .....	25
6.2. Study design .....	25
6.3. Study population .....	25
6.3.1. Data collected from each patient.....	26
6.4. Statistical analysis.....	27
7. Results .....	28
7.1 Patient with IFI .....	30
7.1.1 Demographics of patients with IFIs.....	30
7.1.2 Patient with invasive aspergillosis.....	31
7. Discussion.....	33
References.....	37

## 1. List of Abbreviations

AE = adverse event

AML = acute myeloid leukemia

BTK = Bruton's tyrosine kinase

CNS = central nervous system

COPD = chronic obstructive pulmonary disease

CLL = chronic lymphocytic leukemia

ECRF = electronic case report form

EMA = European Medicines Agency

EORTC/MSG = European Organization for Research and Treatment of Cancer/Mycoses Study Group

FDA = Food and Drug Administration

HSCT = hematopoietic stem cell transplantation

IA = invasive aspergillosis

ICU= intensive care unit

IFI = invasive fungal infection

IQR = interquartile range

MG = milligram

NHL = non-Hodgkin's lymphoma

PAMP = pathogen associated molecular pattern

PRR = pathogen recognition receptor

SD = standard deviation

SMKI = small molecule kinase inhibitor

WM = Waldenstroms macroglobulinemia

## 2.1 List of Tables

Table 1 Discussion of potential mechanisms how Ibrutinib enhances susceptibility to IFIs .....	20
Table 2 Criteria for proven invasive fungal infection .....	22
Table 3 Criteria for probable invasive mold infection.....	22
Table 4 Baseline characteristics of patients.....	28
Table 5 Underlying hematological malignancy .....	30
Table 6 Characteristics of Patients with IFIs.....	32

## 2.2 List of Figures

Figure 1 a-c.....	32
-------------------	----

## 3. Abstract

### 3.1 Background

Since the broad implementation of ibrutinib, an irreversible Bruton's tyrosine kinase (BTK) inhibitor approved in 2013 for the treatment of non-Hodgkin's lymphoma (NHL) including chronic lymphocytic leukemia (CLL), an increasing number of case series and retrospective studies reporting on infectious complications like invasive fungal infections (IFIs) associated with ibrutinib administration have emerged. Epidemiologic data, as well as the exact mechanisms and extent of ibrutinib enhancing susceptibility to IFIs, is fragmentary. We tried to shed a little more light on the role of ibrutinib acting as an independent risk factor for IFIs, and the dynamics of infection events in patients receiving it.

### 3.2 Methods

We conducted a two-centered retrospective study by analyzing electronic medical records of patients who received ibrutinib between October 2020 and August 2021. Presence of IFI was identified by examination of laboratory, radiological and microbiology findings according to EORTC/MSG diagnostic criteria. Prevalence for IFIs in investigated patients was calculated.

### 3.3 Results

Ninety-seven patients with NHL and ibrutinib therapy were retrospectively analyzed. CLL was the most frequent underlying hematological malignancy in 76 cases. Forty-eight percent of patients received previous treatment lines for their underlying diseases. One patient developed a probable IFI according to 2019 EORTC/MSG diagnostic criteria implying a prevalence of 0.97%. Invasive pulmonary aspergillosis was the underlying type of IFI, with *Aspergillus fumigatus* being the causative pathogen. The patient with IFI had concomitant established risk factors (e.g. neutropenia, corticosteroid usage) and received one previous treatment line. The patient died approximately 2.5 years after the IPA episode due to a coronavirus-19 associated ARDS episode.

### 3.4 Conclusion

Incidence of IFIs was low in this study, with only a single case diagnosed with invasive aspergillosis. The case of invasive fungal infection was diagnosed in a patient who had concomitant risk factors for IFIs, besides ibrutinib therapy. Thus, standardized approaches

for the appropriate risk stratification of IFI occurrence, including baseline risk factors, stage of the underlying diseases, etc., in patients receiving ibrutinib or other small molecule kinase inhibitors (SMKI) are recommended.

## 4. Zusammenfassung

### 4.1 Hintergrund

Seit der weitläufigen Einführung von Ibrutinib, einem irreversiblen Hemmer der Bruton Tyrosin Kinase welcher im Jahr 2013 für die Therapie von „Non-Hodgkin“ Lymphomen einschließlich der chronisch lymphatischen Leukämie zugelassen wurde, kam eine zunehmende Anzahl von Fallserien und retrospektiven Studien auf, welche von infektiologischen Komplikationen wie invasiven Pilzerkrankungen berichteten, die scheinbar mit der Gabe von Ibrutinib assoziiert waren. Epidemiologische Daten, sowie die genauen Mechanismen wie und wie stark Ibrutinib die Anfälligkeit für invasive Pilzerkrankungen beeinflusst, sind lückenhaft. Wir haben versucht Aufschluss zu geben über die Rolle von Ibrutinib als Risikofaktor für invasive Pilzerkrankungen und die Dynamik von Infektionsgeschehen in Patient\*innen die es erhalten.

### 4.2 Methoden

Wir haben eine retrospektive Datenanalyse in zwei Krankenhäusern durchgeführt und die elektronischen Fieberkurven von Patient\*innen, welche zwischen Oktober 2020 und August 2021 Ibrutinib erhielten, analysiert. Das Vorliegen einer invasiven Pilzerkrankung wurde durch die Durchsicht von Laborergebnissen, bildgebenden Verfahren und mikrobiologischen Befunden anhand der diagnostischen Kriterien der EORTC/MSG aus 2019 nachgewiesen. Die Prävalenz von invasiven Pilzerkrankungen in den untersuchten Patient\*innen wurde berechnet.

### 4.3 Ergebnisse

Siebenundneunzig Patient\*innen mit Non-Hodgkin Lymphomen und Ibrutinib als Therapie wurden rückblickend untersucht. Die chronisch lymphatische Leukämie stellte die am häufigsten vorliegende bösartige hämatologische Erkrankung dar mit 76 Fällen. 48% der Patient\*innen erhielten bereits vorangehende Therapien für ihre zugrundeliegenden Erkrankungen. Eine Patientin entwickelte eine wahrscheinliche invasive Pilzerkrankung nach den diagnostischen Kriterien der EORTC/MSG, was eine Prävalenz von 0.97% impliziert. Die Art einer invasiven Pilzerkrankung war eine invasive pulmonale Aspergillose und *Aspergillus fumigatus* das ursächliche Pathogen. Die Patientin mit invasiver Pilzerkrankung wies gleichzeitig etablierte Risikofaktoren (z.B. Neutropenie, Gabe von

Kortikosteroiden) auf und erhielt zusätzlich eine vorangehende Therapie für die Grunderkrankung. Die Patientin ist ca. zweieinhalb Jahre nach der invasiven Pilzerkrankung an einem Coronavirus assoziiertem Lungenversagen verstorben.

#### 4.4 Konklusion

Die Inzidenz von invasiven Pilzerkrankungen in unsere Studie war niedrig, mit nur einem singulären Fall einer invasiven Mykose. Die Patientin bei der eine invasive Pilzerkrankung diagnostiziert wurde wies neben der Therapie mit Ibrutinib zusätzliche Risikofaktoren auf. Demnach sind standardisierte Herangehensweisen notwendig um das Risiko für invasive Pilzerkrankungen bei Patient\*innen die Ibrutinib oder andere kleinmolekulare Kinasehemmer erhalten einschätzen und dementsprechend agieren zu können.

## 5. Introduction

### 5.1 Invasive fungal infections in patients with hematologic malignancies

With no less than 1.7 billion people globally suffering from fungal infections, comprising superficial fungal infections and invasive fungal infections (IFIs), the enormous burden for healthcare systems caused by those diseases gets overt (1). Although IFIs account for far less infections than superficial fungal infections, they depict highly complex to treat entities associated with mortality rates as high as 45% (2). Globally, they are held responsible for an approximate estimate of 1.5 million deaths annually (3). These numbers partially exceed infection related deaths caused by respective names like tuberculosis or malaria (3, 4). Vaguely, invasive disease means that fungi managed to evade host defense mechanisms and hence being capable of migrating and infiltrating deep tissues from where they cause potential life-threatening diseases. The number of patients developing IFIs is rising globally (5). This development is paralleled by the emergence of multi resistant species (e.g. *Candida auris*, *Lomentospora prolificans*) and azole-resistant *Aspergillus spp.*, further limiting already narrow therapeutic options (6, 7). Regarding IFIs, patients with hematologic malignancies are most frequently affected (5). In this cohort, a number of reasons resulting in an impairment of host defense mechanisms are responsible for this high vulnerability. These mainly include the intensified administration and usage of treatment options, comprising monoclonal antibodies, new immunosuppressive agents, corticosteroids, cytotoxic chemotherapies and hematopoietic stem cell transplantations (HSCT) (8). Occurrence of IFIs is accompanied by a high rate of infection related death and interference with outcome of the underlying hematological disease (8). Primarily, molds and yeasts have been identified to cause severe IFIs. Concerning mold infections, *Aspergillus spp.* are the major representative, whereas *Candida spp.* are the cause of most yeast infections (9). Importantly, other opportunistic fungal pathogens such as the *Mucorales*, *Fusarium spp.* and *Scedosporium spp.* are emerging, due to the selective pressure caused by the surge of antifungal usage in prophylaxis, empirical therapy and agriculture (10). The mentioned pathogens are accompanied by a substantial increase in mortality when causing invasive disease (5). Assessing the exact incidence of IFIs in patients with hematological malignancies is challenging but essential for clinicians diagnostic, preventive and

therapeutic approaches. Amongst many others, epidemiologic data facilitates risk stratification for IFI development, influencing potential preventive measures. The facts that different studies focus on different patient subgroups, treatment regimens and underlying diseases, is aggravating general statements about epidemiologic developments. The following sections will mainly focus on the hematologic setting.

### 5.1.1. Invasive Aspergillosis

#### 5.1.1.1. Pathogenesis

As a result of the ubiquitous existence of *Aspergillus* spores in the environment, we face permanent inhalation. Due to a number of defense mechanisms, immune competent hosts usually do not develop fungus related invasive disease. Hence, invasive Aspergillosis (IA) depicts a disease most commonly related to conditions causing high immunosuppression (11). After *Aspergillus* conidia, the infectious part of *Aspergillus species*, enter the respiratory tract and reach the alveoli, the following immune cells and subsequent immune responses play a key role in host defense. Alveolar macrophages represent the first immune cells with phagocytic properties and are responsible for fast clearance of *conidia* (12). Macrophages get activated via recognition of pathogen-associated molecular patterns (PAMPs) by pathogen recognition receptors (PRRs) expressed on their surface (13). This activation initializes secretion of inflammatory mediators leading to neutrophil recruitment, which are essential for killing potential tissue-invasive hyphae and regulation of further immune responses. After conidial germination into hyphal forms, neutrophils are the main cells in fungal defense mechanisms. T-cell immunity, and concomitant complex cytokine and chemokine regulation, is involved by activation of PRRs. Impairment of these defense mechanisms due to various reasons can result in potential pulmonary infection with angio- and/or tissue invasion, intravascular thrombosis, tissue infarction and secondary dissemination, which is characteristic for IA (11).

#### 5.1.1.2. Epidemiology

As aforementioned, studies investigating the epidemiology of IFIs in patients with hematologic malignancies often differentiate between several sub-cohorts like patients

with acute myeloid leukemia (AML) or patients receiving HSCT. Hence, determining exact numbers representing the overall epidemiology remains difficult. Local epidemiology can be highly variable and is therefore essential for considerations regarding therapeutic approaches, prophylaxis and diagnostic procedures.

A major study including more than 11 000 patients with hematological malignancies has been conducted in Italy in 2004. Patients receiving HSCT were excluded. An overall incidence for IFIs of 4.6% was found (5). In accordance with other epidemiologic studies, molds represented the most frequent pathogen with 90% being caused by *Aspergillus spp.*, followed by *Mucor spp.* and *Fusarium spp.* (5, 8). Invasive pulmonary aspergillosis was the most common clinical presentation of IA, accounting for about 99% of the cases. In the *Aspergillus spp.* cohort, *Aspergillus fumigatus* was the main representative. Patients with AML were at highest risk for developing an IFI, while patients with CLL only represented 1.5% of total cases found. This underlines the consensus that patients with CLL are generally considered low risk for the occurrence of IFIs (14). In general, the attributable mortality rate (AMR) was 39% for IFIs. Interestingly, AMR was 80% in CLL patients and 43% in AML patients (5).

## 5.1.2. Invasive Candidiasis

### 5.1.2.1. Pathogenesis

Both Candidemia, meaning *Candida spp.* accessing the bloodstream, and deep seated tissue infection are referred to as invasive candidiasis. Of note, this implies that evidence of *Candida* growth in blood cultures should never be considered as contamination, even if only one bottle turns out positive. *Candida spp.* are considered to be physiologic commensals in human bowel microbiota, the genitourinary tract, the respiratory tract and the skin. A number of reasons can potentially result in invasiveness (15). On the one hand, factors like critical illness, particularly in intensive care unit (ICU) patients, administration of broad spectrum antibiotics, total parenteral nutrition and patients undergoing hemodialysis, are associated with excessive fungal growth and hence increased risk for fungi invading through the mucosal barrier of the gut (15). On the other side, therapies negatively affecting integrity of intestinal mucosa like the application of chemotherapy,

especially in combination with glucocorticoids, can lead to facilitated fungal invasion as well (16). Aside from patients in the ICU, patients with hematological malignancies represent another high risk group. Hematologic malignancies on their own, and particularly in combination with chemotherapeutics, substantially predispose patients for developing invasive disease (17). Another major risk factor is the presence of intravascular catheters, most notably central venous catheters. Once *Candida* has entered the bloodstream, this medical devices can become colonized leading to biofilm formation and potential permanent release of fungi and subsequent candidemia (18). Secondary candidemia can also be caused by deep seated infections constantly leading to fungal release into the bloodstream and possible dissemination with metastatic infection of various body sites (e.g. lung, bone, eye, liver, spleen, urinary tract) (15). In case this becomes a chronic condition, it is referred to as chronic disseminated candidiasis, which patients with hematological malignancies are particularly prone to.

#### 5.1.2.2. Epidemiology

In accordance with before mentioned variability in epidemiologic numbers concerning *Aspergillus* infections, the same applies for *Candida*. Assuredly, *Candida spp.* still account for the vast majority of invasive yeast infections. In contrast, a trend towards *non-albicans Candida spp.* causing invasive disease is unmistakably notable (19, 20). Several studies found at least 50% of *non-albicans Candida spp.*, like *Candida glabrata* or *Candida parapsilosis*, being the causative pathogen alongside *Candida albicans*. The shift towards *non-albicans spp.* has been shown to be closely related to application of fluconazole and echinocandins (21). Noteworthy, literature is increasingly reporting on a multi drug resistant *Candida spp.* emerging, namely *Candida auris* (22). Infections have been accompanied by high mortality rates and virtually absent therapeutic options (22). These findings implicate necessary considerations concerning the management of *Candida* infections. Species substantially differ in virulence and susceptibility to antifungal drugs, hence adjusted diagnostic and therapeutic strategies are required (23). Regarding the epidemiology in patient with hematologic malignancies, yeasts were held responsible for 1.6% of the overall IFI incidence of 4.6% (5). Primarily *Candida spp.* were reported to be the second most common fungal pathogen found after molds, particularly *Aspergillus spp.* Equal to *Aspergillus spp.*, patients with AML were at highest risk accounting for 71% of cases

found. Regarding patients with CLL, only one case of invasive candidiasis was noted. Besides *Candida*, a noteworthy number of infections caused by *Cryptococcus spp.* and *Trichosporon spp.* were registered. Taking a closer look on AMRs, 33% were reported for invasive Candidiasis. Divided into species specific AMRs, AMR for was 6% for *C.tropicalis* and 54% for *C.glabrata* (5), emphasizing the difference in virulence (23).

### 5.1.3. Others

Concerning other opportunistic fungal infections, two emerging molds are increasingly observed in patients with hematological malignancies. First, mucormycosis, a mold infection primarily found in immunocompromised patients and patients with uncontrolled diabetes mellitus, has been reported to be the second most frequent mold infection after aspergillosis (5). Different countries report an increase in incidence, without clear evidence of underlying factors (24). The administration of voriconazole prophylaxis has been shown to be an independent risk factor (25). Combined with the general rise of intensified immunosuppressive treatment regimens being applied, those two factors might substantially contribute to recent findings. Concerning mycology, mucormycosis refers to fungal infections caused by representatives of the genus *Mucor*. The overlying order is *Mucorales*, comprising the most commonly found genera *Rhizopus*, *Mucor*, *Cunninghamella* and *Lichtheimia*. The latter are held responsible for causing the vast majority of infections in humans (26). Several clinical presentations can manifest as mucormycosis, including the two most important, rhino-orbital-cerebral mucormycosis and pulmonary mucormycosis. The latter is accompanied by mortality rates as high as 87% (27), probably resulting from substantial difficulties in diagnosis and therapy, combined with the rapid and aggressive growth.

Second, *Fusarium spp.*, another ubiquitous mold which potentially causes severe invasive infections in immunocompromised hosts. In the reference study, *Fusarium spp.* were the third most frequent reported mold pathogen in patients with hematologic malignancies (5). These findings are confirmed by a recent study conducted in Brazil (28). Invasive fusariosis primarily develops via airborne infections or direct entry at the various sites of preexisting weakness spots, including damaged skin or intravascular catheters. Innate

immunity, particularly macrophages and neutrophils, have been shown to play a pivotal role in immune defense mechanisms (29).

## 5.2. Risk factors for IFIs in patients with hematologic malignancies

Considering the pathogenesis of IFIs, several risk factors have been identified, resulting in higher susceptibility for invasive disease. Being aware of these factors is essential for risk stratification and potential consequent administration of antifungal prophylaxis, as well as early treatment or intensified surveillance. Patients with hematologic malignancies are considered to be at highest risk for developing IFIs. Particularly, those with prolonged neutropenia, those with AML undergoing myeloablative chemotherapy, and patients receiving HSCT are highly vulnerable for infections due to immunosuppression either caused by the underlying disease itself or aggressive treatment regimens (30-32). Based on the high incidence of IFIs and poor outcome in case of IFI development in these highly vulnerable patients, antifungal prophylaxis is recommended in this setting (32). Compared with this high risk cohort, evidence is growing that IFIs do also emerge in patients with less aggressive diseases like chronic leukemia, lymphoma and multiple myeloma, probably due to therapy associated effects (14). Therapies affecting T-cell immunity like the antithymocyte globulin alemtuzumab or purine analogs (e.g. pentostatin, cladribine, fludarabine), make hosts prone to develop IFIs (33, 34). Another potential candidate acting as a risk factor is ibrutinib, which was approved by the European Medicines Agency (EMA) in 2014 and the US Food and Drug Administration (FDA) in 2013 for the treatment of B-cell malignancies (35). Considering the mechanism of action, an increased incidence of IFIs possibly caused by ibrutinib therapy was unanticipated and could not be completely explained by obvious reasons (36). Ibrutinib and small molecule kinase inhibitors (SMKIs) in general experience rapidly growing application and may represent the future of individual therapeutic approaches in hematologic- and autoimmunologic diseases. Hence, probing the causes of those increased incidence of IFIs observed is essential in order to correctly estimate the risk associated with ibrutinib administration.

### 5.3. Bruton's Tyrosine Kinase

In 1992, BTK was identified being the genetic cause of X-linked agammaglobulinemia (37), a condition which was first described in 1950 by OC Bruton. It is a cytosolic tyrosine kinase and member of the TEC kinase family, which itself is a subgroup of non-receptor protein kinases (PTKs) (38). Its fundamental role for the human immune system has been underlined as the presence of loss-of-function mutations in BTK results in nearly absolute absence of B-cells and hence antibody production (38). This small molecule kinase has been studied extensively and its crucial role in adaptive immunity by acting as an important mediator in B-cell receptor (BCR) signaling and cytokine pathways has been established (38). Due to the BCR pathway being substantially involved in the pathogenesis of B-cell malignancies, BTK was highly interesting acting as a crucial point for therapeutic strategies. Today, drug agents inhibiting this exact pathway are approved and intensive research is taking place in this field (39). Remarkably, recent data emerges suggesting BTK also being involved in several processes in mononuclear cells of the innate immunity, comprising dendritic cells and macrophages (40). More precisely, besides contribution in recognition of infectious pathogens by functioning in TLRs, BTK has also shown regulatory effects on cellular recruitment and hence induction of inflammatory responses (40). These findings suggest a much more profound role of BTK in immunologic sequences and may be important for the better understanding of adverse events associated with therapeutic inhibition.

### 5.4 Ibrutinib

The treatment of hematological malignancies with conventional chemotherapeutics is accompanied by marked toxicity and unspecific mechanisms of action, frequently resulting in severe adverse events. To avoid these downsides, extensive efforts for the finding and developing of more specific and hence less toxic therapeutic options has been conducted. With ibrutinib, an irreversible BTK inhibitor, an agent potentially living up to this demands, was found. Its accelerated approval by the FDA in 2013 for B-cell malignancies, including chronic lymphocytic leukemia (CLL), Waldenstroms macroglobulinemia (WM), mantle cell lymphoma, primary central nervous system (CNS) lymphoma and diffuse large B-cell lymphoma, was considered to be "game-changing" (35). In fact, ibrutinib has achieved

enormous treatment success and primarily revolutionized CLL therapy (41-43). With a global annual incidence of 114 000 (95% CI, 108 000 to 121 000) and 35 000 deaths (95% CI, 34 000 to 37 000), CLL depicts one of the most common forms of leukemia in western countries and the by far most common indication for ibrutinib therapy (44). Since older individuals with comorbidities are the main cohort of CLL patients, conventional chemotherapy and associated toxicity was frequently limiting therapeutic strategies, hence new treatment options in this cohort was more than welcome. Ibrutinib is a once daily orally administered drug with a common dosage of 420 milligrams (mg). Besides thrombocytopenia, increased risk of bleeding, neutropenia, diarrhea, atrial fibrillation, nausea and fatigue representing the major adverse events, the small molecule kinase inhibitor is generally well tolerated (42, 43). AE particularly occur in the first treatment year and are usually sufficiently manageable. Incidence of grade > 3 AE have been found to decrease over time. O'Brien et al. shared five years of experience with ibrutinib as a single agent in treatment naïve and relapsed/refractory CLL. In summary, no new safety issues were reported with well tolerated long term use and less appearing grade >3 cytopenias. Maintained efficacy, with treatment duration related increased rate of complete responses was observed (45).

#### 5.4.1 Invasive fungal infections in patients receiving ibrutinib therapy

##### 5.4.1.1 Current Data

Since the widespread usage of ibrutinib therapy, an increasingly number of reports about the occurrence of opportunistic fungal infections after treatment initiation has emerged. Causative pathogens include invasive mold infections (predominantly caused by *Aspergillus spp.* but also *Mucor spp.* and *Fusarium spp.*), *Pneumocystis jirovecii* and *Cryptococcus spp.* (46, 47). Interesting is the occurrence of atypical presentations of IFIs (e.g. extrapulmonary pneumocystosis, CNS aspergillosis, disseminated cryptococcosis) and varying incidences between 0% and 39% being reported in different studies (48-50). As mentioned above, these findings were unanticipated as CLL per se is usually accompanied by low risk for developing IFIs and ibrutinib induced lack of normal B-cell function is not considered to be associated with higher susceptibility to fungal infections (14, 51). Taking a closer look on above mentioned findings, some similarities are objectifiable. Invasive mold infections,

particularly IA, are overrepresented. With few exceptions, the vast majority of opportunistic fungal infections occurred early after treatment initiation within the first 6 months.. Different studies showed the presence of concomitant established risk factors, when patients developed IFIs during ibrutinib therapy. Yet, in a recent retrospective analysis of Varughese et al. the majority of patients were lacking classical risk factors (52). The extent of ibrutinib influencing the susceptibility to IFIs and whether or not ibrutinib has the potential to be a sole driver for developing IFIs is difficult to assess and remains unclear for now. This is based on a vast number of possible predisposing/influencing factors associated with an increased risk for IFIs might act a part, but are difficult to be entirely determined.

#### 5.4.1.2 Potential mechanisms how ibrutinib enhances susceptibility to IFIs

To date, the inconsistency of reported incidences of IFIs during ibrutinib therapy and the current lack of established risk stratifications reliably indicating an increased risk for developing an IFI during therapy, imply that ibrutinib related IFIs arise from a many-faceted immunodeficiency, affecting both innate and adaptive immunity. Potential mechanisms being at play will be displayed in the following table.

*Table 1 Discussion of potential mechanisms how Ibrutinib enhances susceptibility to IFIs*

<i>Suspected mechanism</i>	<i>Details</i>
<i>Interindividual environmental exposure</i>	Fungal conidia are ubiquitous. Yet, variability in exposure depending on travel history, nosocomial factors, profession and previous lung colonization seems to influence execution of interindividual host defense mechanisms (53).
<i>Other kinases being effected by ibrutinib</i>	BTK is not the only small molecule kinase being inhibited by ibrutinib. Other members of the TEC family including TEC, BMX, ITK, TXK are also affected, impairing sufficient functioning of related immune processes (35, 54, 55). Aside from that, kinases from the SRC-family, which hold essential roles in immune signaling and platelet functioning are bound off-target as well (56). As mentioned in the pathogenesis of IA, platelets contribute to immune response against fungal pathogens (57).
<i>Underlying host factors</i>	A broad range of underlying host factors, having an effect on the overall host immune state, are involved. These include, any comorbid conditions ((e.g. metabolic diseases, structural lung diseases, previous viral infections like CMV, Influenza (53, 58)) not only influencing antifungal host defense mechanisms, but also PK/PD properties. Depending on the type and state

of the underlying malignancy (59) in combination with genetic polymorphisms leading to increased risk for IFI development (60), a variable degree of immunodeficiency is present. Many more factors may play a role, aggravating the precise assessment of the present host immune status.

*Synergistic effects*

Concomitant, previous, or consecutive treatment regimens with other immunosuppressive drugs (e.g. corticosteroids, CD antibodies) and potentially underappreciated drug-drug interactions may result in increased susceptibility to IFIs (53, 58). These variables are further accompanied by the interindividual, genetically affected, functioning of transporters and metabolizing enzymes involved in different PK/PD procedures (e.g. absorption, metabolism, elimination) implying presence of additional factors which need consideration when assessing the risk for IFI development (61).

### 5.5. Diagnostic criteria for IFI

IFI classification is based on the revised and updated consensus definitions of IFIs from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG), which has been published in 2019 (62). Table 2 displays criteria for proven IFIs, Tables 3,4,5 display definitions of probable and possible invasive mold diseases and Figure 6 displays definitions of probable and possible candidiasis, cryptococcosis and pneumocystosis (62).

Table 2 Criteria for proven invasive fungal infection

Fungus	Microscopic analysis: sterile material	Culture: sterile material	Blood	Serology	Tissue nucleic acid diagnosis
<b>Molds<sup>a</sup></b>	Histopathologic, cytopathologic, or direct microscopic examination <sup>b</sup> of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage	Recovery of a hyaline or pigmented mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL fluid, a paranasal or mastoid sinus cavity specimen, and urine	Blood culture that yields a mold <sup>c</sup> (eg, <i>Fusarium</i> species) in the context of a compatible infectious disease process	Not applicable	Amplification of fungal DNA by PCR combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue
<b>Yeasts<sup>a</sup></b>	Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells, for example, <i>Cryptococcus</i> species indicating encapsulated budding yeasts or <i>Candida</i> species showing pseudohyphae or true hyphae <sup>d</sup>	Recovery of a yeast by culture of a sample obtained by a sterile procedure (including a freshly placed [ $<24$ hours ago] drain) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process	Blood culture that yields yeast (eg, <i>Cryptococcus</i> or <i>Candida</i> species) or yeast-like fungi (eg, <i>Trichosporon</i> species)	Cryptococcal antigen in cerebrospinal fluid or blood confirms cryptococcosis	Amplification of fungal DNA by PCR combined with DNA sequencing when yeasts are seen in formalin-fixed paraffin-embedded tissue
<b>Pneumocystis</b>	Detection of the organism microscopically in tissue, BAL fluid, expectorated sputum using conventional or immunofluorescence staining	Not applicable	Not applicable	Not applicable	Not applicable
<b>Endemic mycoses</b>	Histopathology or direct microscopy of specimens obtained from an affected site showing the distinctive form of the fungus	Recovery by culture of the fungus from specimens from an affected site	Blood culture that yields the fungus	Not applicable	Not applicable

**Abbreviations:** BAL, bronchoalveolar lavage; PCR, polymerase chain reaction.

<sup>a</sup> If culture is available, append the identification at the genus or species level from the culture results.

<sup>b</sup> Tissue and cells submitted for histopathologic or cytopathologic studies should be stained using Grocott-Gomori methenamine silver stain or periodic acid Schiff stain to facilitate inspection of fungal structures. Whenever possible, wet mounts of specimens from foci related to invasive fungal disease should be stained with a fluorescent dye (eg, calcofluor or blankophor).

<sup>c</sup> Recovery of *Aspergillus* species from blood cultures rarely indicates endovascular disease and almost always represents contamination.

<sup>d</sup> *Trichosporon* and yeast-like *Geotrichum* species and *Blastoschizomyces capitatus* may also form pseudohyphae or true hyphae.

Table 3 Criteria for probable invasive mold infection

#### Host factor

- Ibrutinib treatment  $> 2$  consecutive weeks

#### Clinical features

- *Pulmonary aspergillosis*
  - The presence of 1 of the following 4 signs on CT:
    - Dense, well-circumscribed lesions(s) with or without a halo sign
    - Air-crescent sign
    - Cavity
    - Wedge-shaped and segmental or lobar consolidation
- *Other pulmonary mold diseases*
  - As for pulmonary aspergillosis but also including a reverse halo sign
- Tracheobronchitis

- Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis
- Sinonasal infection: Imaging showing sinusitis plus at least 1 of the following 3 signs:
  - Acute localized pain (including pain radiating to the eye)
  - Nasal ulcer with black eschar
  - Extension from the paranasal sinus across bony barriers, including into the orbit
- CNS infection: 1 of the following 2 signs:
  - Focal lesions on imaging
  - Meningeal enhancement on MRI or CT

### **Mycological criteria**

- Any mold, for example, *Aspergillus*, *Fusarium*, *Scedosporium* species or Mucorales recovered by culture from sputum, BAL, bronchial brush, or aspirate
- Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold
- Tracheobronchitis
  - *Aspergillus* recovered by culture of BAL or bronchial brush
  - Microscopic detection of fungal elements in BAL or bronchial brush indicating a mold
- Sinonasal diseases
  - Mold recovered by culture of sinus aspirate samples
  - Microscopic detection of fungal elements in sinus aspirate samples indicating a mold

### **Aspergillosis only**

- Galactomannan antigen
- Antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF
- Any 1 of the following:
  - Single serum or plasma:  $\geq 1.0$  - BAL fluid:  $\geq 1.0$
  - Single serum or plasma:  $\geq 0.7$  and BAL fluid  $\geq 0.8$
  - CSF:  $\geq 1.0$
- *Aspergillus* PCR
- Any 1 of the following:
  - Plasma, serum, or whole blood 2 or more consecutive PCR tests positive
  - BAL fluid 2 or more duplicate PCR tests positive
  - At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid
  - *Aspergillus* species recovered by culture from sputum, BAL, bronchial brush, or aspirate

---

### **Note:**

Probable IFI = presence of a host factor, a clinical factor and a mycological criterion

Possible IFI = presence of a host factor and a clinical factor but absence of a mycological criteria

---



---

### **Candidiasis**

- Host factors
  - Ibrutinib treatment
- Clinical features
- At least 1 of the following 2 entities after an episode of candidemia within the previous 2 weeks
  - Small, target-like abscesses in liver or spleen (bull's-eye lesions) or in the brain, or, meningeal enhancement
  - Progressive retinal exudates or vitreal opacities on ophthalmologic examination
- Mycological evidence
  - $\beta$ -D-glucan (Fungitell)  $\geq 80$  ng/L (pg/mL) detected in at least 2 consecutive serum samples provided that other etiologies have been excluded
  - Positive T2Candida

### Cryptococcosis

- Host factors
  - Ibrutinib treatment
- Clinical features
  - Meningeal inflammation
  - Radiological lesion consistent with cryptococcal disease
- Mycological evidence
  - Recovery of *Cryptococcus* from a specimen obtained from any nonsterile site

### Pneumocystosis

- Host factors
  - Ibrutinib treatment
- Clinical features
  - Any consistent radiographic features particularly bilateral ground glass opacities, consolidations, small nodules or unilateral infiltrates lobar infiltrate, nodular infiltrate with or without cavitation, multifocal infiltrates, miliary pattern
  - Respiratory symptoms with cough, dyspnea, and hypoxemia accompanying radiographic abnormalities including consolidations, small nodules, unilateral infiltrates, pleural effusions, or cystic lesions on chest X-ray or computed tomography scan
- Mycological evidence
  - $\beta$ -D-glucan (Fungitell)  $\geq 80$  ng/L (pg/mL) detection in  $\geq 2$  consecutive serum samples provided other etiologies have been excluded
  - Detection of *Pneumocystis jirovecii* DNA by quantitative real-time polymerase chain reaction in a respiratory tract specimen

### Endemic Mycosis

- Host factors
  - Not applicable as these diseases affect both healthy and less healthy hosts
- Clinical features
  - Evidence for geographical or occupational exposure (including remote) to the fungus and compatible clinical illness
- Mycological evidence
  - Histoplasma or Blastomyces antigen in urine, serum, or body fluid
  - Antibody to *Coccidioides* in cerebrospinal fluid or 2-fold rise in 2 consecutive serum samples

---

#### Note:

Probable IFI = presence of a host factor, a clinical factor and a mycological criterion

Possible IFI = presence of a host factor and a clinical factor but absence of a mycological criteria

---

## 5.6 Importance of local epidemiology

Considering the wide variation in incidence rates worldwide of IFIs during ibrutinib treatment, our aim for this study was to determine the local epidemiology in Styria. As appropriate for every infectious disease, understanding of local epidemiology is key in order to define reasonable approaches for disease management. To date, there is no epidemiologic data available in Austria facilitating attending physicians in their decision making on which approach would be optimal for the individual patient. With the data of this study, we tried to improve management for patients with ibrutinib therapy, who might potentially develop an IFI.

## 6. Material and Methods

### 6.1. Aim of the study

The primary aim of this study was to determine the prevalence of IFIs in patients receiving ibrutinib mono- or combination treatment for underlying hematological malignancies. Secondary objective were the identification of potential risk factors associated with IFIs (e.g. glucocorticoid treatment, concomitant diseases), outcome of IFIs during ibrutinib treatment, epidemiology of IFIs and antifungal treatment regimes.

### 6.2. Study design

This study was a retrospective, two-center study conducted at the Medical University of Graz, Austria, and the State Hospital Leoben, Austria. By chart review, clinical data from participating patients have been retrospectively analyzed between October 2020 and August 2021.

### 6.3. Study population

All adult patients with an underlying hematological disease who have received or who have been receiving ibrutinib as mono or combination therapy were considered eligible for study inclusion. Patients were included in this study if they received ibrutinib for at least two consecutive weeks. Patients who did not receive ibrutinib therapy for at least two consecutive weeks were excluded. In case patients had more than one episode of ibrutinib treatment (each need to be ongoing for at least 2 weeks as mentioned above) these additional episodes have also been included in the study.

#### Inclusion criteria:

- 1.) Patient is aged 18 years or older
- 2.) Patient has underlying hematological malignancy
- 3.) Patient received ibrutinib therapy for at least 2 consecutive weeks (mono- or combination therapy)

- 4.) Patient received first dose of ibrutinib  $\geq 1$  year before the start of data chart abstraction to guarantee adequate follow up time

Exclusion criteria:

- 1.) Patient death under ibrutinib treatment before 2 weeks of treatment were reached

Every patient who fulfilled inclusion criteria during the observational period between October 2020 and August 2021 was included in the analysis.

6.3.1. Data collected from each patient

Following data were entered in the electronic case report form:

- 1.) Demographic data
- 2.) Type and status of underlying hematological malignancy
- 3.) Ibrutinib treatment regime (dose, duration, episodes)
- 4.) Concomitant immunosuppressive treatment and/or chemotherapy
- 5.) Risk factors for IFIs besides ibrutinib treatment
- 6.) Details on previous lines of treatment for underlying hematological malignancy
- 7.) Cause of death (if applicable)
- 8.) Presence of IFI within observation period

**For patients with documented IFI only**

- 1.) Type of IFI
- 2.) Medical and surgical antifungal treatment
- 3.) Outcome of IFI

Patients attending the outpatient clinic of the division for hematology of each participating center were screened for ibrutinib treatment and inclusion criteria. In these patients, electronic medical records, including laboratory data, imaging studies and, if available, histopathologic- or cytology studies were screened for evidence of IFI according to the EORTC/MSG diagnostic criteria (62). Study data were collected anonymized by using the online based eCRF (electronic case report form) Research Electronic Data Capture (REDCap) hosted at the Medical University of Graz. REDCap is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources (63, 64). Every participating center was provided with a study specific link in order to enter the survey. Participating centers have received a manual with instructions how to use the online eCRF. In case additional support was necessary, assistance via mobile phone or e-mail provided by our local institution was applied. All institutions were responsible for identifying their patient collective for eligible patients by themselves.

#### 6.4. Statistical analysis

IBM SPSS Statistics 26 (IBM Corporation, Armonk, NY) was used for the statistical analysis of the collected data. For the primary analysis, the prevalence of IFI was estimated by calculating the relative number of patients with IFI in the whole study cohort. For the secondary analysis patients with IFIs and patients who did not develop IFI were characterized by descriptive statistics using absolute and relative numbers, median [interquartile range (IQR)], mean [(standard deviation (SD))] and range as appropriate. This study was approved by the local ethics committee (32-127 ex 19/20).

## 7. Results

Ninety-seven patients were included in the retrospective analysis. The primary underlying hematologic malignancy was CLL in 77.3% and NHL in 21.6 % (Table 5). The mean age of patients included was 73.4 years (SD 10.4) with 64.9 % being male (Table 4). The most frequent dosage of ibrutinib was 420mg (74.2%). Ibrutinib was the first line treatment in 51.5% of patients and administered as monotherapy in 88.7%. Previous treatment lines were present in 48.4% with bendamustin, rituximab and both as combination therapy being the most frequent. Twenty-eight patients (28.8%) received >1 previous treatment line. Ten percent of patients had concurrent treatment lines aside ibrutinib with rituximab taking first place. Three patients received >1 concomitantly administered immunosuppressive/ immunomodulatory drug.

Concerning classical risk factors for IFIs at the time of ibrutinib administration, neutropenia (<500/ $\mu$ L) was present in two patients, chronic pulmonary disease in six patients , HSCT in four patients with a median delay from HSCT to ibrutinib initiation of 11.5 months, and cortisone therapy in 10 patients. Aside from 10 patients who had PJP prophylaxis with trimethoprim/sulfamethoxazole only one patient received further antifungal prophylaxis with posaconazole.

According to EORTC/MSG criteria, one patient developed a probable IFI. IFIs was diagnosed 42 days after ibrutinib initiation.

Table 4 Baseline characteristics of patients

	Study cohort (N = 97) (%)
<b>Age, mean (SD), y</b>	73.4 (10.4)
<b>Sex – no. (%)</b>	
Male	63 (64,9)
Female	34 (35,1)
Others	0
<b>Ibrutinib daily dosage, mg</b>	
140	7
280	10
420	72
560	8

*≥1 episode of ibrutinib therapy*

17

*Ibrutinib as monotherapy*

86

*Ibrutinib as 1<sup>st</sup> line treatment*

50

***Concurrent treatment regimens***

*Rituximab*

9

*Human normal immunoglobulin*

4

*Methotrexate*

2

*Venetoclax*

1

*DA-TEDDI-R*

1

*≥1 concurrent treatment line*

1

3

***Previous treatment regimens***

47

*Alemtuzumab*

1

*Bendamustin*

7

*Obinutuzumab*

1

*Rituximab*

7

*Chlorambucil*

4

*Fludarabin*

2

*FCM*

1

*R-Benda*

8

*R-CHOP*

2

*R-COMP*

1

*R-FC*

4

*R-FCM*

1

*R-CP*

1

*Obinutuzumab/chlormabucil*

3

*VR-CAP*

1

*Methotrexate/cytarabine/thiotepa*

1

*≥1 previous treatment line*

28

***Risk factors for IFIs***

*HIV/AIDS*

0

*Neutropenia (<500/ $\mu$ L)*

2

*Solid organ transplantation*

0

*Chronic pulmonary disease*

6

*COPD*

6

*GOLD Stadium I*

2

*GOLD Stadium II*

1

*unknown*

3

*Hematopoietic stem cell transplantation*

4

*Allogenic*

1

*Autologous*

3

<i>Cortisone therapy</i>	10
<i>Diabetes mellitus</i>	10
<b><i>Antimicrobial prophylaxis during ibrutinib treatment</i></b>	
<i>Antifungal prophylaxis</i>	1
<i>PJP prophylaxis</i>	10
<b><i>IFIs</i></b>	1

Abbreviations: DA-TEDDI-R = dose adjusted temozolomide, etoposide, doxil, dexamethasone, ibrutinib, rituximab, FCM = fludarabine, cyclophosphamide, mitoxantrone, R = rituximab, Benda = bendamustine, CHOP = cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone, COMP = cyclophosphamide, doxorubicin, vincristine, prednisone, FC = fludarabine, cyclophosphamide, CP = cyclophosphamide, prednisone, VR-CAP = bortezomib, rituximab, cyclophosphamide, doxorubicin, prednisone, HIV = human immunodeficiency virus, AIDS = acquired immunodeficiency syndrome, PJP = pneumocystis jirovecii

Table 5 Underlying hematological malignancy

<i>Hematologic malignancy</i>	<i>Patients, No.</i>
<b><i>Chronic lymphocytic leukemia (CLL)</i></b>	75
<b><i>Non-Hodgkin lymphoma (NHL)</i></b>	21
<i>Mantle cell lymphoma</i>	7
<i>Diffuse large B-cell lymphoma</i>	1
<i>Waldenstrom macroglobulinemia</i>	8
<i>Primary central nervous lymphoma</i>	1
<i>Prolymphocytic leukemia</i>	3
<i>Unknown</i>	1

## 7.1 Patient with IFI

### 7.1.1 Demographics of patients with IFIs

One female patient developed an IFI during ibrutinib treatment. The underlying hematologic malignancy was CLL (Table 6).

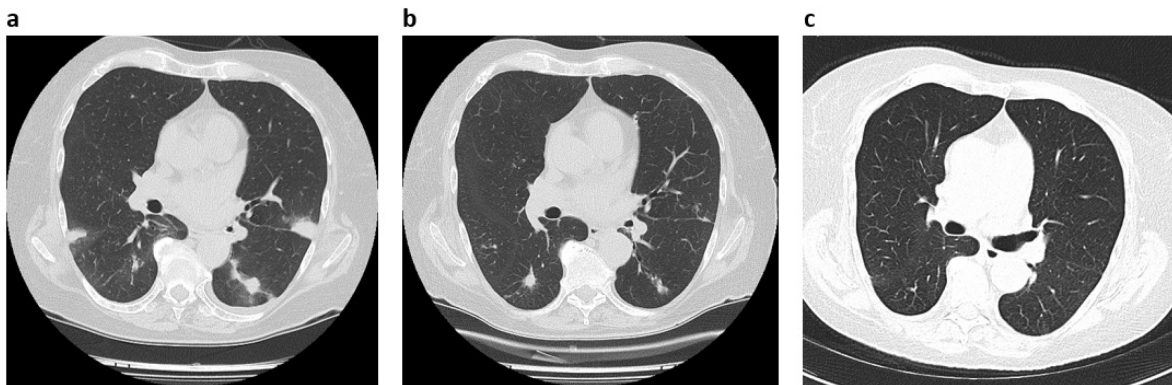
Underlying hematologic malignancy was diagnosed three years before IFI diagnosis. The patient received 420mg of ibrutinib with 42 days between ibrutinib initiation and occurrence of IFI. A previous line of treatment for the hematological malignancy (=rituximab) was present. Based on the EORTC criteria, the patient was diagnosed with probable invasive aspergillosis.

### 7.1.2 Patient with invasive aspergillosis

The IPA case was diagnosed in a 68-year-old female patient who presented with acute onset of fever and cough for three days in the emergency department. Chest CT scan revealed multiple bi-pulmonary nodular infiltrates with a maximum diameter of up to 4 cm and surrounding ground-glass opacities, compatible with a halo-sign (see figure 1a). Thus, bronchoscopy was performed two days after the CT scan revealing signs of chronic bronchitis with no evidence for ulcerative disease. Microbiological work-up of BAL fluid showed a positive GM (2.02 ODI) and growth of *Aspergillus fumigatus* in fungal culture. Besides *Candida albicans*, no further pathogen was detected in BAL fluid culture. Serum GM was unfortunately not available, but serum BDG turned out positive on the day of bronchoscopy (94.4 pg/mL).

A “classical” risk factor for invasive disease was present at the time of IFI diagnosis. The patient had concomitant treatment with corticosteroids with a dose of  $\geq 0.3$  mg/kg corticosteroids for  $\geq 3$  weeks but the patient was not neutropenic at the time of IPA diagnosis. The patient was on ongoing *pneumocystis* prophylaxis with sulfamethoxazole and trimethoprim but no antifungal prophylaxis. The patient received antifungal treatment with isavuconazole (loading dose of 200mg three times a day for two days, followed by a maintenance dose of 200mg once daily) for a total duration of five weeks. Therapeutic drug monitoring revealed isavuconazole trough levels between 2.0 and 3.0  $\mu\text{g/mL}$ . Follow-up CT scan three weeks after initiation of isavuconazole showed significant reduction of the infiltrates (see figure 1b) with a complete resolution of infiltrates 14 month after isavuconazole initiation (see figure 1c). After IFI episode, ibrutinib was administered again, but there was no recurrence of IFIs after second line ibrutinib treatment. Unfortunately, the patient died approximately 2.5 years after the IPA episode due to a coronavirus-19 associated ARDS episode. However, there was no recurrence of IPA within this observational period.

Figure 1 a-c



- (a) Initial CT scan with multiple bi-pulmonary nodular infiltrates and surrounding ground-glass opacities, compatible with a halo-sign
- (b) Follow-up CT scan three weeks after isavuconazole therapy showing significant reduction of infiltrates
- (c) Complete resolution of infiltrates 14 months after isavuconazole initiation

Table 6 Characteristics of Patients with IFIs

	Patient with IFI (N=1)
<b>Female</b>	1
<b>Underlying hematological malignancy</b> CLL	1
<b>Ibrutinib dosage 420mg</b>	1
<b>Previous treatment line</b> ≥1 previous treatment lines	1
<b>PJP prophylaxis</b>	1
<b>Typ of IFI</b> Invasive pulmonary aspergillosis	1
<b>Underlying fungal pathogen</b> Aspergillus fumigatus	1
<b>Risk factors present</b> Concomitant therapy with corticosteroids	1

Abbreviations: CLL = chronic lymphocytic leukemia, IFI = invasive fungal infection, PJP = pneumocystis jirovecii

## 7. Discussion

Within the last year, the therapeutic landscape in hemato-oncological diseases has undoubtedly been shaped by the rapid development and approval of various target specific compounds with the outlook of eluding, or at least mitigating toxicity and severe complications of conventional chemotherapeutics. In fact, many of these addressed small molecule kinase inhibitors (SMKIs) have provided the before mentioned expectations and register enormous treatment success and clinical benefit for a number of hematologic malignancies (42, 43). To date, we look back on more than five years of clinical experience with ibrutinib in the treatment of CLL and NHL. Conducted observational research and follow up investigations continue to underscore the efficacy and tolerability of long term usage (45).

Since the broad implementation of ibrutinib in therapeutic strategies, a number of case reports, case series and, later on, retrospective studies have reported an increasing incidence of serious infections including IFIs and communicated a potential association between ibrutinib therapy and the occurrence of invasive infections (46, 48-50, 65-69). These findings were somewhat unexpected as the incidence of IFIs in patients with chronic lymphoproliferative disorders was considered to be low and no such events were anticipated by the mechanisms of action in initial clinical studies (5, 36, 70). Most of the articles analyzing this concern found an average prevalence around 2 – 3% with some outliers reporting 11% - 39% (49, 71). A caveat is the partially small number of cases investigated in these retrospective studies and the general lack of prospective study designs evaluating convincing incidences. In the study of Lionakis et al, which reported a prevalence of 39%, several factors may contribute to this relatively high number. The study compared ibrutinib treatment alone versus ibrutinib combined with chemotherapy for primary central nervous system lymphoma. Eighteen patients were included in this study, of which seven patients developed an IFI. Importantly, only two of this patients developed an IFI on ibrutinib alone, whereas the other five IFIs occurred during combination treatment with other chemotherapy regimens. Additionally, concomitant dexamethasone treatment was present in the two IFI cases and higher dosage of ibrutinib (mainly 840mg) was administered. In summary, this study and the prevalence reported is not comparable to most other studies which stated much lower prevalence.

Taking a closer look on the findings of Byrd et al. and Varughese et al, who included the largest cohorts with 391 and 378 patients, respectively, a prevalence of 0,5% and 4,1%, respectively, for IFIs was found (52, 66). In our two-centered, retrospective study we included 97 patients and found a prevalence of 0.97%, which is in line with the previous reported findings. Concerning the study by Varughese et al, it is noteworthy that they reported the lack of typical risk factors for IFIs in the majority of patients (e.g. neutropenia, corticosteroids, HSCT) besides ibrutinib administration in treatment naïve patients (52), whereas most other investigations showed the invariable presence of concomitant classical risk factors and occurrence of IFIs only in patients who were treated for relapsed/refractory disease (36, 43, 66, 69) . The latter findings are in line with our observations, even though the number of IFI cases in our study were too little to conclude whether ibrutinib is a significant additional risk factor for the development of IFIs.

Diagnosis of IPA in the patient reported here was based on suspicion of IPA in chest CT scan with concomitant bronchoscopy and positive GM and culture on BAL fluid. Patients with ibrutinib therapy who develop IPA usually present with “typical” findings in CT scans with consolidations +/- surrounding ground-glass opacities representing the most prominent findings (52) as also observed in the patient reported in this study . Unfortunately, serum GM was not performed in our patient, but positive serum GM levels in these cohort of patients seem to be relatively rare, whereas positive BDG levels are more frequently reported (52). The limited sensitivity of serum GM may be, at least partly, explained by the fact that some of the patients do not present with underlying neutropenia at the time of diagnosis. Lack of neutropenia is associated with reduced or even missing angio-invasive growth of *Aspergillus* and therefore negative serum GM levels (11, 72). This implicates, that a negative serum GM is not able to rule out IPA in such patients and that further investigations, including bronchoscopy, is highly recommended in case of IPA suspicion. In addition, positive fungal biomarkers in blood (GM or BDG) should always trigger suspicion for underlying IFI and consecutively further diagnostic work-up as in our patient.

In general, the population included in the trials mentioned above, was similar in regards of underlying diseases, previous treatment lines and additional risk factors for IFIs as the cohort described in this study. Whether or not ibrutinib is capable of being a sole drive for the development of an IFI in the absence of other predisposing-and risk factors is, however,

questionable. Ibrutinib might rather be the tip of the iceberg, which is built of numerous influential variables. This assumption is further encouraged by the overall rarity of IFIs in light of the huge number of patients who are treated with ibrutinib. These findings indicate that Ibrutinib should demand consideration and weighting in risk evaluation for selected patients which are already considered high risk and ibrutinib might act as the final straw.

In respect of the literature, data indicates a vague association between ibrutinib usage and the occurrence of IFIs. Taking account of the broad spectrum of immunologic sequences BTK is involved in and the knowledge of ibrutinib manipulating by far more components of the immune system than solely BTK, this association seems plausible. More recent data underscored the broad and partially, to date, unknown activities of BTK, as well as the influence on susceptibility to IA (40, 49). Another potential key factor is the enhancement of susceptibility taking place at the genetic level. These factors are not yet screened for in clinical routine, but studies showed genetic defects, including polymorphisms in immune related genes, which if present, made patients more prone to IFIs (60, 73) and may not only explain the geographic variances but also variation of incidence rates among different studies. Among others, this knowledge could potentially refine approaches assessing the individual risk of disease.

An important limitation of our study is the retrospective design and lack of general IFI screening protocols, which may have led to underestimation of IFI incidence in patients receiving ibrutinib therapy. Additionally, the number of IFI cases is small, hence no sufficient analysis regarding potential risk factors was possible.

To conclude, infectious complications like IFIs are major contributors to mortality rates in patients with hematological malignancies and demand attention. Thus, the aim of all studies, including our study, was the assessment of IFI prevalence and potential risk factors in this patient cohort in order to provide essential information to clinicians, facilitating diagnostic and therapeutic decision making. High vigilance of clinicians in patients at risk is necessary. In order to support clinicians evaluations, tools for the exact risk stratification would be most helpful. Depending on the assessments results, consequent standardized diagnostic and preventive approaches, including antifungal prophylaxis, could be implemented. As aforementioned, currently available tools are not yet sufficient, but this issue is subject of latest research. Concerning Ibrutinib and SMKIs, they should demand

consideration and weighting in risk evaluation as current data indicates an association between ibrutinib and the development of IFIs. However, apart from sparse aberrations, the occurrence of IFIs was largely accompanied by the presence of other established risk factors (i.e. neutropenia, corticosteroid use,  $\geq 1$  previous treatment lines) and influencing variables, implying the aforementioned tip of the iceberg picture. Taking account of the vast number of patients for whom ibrutinib depicts the best therapeutic approach and necessary treatment for their underlying hematological malignancy, IFIs claim particular attention and a high level of alertness in selected patients, but should not unfoundedly detain ibrutinib usage. Additionally, general antifungal prophylaxis would be disproportionate given the observed prevalence and would come along with high costs and limiting drug-drug interactions. Amongst others, our study elaborates the dynamics of infection events in patients treated with ibrutinib, which optimally results in an increased awareness.

## References

1. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med*. 2012;4(165):165rv13.
2. Drgona L, Khachatryan A, Stephens J, Charbonneau C, Kantecki M, Haider S, et al. Clinical and economic burden of invasive fungal diseases in Europe: focus on pre-emptive and empirical treatment of *Aspergillus* and *Candida* species. *Eur J Clin Microbiol Infect Dis*. 2014;33(1):7-21.
3. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. *J Fungi (Basel)*. 2017;3(4).
4. Pasqualotto AC, Quieroz-Telles F. Histoplasmosis dethrones tuberculosis in Latin America. *Lancet Infect Dis*. 2018;18(10):1058-60.
5. Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, Martino B, et al. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. *Haematologica*. 2006;91(8):1068-75.
6. Forsberg K, Woodworth K, Walters M, Berkow EL, Jackson B, Chiller T, et al. *Candida auris*: The recent emergence of a multidrug-resistant fungal pathogen. *Med Mycol*. 2019;57(1):1-12.
7. Chowdhary A, Sharma C, Meis JF. Azole-Resistant Aspergillosis: Epidemiology, Molecular Mechanisms, and Treatment. *J Infect Dis*. 2017;216(suppl\_3):S436-S44.
8. Auberger J, Lass-Flörl C, Ulmer H, Nogler-Semenitz E, Clausen J, Gunsilius E, et al. Significant alterations in the epidemiology and treatment outcome of invasive fungal infections in patients with hematological malignancies. *Int J Hematol*. 2008;88(5):508-15.
9. Donhuijsen K, Petersen P, Schmid WK. Trend reversal in the frequency of mycoses in hematological neoplasias: autopsy results from 1976 to 2005. *Dtsch Arztebl Int*. 2008;105(28-29):501-6.
10. Rauseo AM, Coler-Reilly A, Larson L, Spec A. Hope on the Horizon: Novel Fungal Treatments in Development. *Open Forum Infect Dis*. 2020;7(2):ofaa016.
11. Segal BH. Aspergillosis. *N Engl J Med*. 2009;360(18):1870-84.
12. Ben-Ami R, Lewis RE, Kontoyiannis DP. Enemy of the (immunosuppressed) state: an update on the pathogenesis of *Aspergillus fumigatus* infection. *Br J Haematol*. 2010;150(4):406-17.
13. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in Invasive Aspergillosis. *Clin Microbiol Rev*. 2009;22(3):447-65.
14. Tisi MC, Hohaus S, Cuccaro A, Innocenti I, De Carolis E, Za T, et al. Invasive fungal infections in chronic lymphoproliferative disorders: a monocentric retrospective study. *Haematologica*. 2017;102(3):e108-e11.
15. Kullberg BJ, Arendrup MC. Invasive Candidiasis. *N Engl J Med*. 2016;374(8):794-5.

16. Velasco E, Bigni R. A prospective cohort study evaluating the prognostic impact of clinical characteristics and comorbid conditions of hospitalized adult and pediatric cancer patients with candidemia. *Eur J Clin Microbiol Infect Dis*. 2008;27(11):1071-8.
17. Hachem R, Hanna H, Kontoyiannis D, Jiang Y, Raad I. The changing epidemiology of invasive candidiasis: *Candida glabrata* and *Candida krusei* as the leading causes of candidemia in hematologic malignancy. *Cancer*. 2008;112(11):2493-9.
18. Chow JK, Golan Y, Ruthazer R, Karchmer AW, Carmeli Y, Lichtenberg DA, et al. Risk factors for albicans and non-albicans candidemia in the intensive care unit. *Crit Care Med*. 2008;36(7):1993-8.
19. Arendrup MC. Epidemiology of invasive candidiasis. *Curr Opin Crit Care*. 2010;16(5):445-52.
20. Cleveland AA, Harrison LH, Farley MM, Hollick R, Stein B, Chiller TM, et al. Declining incidence of candidemia and the shifting epidemiology of *Candida* resistance in two US metropolitan areas, 2008-2013: results from population-based surveillance. *PLoS One*. 2015;10(3):e0120452.
21. Lortholary O, Desnos-Ollivier M, Sitbon K, Fontanet A, Bretagne S, Dromer F, et al. Recent exposure to caspofungin or fluconazole influences the epidemiology of candidemia: a prospective multicenter study involving 2,441 patients. *Antimicrob Agents Chemother*. 2011;55(2):532-8.
22. Sultan AS, Vila T, Montelongo-Jauregui D, Jabra-Rizk MA. The Global Emergence of the Fungal Pathogen *Candida auris*. *Clin Infect Dis*. 2021;72(1):178-9.
23. Arendrup M, Horn T, Frimodt-Moller N. In vivo pathogenicity of eight medically relevant *Candida* species in an animal model. *Infection*. 2002;30(5):286-91.
24. Bitar D, Van Cauteren D, Lanternier F, Dannaoui E, Che D, Dromer F, et al. Increasing incidence of zygomycosis (mucormycosis), France, 1997-2006. *Emerg Infect Dis*. 2009;15(9):1395-401.
25. Kontoyiannis DP, Lionakis MS, Lewis RE, Chamilos G, Healy M, Perego C, et al. Zygomycosis in a tertiary-care cancer center in the era of Aspergillus-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis*. 2005;191(8):1350-60.
26. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin Infect Dis*. 2005;41(5):634-53.
27. Almyroudis NG, Sutton DA, Linden P, Rinaldi MG, Fung J, Kusne S. Zygomycosis in solid organ transplant recipients in a tertiary transplant center and review of the literature. *Am J Transplant*. 2006;6(10):2365-74.
28. Souza L, Nouer SA, Morales H, Simoes B, Solza C, Queiroz-Telles F, et al. Epidemiology of invasive fungal disease in haematologic patients. *Mycoses*. 2021;64(3):252-6.
29. Shoham S, Levitz SM. The immune response to fungal infections. *Br J Haematol*. 2005;129(5):569-82.

30. Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med*. 2007;356(4):348-59.
31. Pagano L, Akova M, Dimopoulos G, Herbrecht R, Drgona L, Blijlevens N. Risk assessment and prognostic factors for mould-related diseases in immunocompromised patients. *J Antimicrob Chemother*. 2011;66 Suppl 1:i5-14.
32. Wingard JR. Empirical antifungal therapy in treating febrile neutropenic patients. *Clin Infect Dis*. 2004;39 Suppl 1:S38-43.
33. Martin SI, Marty FM, Fiumara K, Treon SP, Gribben JG, Baden LR. Infectious complications associated with alemtuzumab use for lymphoproliferative disorders. *Clin Infect Dis*. 2006;43(1):16-24.
34. Morrison VA, Rai KR, Peterson BL, Kolitz JE, Elias L, Appelbaum FR, et al. Impact of therapy With chlorambucil, fludarabine, or fludarabine plus chlorambucil on infections in patients with chronic lymphocytic leukemia: Intergroup Study Cancer and Leukemia Group B 9011. *J Clin Oncol*. 2001;19(16):3611-21.
35. Wu P, Nielsen TE, Clausen MH. Small-molecule kinase inhibitors: an analysis of FDA-approved drugs. *Drug Discov Today*. 2016;21(1):5-10.
36. Ghez D, Calleja A, Protin C, Baron M, Ledoux MP, Damaj G, et al. Early-onset invasive aspergillosis and other fungal infections in patients treated with ibrutinib. *Blood*. 2018;131(17):1955-9.
37. Vetrie D, Vorechovsky I, Sideras P, Holland J, Davies A, Flinter F, et al. The gene involved in X-linked agammaglobulinaemia is a member of the Src family of protein-tyrosine kinases. 1993. *J Immunol*. 2012;188(7):2948-55.
38. Woyach JA, Johnson AJ, Byrd JC. The B-cell receptor signaling pathway as a therapeutic target in CLL. *Blood*. 2012;120(6):1175-84.
39. Reinwald M, Silva JT, Mueller NJ, Fortun J, Garzoni C, de Fijter JW, et al. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies: an infectious diseases perspective (Intracellular signaling pathways: tyrosine kinase and mTOR inhibitors). *Clin Microbiol Infect*. 2018;24 Suppl 2:S53-S70.
40. Weber ANR, Bittner Z, Liu X, Dang TM, Radsak MP, Brunner C. Bruton's Tyrosine Kinase: An Emerging Key Player in Innate Immunity. *Front Immunol*. 2017;8:1454.
41. Scott DE, Bayly AR, Abell C, Skidmore J. Small molecules, big targets: drug discovery faces the protein-protein interaction challenge. *Nat Rev Drug Discov*. 2016;15(8):533-50.
42. Burger JA, Styles L, Kipps TJ. Ibrutinib for Chronic Lymphocytic Leukemia. *N Engl J Med*. 2016;374(16):1594-5.
43. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2013;369(1):32-42.
44. Global Burden of Disease Cancer C, Fitzmaurice C, Abate D, Abbasi N, Abbastabar H, Abd-Allah F, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-

- Years for 29 Cancer Groups, 1990 to 2017: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol.* 2019;5(12):1749-68.
45. O'Brien S, Furman RR, Coutre S, Flinn IW, Burger JA, Blum K, et al. Single-agent ibrutinib in treatment-naive and relapsed/refractory chronic lymphocytic leukemia: a 5-year experience. *Blood.* 2018;131(17):1910-9.
  46. Arthurs B, Wunderle K, Hsu M, Kim S. Invasive aspergillosis related to ibrutinib therapy for chronic lymphocytic leukemia. *Respir Med Case Rep.* 2017;21:27-9.
  47. Kreiniz N, Bejar J, Polliack A, Tadmor T. Severe pneumonia associated with ibrutinib monotherapy for CLL and lymphoma. *Hematol Oncol.* 2018;36(1):349-54.
  48. Ahn IE, Jerussi T, Farooqui M, Tian X, Wiestner A, Gea-Banacloche J. Atypical *Pneumocystis jirovecii* pneumonia in previously untreated patients with CLL on single-agent ibrutinib. *Blood.* 2016;128(15):1940-3.
  49. Lionakis MS, Dunleavy K, Roschewski M, Widemann BC, Butman JA, Schmitz R, et al. Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary CNS Lymphoma. *Cancer Cell.* 2017;31(6):833-43 e5.
  50. Ruchlemer R, Ben Ami R, Lachish T. Ibrutinib for Chronic Lymphocytic Leukemia. *N Engl J Med.* 2016;374(16):1593-4.
  51. Lionakis MS, Netea MG, Holland SM. Mendelian genetics of human susceptibility to fungal infection. *Cold Spring Harb Perspect Med.* 2014;4(6).
  52. Varughese T, Taur Y, Cohen N, Palomba ML, Seo SK, Hohl TM, et al. Serious Infections in Patients Receiving Ibrutinib for Treatment of Lymphoid Cancer. *Clin Infect Dis.* 2018;67(5):687-92.
  53. Bodey GP. Managing infections in the immunocompromised patient. *Clin Infect Dis.* 2005;40 Suppl 4:S239.
  54. Dubovsky JA, Beckwith KA, Natarajan G, Woyach JA, Jaglowski S, Zhong Y, et al. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood.* 2013;122(15):2539-49.
  55. Sagiv-Barfi I, Kohrt HE, Czerwinski DK, Ng PP, Chang BY, Levy R. Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both BTK and ITK. *Proc Natl Acad Sci U S A.* 2015;112(9):E966-72.
  56. Rotin LE, Gronda M, MacLean N, Hurren R, Wang X, Lin FH, et al. Ibrutinib synergizes with poly(ADP-ribose) glycohydrolase inhibitors to induce cell death in AML cells via a BTK-independent mechanism. *Oncotarget.* 2016;7(3):2765-79.
  57. Perkhofer S, Kehrel BE, Dierich MP, Donnelly JP, Nussbaumer W, Hofmann J, et al. Human platelets attenuate *Aspergillus* species via granule-dependent mechanisms. *J Infect Dis.* 2008;198(8):1243-6.
  58. Kontoyiannis DP, Patterson TF. Diagnosis and treatment of invasive fungal infections in the cancer patient: recent progress and ongoing questions. *Clin Infect Dis.* 2014;59 Suppl 5:S356-9.
  59. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. *Blood.* 2015;126(5):573-81.
  60. Fisher CE, Hohl TM, Fan W, Storer BE, Levine DM, Zhao LP, et al. Validation of single nucleotide polymorphisms in invasive aspergillosis following hematopoietic cell transplantation. *Blood.* 2017;129(19):2693-701.

61. Pander J, Guchelaar HJ, Gelderblom H. Pharmacogenetics of small-molecule tyrosine kinase inhibitors: Optimizing the magic bullet. *Curr Opin Mol Ther.* 2010;12(6):654-61.
62. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis.* 2020;71(6):1367-76.
63. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform.* 2019;95:103208.
64. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009;42(2):377-81.
65. Baron M, Zini JM, Challan Belval T, Vignon M, Denis B, Alanio A, et al. Fungal infections in patients treated with ibrutinib: two unusual cases of invasive aspergillosis and cryptococcal meningoencephalitis. *Leuk Lymphoma.* 2017;58(12):2981-2.
66. Byrd JC, Brown JR, O'Brien S, Barrientos JC, Kay NE, Reddy NM, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med.* 2014;371(3):213-23.
67. Faisal MS, Shaikh H, Khattab A, Albrethsen M, Fazal S. Cerebral aspergillosis in a patient on ibrutinib therapy-A predisposition not to overlook. *J Oncol Pharm Pract.* 2019;25(6):1486-90.
68. Messina JA, Maziarz EK, Spec A, Kontoyiannis DP, Perfect JR. Disseminated Cryptococcosis With Brain Involvement in Patients With Chronic Lymphoid Malignancies on Ibrutinib. *Open Forum Infect Dis.* 2017;4(1):ofw261.
69. O'Brien S, Jones JA, Coutre SE, Mato AR, Hillmen P, Tam C, et al. Ibrutinib for patients with relapsed or refractory chronic lymphocytic leukaemia with 17p deletion (RESONATE-17): a phase 2, open-label, multicentre study. *Lancet Oncol.* 2016;17(10):1409-18.
70. Steinbach WJ, Marr KA, Anaissie EJ, Azie N, Quan SP, Meier-Kriesche HU, et al. Clinical epidemiology of 960 patients with invasive aspergillosis from the PATH Alliance registry. *J Infect.* 2012;65(5):453-64.
71. Chamoun K, Choquet S, Boyle E, Houillier C, Larrieu-Ciron D, Al Jijakli A, et al. Ibrutinib monotherapy in relapsed/refractory CNS lymphoma: A retrospective case series. *Neurology.* 2017;88(1):101-2.
72. Cordonnier C, Botterel F, Ben Amor R, Pautas C, Maury S, Kuentz M, et al. Correlation between galactomannan antigen levels in serum and neutrophil counts in haematological patients with invasive aspergillosis. *Clin Microbiol Infect.* 2009;15(1):81-6.

73. Cunha C, Aversa F, Bistoni G, Casagrande A, Rodrigues F, Romani L, et al. Immunogenetic profiling to predict risk of invasive fungal diseases: where are we now? *Immunol Invest.* 2011;40(7-8):723-34.