Diploma thesis

Influence of open and laparoscopic abdominal surgery involving the intestinal tract on serum 1,3-ß-D-glucan (BDG) values

submitted by

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for the academic degree of

Doktorin der gesamten Heilkunde

(Dr. med. univ.)

at the

Medical University of Graz

conducted at the

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Graz, 6th of November 2018

Statutory declaration

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

Graz, 6th of November 2018 Anna Sophie Herzeg m.p.

Acknowledges

I would like to express my deepest gratitude and respect to my supervisor Prof. Robert Krause. He did not only manage to arouse my interest in the topic of fungal diseases, but also showed me a passion, motivation and immense knowledge, that inspired me to go even further in my academic career. Thank you for your guidance, assistance and understanding throughout the process of writing this thesis.

Also, I would like to thank my whole family for not only supporting me financially throughout my studies, but also emotionally. I would like to especially thank my mom, who will always be my guidance in whatever I do, my greatest critic and fan. Without you I would not be where I stand today.

I am also very grateful to all my friends and colleagues who supported me during my studies and will hopefully accompany me also in the following years.

Zusammenfassung

Hintergrund:

Invasive *Candida* Infektionen treten sowohl bei immunsupprimierten Patientinnen und Patienten, als auch bei Patientinnen und Patienten der Intensivstation auf und stellen die häufigste schwerwiegende und potentiell lebensbedrohliche Mykose bei dieser Patientengruppe dar.

1,3-β-D-Glucan, ein Zellwandbestandteil von vielen Pilzen, kann hierbei als Marker für den Nachweis oder Ausschluss invasiver Pilzerkrankungen verwendet werden.

Jedoch können andere Glucane, wie beispielsweise Cellulose, mit dem 1,3-β-D-Glucan-Test interferieren und zu falsch positiven Ergebnissen führen. Außerdem kann 1,3-β-D-Glucan als Bestandteil der Zellwände von *Candida spp*., welche im Intestinaltrakt als Kolonisationskeime vorhanden sein können, durch Mukosaschädigungen in den Blutkreislauf übertreten. Solche Mukosaschäden treten u.a. bei chirurgischen Eingriffen des Intestinaltraktes auf. Abdominelle operative Eingriffe könnten daher die Wertigkeit des 1,3-β-D-Glucan-Tests beeinflussen.

Ziel dieser Studie war die Beurteilung des Einflusses von offenen und laparoskopischen abdominellen Eingriffen auf peri- und post-operative 1,3-β-D-Glucan Werte im Serum.

Hypothese:

Da *Candida spp.* zur natürlichen mikrobiellen Darmflora gehören und sowohl 1,3-β-D-Glucan als auch andere Glucane mit der Nahrung zugeführt werden, könnte bei Durchtrennung des Darms ein Anstieg der 1,3-β-D-Glucan Werte sowohl nach laparoskopisch als auch offen durchgeführten, abdominellen Eingriffen auftreten. Außerdem könnten bei der Operation eingesetzte Materialien wie Cellulose Tupfer zu einer Erhöhung der 1,3-β-D-Glucan Werte führen.

Methoden:

Bei 50 Patientinnen und Patienten mit elektiven laparoskopischen oder offenen abdominellen chirurgischen Eingriffen wurden zu definierten Zeitpunkten jeweils 7 Blutproben abgenommen. 24 dieser Patientinnen und Patienten wurden laparoskopisch und 26 offen operiert. Aus diesen Proben wurde mittels modifiziertem *Fungitell*© Test

(automated BDG Test) 1,3-β-D-Glucan Werte bestimmt. Eine Konzentration von ≥80pg/ml wurde als positiv, eine Konzentration von <60pg/ml als negativ definiert.

Resultate:

Es konnte gezeigt werden, dass 1,3-β-D-Glucan Werte während und nach offener und laparoskopischer Operation signifikant ansteigen. In 54% (Gruppe: Laparoskopie) bis 61% (Gruppe: offene OP) wurde ein 1,3-β-D-Glucan Wert von ≥80pg/ml während der Operation nachgewiesen. 1,3-β-D-Glucan Werte steigen bei offenen Operationen höher an im Vergleich zu laparoskopischen Operationen. Es konnte kein einzelner Faktor für die Erhöhung des 1,3-β-D-Glucan Wertes identifiziert werden.

Conclusio:

1,3-β-D-Glucan Werte steigen signifikant während und nach einer bauchchirurgischen Operation an. Dieser Anstieg kann bis zu mehrere Tage beobachtet werden, weswegen die Aussagekraft über ein positives 1,3-β-D-Glucan Ergebnis bis zu 5 Tage nach der Operation limitiert sein kann.

Abstract

Background:

Invasive *Candida* infections occur in immunocompromised patients as well as in patients treated in intensive care units. Furthermore, invasive candidiasis is the most common serious and potentially life threatening fungal infection amongst these patients.

1,3-β-D-Glucan, a major cell wall component of many fungi, is used as a biomarker for invasive fungal diseases. Other glucans, such as cellulose, can interfere with 1,3-β-D-glucan testing and may lead to false positive results.

1,3-β-D-glucan is part of *Candida* species colonizing the intestinal tract and can reach the bloodstream during or after disruption of the intestinal mucosa. Abdominal surgery involving the intestinal tract is usually associated with disintegration of the intestinal mucosa. Abdominal surgery might therefore influence the impact of 1,3-β-D-glucan testing. The aim of this study was to evaluate the influence of open and laparoscopic abdominal surgery on peri- and post-operative 1,3-β-D-glucan values.

Hypothesis:

Candida spp. are colonizing the intestinal tract. In addition, food contains 1,3-β-D-glucan as well as other glucans. Therefore, we hypothesize that laparoscopic and open abdominal surgery leads to elevated 1,3-β-D-glucan values due to disintegration of the intestinal mucosa and/or use of cellulose during surgery.

Methods:

50 patients were included in the study, 24 underwent laparoscopic surgery and 26 open abdominal surgery. In each patient 7 blood samples were drawn at scheduled time points. By using the modified *Fungitell*© assay (automated BDG testing) 1,3-β-D-glucan values were determined in these samples. 1,3-β-D-glucan values of ≥80pg/ml were considered positive and values of < 60pg/ml were considered negative.

Results:

1,3-β-D-Glucan values significantly rose during and after open and laparoscopic abdominal surgery. In 54% (laparsoscopic) to 61% (open) of patients 1,3-β-D-glucan values were ≥80pg/ml during surgery. 1,3-β-D-glucan values were higher in open abdominal surgery compared to laparoscopic abdominal surgery. No single factor for 1,3-β-D-glucan elevation could be identified.

Conclusion:

1,3-β-D-Glucan values significantly rise during and after abdominal surgery. This elevation can be observed until day 5 after surgery. Therefore the value of a positive 1,3-β-D-glucan test in a perioperative setting seems to be limited up to 5 days due to 1,3-β-D-glucan elevations by surgical procedures.

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List of abbreviations

AG	Antigen
AIDS	Acquired Immune Deficiency Syndrome
AML	Acute Myeoloid Leukemia
AMR	Antimicrobial Resistances
BAL	Bronchoalveolar Lavage
BDG	1,3-β-D-glucan
C.	*Candida*
CA	*Candida* Antigen
CAGTA	*Candida albicans* germ tube antibody assays
CDAD	*Clostridium difficile*-associated diarrhea
CI	Confidence Interval
CNS	Central Nervous System
CRC	Colorectal Cancer
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EORTC	European Organization of Research and Treatment of Cancer
EPIC	European Study on the Prevalence of Nosocomial Infections in Critically Ill patients
FDA	Food and Drug Agency
GIT	Gastrointestinal tract
HIV	Human Immunodeficiency Virus
HSCT	Hematopoietic stem cell transplantation
IBD	Inflammatory Bowel Disease
ICU	Intensive care unit
IFD	Invasive fungal disease
IFI	Invasive fungal infection
IFICG	Invasive Fungal Infection Cooperative Group
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IVIG	Intravenous Immunoglobulin
LPS	Lipopolysaccharides
MA	Massachusetts

MALDI	Matrix-Assisted Laser Desorption/Ionization
MB	Megabase
MDS	Myelodysplastic syndrome
MSG	Mycoses Study Group
NIAID	National Institute of Allergy and Infectious Diseases
NNIS	Nosocomial Infection Surveillance system
NOS	Not otherwise specified
NPV	Negative Predictive Value
PAS	Periodic Acid-*Schiff* Reaction
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
RPS	Reduced-port laparoscopic Surgery
RVC	reconstituted-vial concentrations
spp.	species
SPS	Single-port laparoscopic Surgery
TOF	Time of Flight
US	United States
vs.	versus

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1. Introduction

Candida species are known to colonize physiologically mucosal surfaces as part of the microbial flora in the human body (microbiota and mycobiota) without causing signs or symptoms of infection and to cause a wide variety of diseases, including mucocutaneous infections and potentially fatal invasive infections of the bloodstream or organs. Throughout the past decades, invasive fungal infections (IFIs) are of increasing importance even in critically ill, non-immunocompromised patients who are in need of treatment in intensive care units (ICU) or have undergone major surgeries. Several factors, including amongst others parenteral nutrition, central venous catheters, broad-spectrum antibiotics admission and disturbance of gastrointestinal mucosa integrity, have been associated with an increased incidence of IFIs. (1)

1,3-ß-D-Glucan (BDG), a major part of the fungal cell wall, can be detected during infections due to several clinical relevant fungi such as *Candida spp.* and *Aspergillus spp.* . Equipped with a good sensitivity but moderate specificity, BDG testing has an excellent negative predictive value. Thus, BDG testing is widely used to assess invasive fungal infections and guide therapeutic decisions, i.e. to withhold antifungal therapy in patients at risk. (2)
Unfortunately, false-positive BDG results have been reported due to several circumstances, such as bacteremia, haemodialysis, blood transfusions, human serum albumin, haemolysed specimen, contact with cellulose based gauze and administration of antimicrobial agents. (3) Moreover, the kinetics of BDG after intestinal mucosal damage (e.g. due to mucositis or gut surgery) is still poorly understood. (4, 5) In abdominal surgery a key concern in serum BDG kinetics is the potential introduction of cellulose, a 1-4-β-glycosidic isomer of BDG, from surgical sponges and gauze or the introduction of BDG via damaged mucosa due to surgical disintegration of mucosa.
All above might cause false positive BDG values limiting the value of BDG testing. (6)

As life-threatening intraabdominal candidiasis occurs in 30 to 40% of high-risk abdominal surgical intensive care unit (ICU) patients it is of utmost importance to obtain reliable BDG values for diagnosis or exclusion of invasive candidiasis (7).

The next few chapters provide a short overview of invasive fungal diseases, *Candida* species, different methods in abdominal surgery and diagnostic testing for fungal diseases, including BDG testing.

1.1. Invasive fungal diseases

Fungi are appearing all over the world and with more than 1 million different species their kingdom is vast. They are eukaryote organism, having a cell membrane containing chitin, glucan and mannan. Moreover, fungi are both saprophytic and heterotrophic and therefore they play an important role in our ecological system.

Around 150 kinds of fungi can colonize and infect humans. However, most fungi are opportunistic and thus mostly induce a local or systemic infection in patients with an immunosuppression or damage of unspecific host defense, like the maintenance of skin and the mucosal integrity. The latter might be impaired by surgical interventions or treatment at ICUs, as intestinal mucosa is disrupted in abdominal surgery and skin is disrupted by placement of catheters used in the ICU. (8)

In clinical practice it is difficult to establish the diagnosis of invasive fungal infection. In 2002 the consensus group of the *European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC)* and the *Mycology Study group (MSG)* of the *National Institute of Allergy and Infectious Diseases (NIAID)* therefore published standard definitions for invasive fungal infections for clinical and epidemiological research.
Invasive fungal infections (IFI's) are described with the presence of fungal elements either as mold or yeast in deep tissues of biopsy or needle aspirates, which has to be confirmed by culture or histopathology.

IFI's are caused by three different major groups of fungi: The first one is composed by **yeasts**, such as *Candida spp*, *Cryptococcus spp* and more rare ones like *Saccharomyces spp, Trichosporo spp, Malassezia spp, Geotrichu candidum, Hansenula anmola, Rhodotorula spp and Picchia spp.* . (9) The term yeast defines an unicellular organism, with some species having the ability of forming strings of connected cells, called pseudohyphae. (8)

The second one is composed by **molds**, such as *Aspergillus spp*, *Fusarium spp*, *Scedosporium prolificans*, *Mucor*, *Rhizopus*, *Rhizomucor* and *Absidia*. (9) Mold is a fungus that grows multicellular, in the form of filaments, called hyphae. (8)
Lastly **dimorphic fungi**, including *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Paracoccidioides spp*, *Sporothrix spp* and *Penicillium marneffii*, can exist in both forms, as mold and yeast. (9)

As already mentioned above, fungi are opportunistic organism. Therefore the outcome of the disease also depends on the host factors in addition to the fungal virulence.
In the last decades, fungi have evolved as a major global health problem, which might be due to increasing frequency of immunosuppression either caused by infections, such as HIV, cancer treatment or metabolic disorders like diabetes. (8)

The definition by the *EORTC/MSG* further contains 3 levels of probability or certainty for the diagnosis of invasive fungal infections: "proven", "probable" and "possible" invasive fungal infection. The definitions for the different levels allow clinicians to define invasive fungal infections with a variable and stepwise certainty of diagnosis. These definitions have been again reviewed and revised by the *EORTC/MSG* in 2008. (9)

1.1.1. Proven invasive fungal disease

A "proven invasive fungal disease" is a fungal infection that can be detected by extracting fungal elements from the patient's tissue from a site of disease. It can therefore be detected by histological analysis or culture, except for *Cryptococcus neoformans*, where a detection of capsular antigen in CSF (cerebrospinal fluid) or a positive result of an India ink preparation of CSF is considered as sufficient to establish a diagnosis of proven cryptococcosis. (9)

1.1.2. Probable invasive fungal disease

By contrast, probable invasive fungal infections are described by three different criteria; a **host factor**, that identifies patients at risk, **clinical signs and symptoms**, and **mycological evidence**, such as a culture, a microscopic analysis and indirect tests, like antigen detection. An overview over these factors is listed in the table below. (9)

Table 1: Criteria for probable IFD (except for endemic mycoses) (9)

Criteria for probable IFD (except for endemic mycoses*)
Host factors: • Recent neutropenia (<0.5 × 10^9 neutrophils/L [<500 neutrophils/mm^3] for >10 days) related to the 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) o Mold in sputum, BAL, bronchial brush or sinus aspirate samples • Indirect tests o Aspergillosis: Galactomannan antigen detected in plasma, serum, BAL or CSF o Invasive fungal disease other than cryptococcosis and zygomycoses: 1,3-β-D-glucan detected in serum

**this chart only provides an overview, detailed information is provided in reference 9.*

1.1.3. Possible invasive fungal disease

The category of possible IFD includes only those cases with the appropriate **host factors** and with sufficient **clinical evidence** consistent with IFD, but for which there was no mycological criteria (direct or indirect tests).

However, this category was not considered appropriate for endemic mycosis, because the host factors and the clinical features are not sufficiently specific and the number of cases is too little to be included into clinical trials, epidemiological studies, or evaluations of diagnostic tests. (9)

1.2. Candida species

Candida species are physiologically colonizing, eukaryotic, commensal yeasts belonging to the phylum *Ascomycota*. With more than 200 kinds of yeasts belonging to the genus *Candida*, the species are very widespread around the world and can be recovered from environmental, human and other mammalian sources. *Candida* species are most commonly found as part of the normal microbial flora within healthy individuals on mucosal surfaces of the oral, gastrointestinal and genitourinary tracts.

Only a rather small number of *Candida* species are of clinical importance including *Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis, Candida parapsilosis* and *Candida dubliniensis. Candida albican*s is by far the most prevalent commensal and pathogenic of the *Candida species*. (8, 10)

Colonies of *Candida spp.* are macroscopically cream-colored to yellowish. Their texture can be, depending on the species, pasty, smooth, glistening or dry, wrinkled, and dull. All species of *Candida* produce blastoconidia, and most produce pseudohyphae. In addition, true hyphae and chlamydospores can be produced by some *Candida* species. (11)

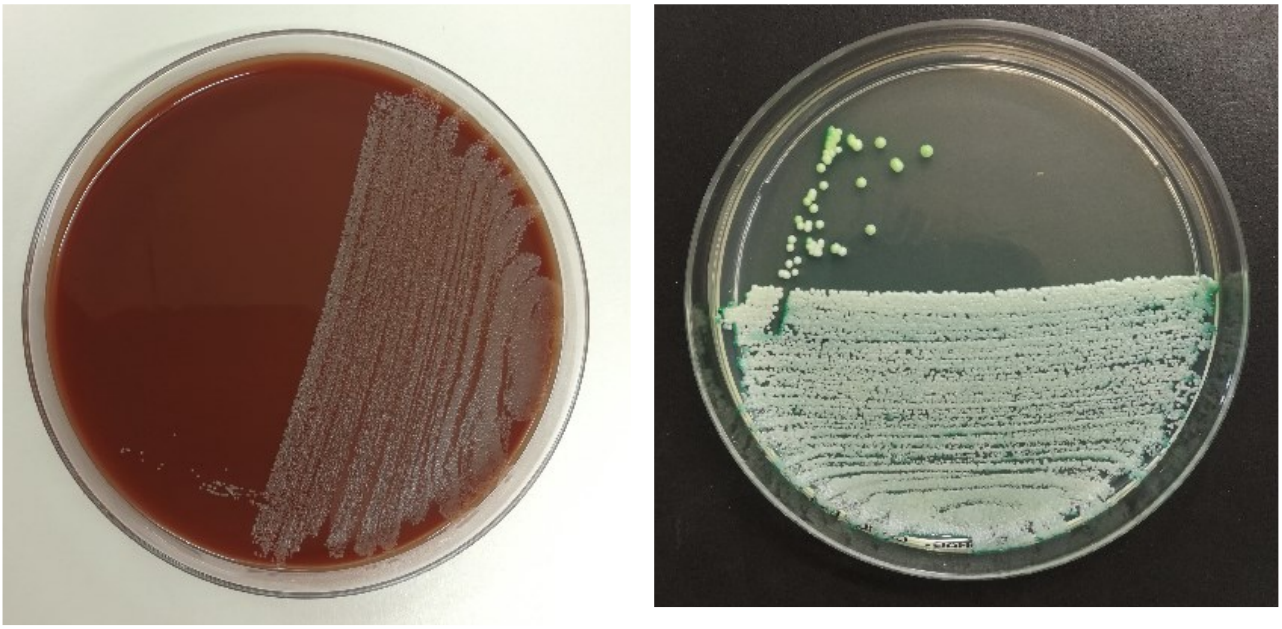

Figure 1: *Candida spp.* derived from a patient suffering from candidemia on blood (left) and chrome agar (right)

1.2.1. *Candida* albicans

C. albicans is a dimorphic fungus, which is enabled to grow as yeast or in a filamentous form. It is one of two *Candida* species capable of forming true hyphae, which is considered to play a big role in processes of infection such as adhesion and tissue invasion.

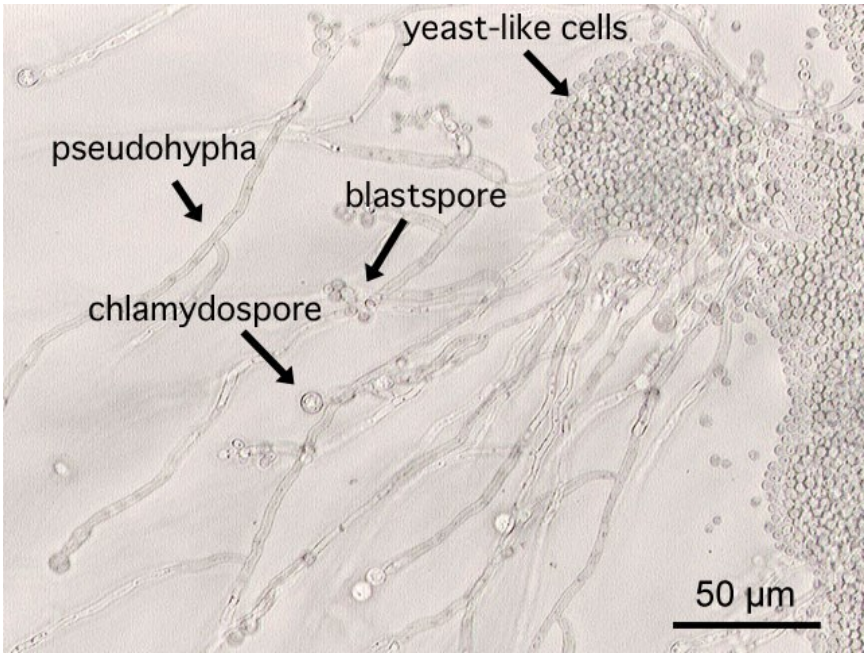

Figure 2: *Candida albicans* microscopically, retrieved from sputum (42)

The genome of *C. albicans* is build by eight pairs of homologs, with a size ranging from 0.95 to 3.3 Mb. The species is normally diploid, however it shows a high range of genome plasticity, seen in the frequent losses of heterozygosis as well as gross chromosomal rearrangements that may result in aneuploidy. The reproduction of *C. albicans* is mostly clonal. It can also utilise a parasexual cycle, which is however very rarely observed in nature. (10)

1.2.2. Clinical aspects of Candidiasis

1.2.2.1. Pathogenesis and Colonization

Potential pathogenic yeasts have a wide repertoire of genes, to adapt to the conditions within the infected organs or tissues. With adhesion-like structures, such as mannoproteins or proteinase molecules, the fungal cells are attaching themselves very tight to epithelial cells, if their receptors are accessible. With lytic enzymes, proteinases and phospholipases the fungus then can overcome the epithelial barrier.

Polymorphonuclear granulocytes and T-lymphocytes with cytokines, which activate macrophages, play an important role to protect the body from colonization by fungi.
As fungi, and therefore also *Candida spp.,* are facultative pathogenic organism, certain factors have to be fulfilled so that the pathogens can spread superficially or invasive within the organs. Those factors are, amongst others, the diminution of bacterial flora due to the use of antibiotics, an elevated pH-level in the vaginal mucosa due to contraception or pregnancy, barrier damages of the skin, due to e.g. combustion, extensive abdominal surgery, parenteral nutrition and immunosuppression, due to illnesses such as HIV or leukemia or to iatrogenic processes such as organ transplantation or chemotherapy. Also metabolic derangements due to diabetes may lead to a better adhesion of fungal cells. (8, 12)

1.2.2.2. Infection

Candida albicans is responsible for most of the oral and systemic candidiasis cases. It can cause, depending on the predisposition and the disease of the patient, either a mucocutaneous infection or a systemic mycosis. (8) Exemplary, *Candida spp*. are causing around 72,8 million cases per year with a case/fatality rate of 33,9%. (38)

The most common mucocutaneous infections include *Candida* vulvovaginitis, infections of the mouth and infections of the skin. *Candida* vulvovaginitis is a frequently recurrent mycosis, with symptoms such as burning and itching and a light vaginal discharge. Infections of the mouth are very common and can also affect the esophagus (oropharyngeal and/or esophageal candidiasis). Those infections occur most frequently in patients with AIDS, diabetes mellitus, leukemia or patients undergoing chemotherapy.
Infections of the skin or the skin appendages, such as infections of nails (onychomycosis), are less frequent.
The most important environmental predisposing factors are humidity, warmth and darkness. (8)

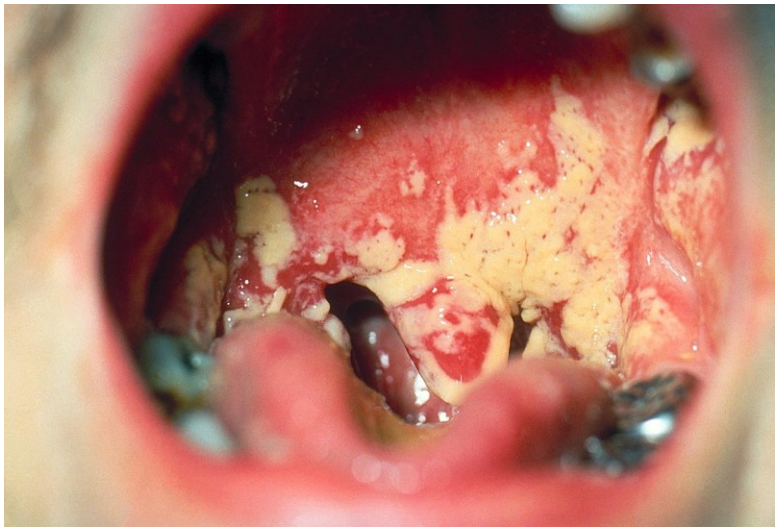

Figure 3: Oropharyngeal Candidiasis (44)

Invasive candidiasis can either be represented as candidemia and/or deep-seated candidiasis. Candidemia mostly derives from translocation of commensal *Candida* from the gastrointestinal tract (GIT) or contamination/colonization of an intravenous catheter. Systemic candidiasis is observed with increased frequency in the past years and has a very high lethality of around 30-40%. (1)
Candida spp. spread via the blood system and mostly infect the liver, kidneys, peritoneum and the endocardium. Less common infected organs are the central nervous system (CNS), the eye and the skeletal system. (12)

Approximately 50% of primary candidemia are causing secondary deep-seated candidiasis involving various organs. However, deep-seated infections can also arise from non-hematogenous introduction of *Candida* into sterile sites, such as the abdominal cavity after

disruption of the gastrointestinal tract. Five to 20% of such primary deep-seated *Candida* infections lead to candidemia (secondary candidemia).

Major predisposing factors for *Candida* infections have been identified by various studies; and *Candida* colonization is the leading one. Furthermore, exposure to antibiotics, neutropenia, vascular access devices, surgical procedures, the presence of renal failure, the use of steroids and H2 blockers, a high severity of the illness score, mucosal or cutaneous barrier disruption, defects in the number and function of neutrophils or in cell-mediated immunity, metabolic dysfunction, advanced age, or a longer ICU stay are proposed as risk factors for invasive candidiasis. An overview of those factors and the associated fungal pathogens can be found in *Table 2*. (1, 38)

Table 2: Factors involved in the development of opportunistic mycoses (38)

Factor	Fungal pathogen(s)*
Mucosal and cutaneous barrier disruption	• *Candida spp.* • *Aspergillus spp.*
Neutrophil dysfunction (quantitative or qualitative)	• *Candida spp.* • *Trichosporon spp.* • *Aspergillus* and other molds
Defects in cell-mediated immunity	• *Cryptococcus spp.* • Endemic mycoses
Metabolic disorders	• Zygomycetes • *Candida* spp.
Extremes of age (<1 and >70 yr)	• *Candida spp.*

**This list is not all-inclusive.*

A spectrum of diseases related to *Candida spp.* can be overviewed in *Table 3*.

Table 3: *Candida* infections in human beings: spectrum of diseases (12)

Hematogenous infections	Non-hematogenous infections
Candidemia Endophthalmitis Vascular-access-related infection Arthritis	Superficial infections: • Cutaneous candidiasis • Oropharyngeal candidiasis

Septic thrombophlebitis Infectious endocarditis Osteomyelitis Pyelonephritis Pulmonary candidiasis Meningitis Hepatosplenic candidiasis Spondylodiscitis	• Vaginitis • <u>Deep-seated infections:</u> • Oesophageal candidiasis • Cystitis • Peritonitis • Tracheitis/bronchitis • Hepatosplenic candidiasis

1.2.2.3. Candida infection in critically ill patients

Although candidiasis is still most frequent amongst immunocompromised patients, data from the last years show an increased importance in critically ill patients.

Data from 790 ICUs from nearly 300 institutions reporting to the *US National Nosocomial Infection Surveillance system* (NNIS) between 1990 and 1999 showed that *Candida spp.* were responsible for 5–10% of all bloodstream infections. Thus, they represent the fourth leading organism causing an infection, preceded only by *coagulase-negative Staphylococci*, *Staphylococcus aureus* and *enterococci*.

Also, according to the *European Study on the Prevalence of Nosocomial Infections in Critically Ill patients* (EPIC), which included 10 038 patients from 1417 intensive care units in 17 countries in 1992, invasive candidiasis accounted for 17% of hospital-acquired infections. In patients generally admitted to the hospital, invasive candidiasis only occurs in 1-8%. (1)

Recently, in a point prevalence study *Candida spp.* were identified as the leading cause of nosocomial blood stream infection. (47)

Furthermore, an early identification of invasive candidiasis within ICU patients is highly challenging due to the patient's various underlying conditions and due to drawbacks of current diagnostic methods, which are not sufficiently sensitive or specific. (22)

All these findings confirm that severe *Candida spp.* infections can no longer be considered as rare infections. Particularly patients with severe underlying diseases or critical illnesses that need aggressive diagnostic or treatment procedures are affected and should therefore be closely monitored. (1)

1.2.2.4. Treatment

For treatment of fungal diseases, there is a spectrum of antifungals with different modes of action. An overview of antimycotic medication can be found in *Table 4* below.

Polyene antifungals have the ability to bind with sterols, mainly ergosterol, which is a major cell wall component of fungi. That causes a pore, which leads to leakage and cell death.

Azole antifungals inhibit the synthesis of ergosterol by binding the heme iron in the cytochrome P450 complex of the enzyme that normally conducts the transformation of lanosterol to ergosterol. Under azole antifungals triazoles, imidazoles and thiazoles are summarized.

Allylamines are inhibiting the squalen peroxidase, which is also required for the ergosterol synthesis.

Echinocandins inhibit the cell wall synthesis via inhibiting the enzyme 1,3-β-glucan-synthase. This enzyme normally produces 1,3-β-D-glucan, a major component of the fungal cell wall.

Flucytosine is an antimetabolite pyrimidin analog that leads to DNA strand breaks. Griseofulvin inhibits the mitosis spindle apparatus of fungi. (28)

Table 4: Classification of Antifungal Drugs (28)

Antifungals
Polyenes: • Nystatin • Natamycin • Amphotericin B
Imidazoles: • Miconazole • Clotrimazole • Ketoconazole
Triazole: • Fluconazole • Isavuconazole

• Itraconazole • Posaconazole • Voriconazole
Allylamine: • Amorolfin • Butenafine • Naftifine • Terbinafine
Echinocandins: • Caspofungin • Micafungin • Anidulafungin
Others: • Griseofulvin • Flucytosine

Cutaneous infections with *Candida spp*. are usually treated with topical application of disinfectants such as ethyl alcohol, betaisodona or octenisept, and the topical or systemic application of antifungal drugs such as polyenes and azoles. Rarely, systemic treatment is needed to treat cutaneous infections.

Systemic candidiasis is treated by high-dose systemic application of Fluconazol or other derives from azoles or with echinocandins. (28)

It is of utmost importance to treat systemic candidiasis as early as possible related to the onset of candidemia. Delays of treatment initiation result in continuously decreasing survival rates. (43)

There are several mechanism of resistances arising in *Candida spp*. . These include, but are not limited to, changes in the cell wall or plasma membrane leading to an impaired uptake of antifungals; efflux pumps that take antifungals outside the cell; overexpression of the antifungal targets; mutations of the antifungal target that decrease its binding ability; activation of alternate pathways that increase the metabolism of the antifungal; sequestration of the antifungal in organelle-like vacuoles; or chromosomal changes to increase the number of copies of the required gene.

Structural changes of the sterol content in the cell wall are associated with a resistance against polyenes. The lack of ergosterol results in a reduced binding of liposomal amphotericin B and nystatin in certain strains of *Candida*.
However, the proportion of *C. albicans* resistant to i.e. triazoles is extremely low, ranging from 0–0,5%; whereas almost all cases were reported in previously exposed, immunocompromised patients. (1)

In the 1980s triazole-based antifungal prophylaxis was introduced to use amongst patients with chemotherapy-induced neutropenia or for conditioning before bone-marrow transplantation. A meta-analysis of 38 randomized, controlled clinical studies including more than 7000 patients showed that prophylaxis reduces the use of parenteral antifungal therapy, the rate of superficial and invasive fungal infection, as well as fungal-infection-related mortality in patients with malignant diseases and prolonged neutropenia.
However, prophylaxis has also been shown to increase the risk of infections due to non-*Candida albicans* strains such as *C. krusei*, which is intrinsically resistant to some triazoles, or *C. glabrata*, which may be sensitive only to higher doses.
Posaconazole is used as first line antifungal prophylaxis in patients with chemotherapy for AML or MDS and with immunosuppression for treatment of graft versus host disease in HSCT patients. (1)

It has been speculated that antifungal prophylaxis in critically ill, non-immunocompromised patients should be considered for selected groups where the incidence of candidiasis is expected to be higher than 10%. However, it was shown that there is no benefit regarding survival rate in critically ill patients receiving antifungal prophylaxis. (1, 45)

1.3. Surgical procedures

1.3.1. Open abdominal surgery

Open abdominal surgery involving the colon and rectum is mostly performed due to colon cancer, diverticulitis, inflammatory bowel disease (IBD) and angiodysplasia.
There are various different techniques applied depending on the underlying disease, the extension and the resected section.

The most common ones are hemicolectomy on the right or left side, subtotal colectomy, resection of the transverse or sigmoid colon and rectum. (13)
A more thorough description of open surgery procedures performed within this study can be found below in section *2. Materials and Methods.*

1.3.2. Laparoscopic abdominal surgery

A laparoscopy (from ancient greek: λαπάρα *(lapara)*, meaning 'flank, side', and σκοπέω *(skopeo)*, meaning 'to see') is the inspection of the abdominal cavity with the aid of a special endoscope.
Laparoscopic surgery is performed in gynecology for adhesiolysis and in visceral surgery for appendectomies, hernia surgery, splenectomies or colon surgery.
In abdominal surgery, laparoscopic surgery is mostly used for colon resection due to diverticulitis, polyps, and benign or malignant colon cancer.

Historically, open laparotomy was the standard surgical procedure in colorectal cancer (CRC) surgery. However, since the introduction of laparoscopy, the proportion of laparoscopic colorectal surgeries has increased. There are some prospective randomized studies of laparoscopic surgical treatment for colon cancer showing better short-term outcomes such as reduced hospital stay, better cosmetic effects, and less pain, as well as comparable long-term outcomes to those of open surgical treatment. Therefore, laparoscopic colon cancer surgery is today a standard surgical treatment. (35)

In laparoscopic surgery, an aeroperitoneum is made after incision of the lower umbilical border by insufflation of air for better sight. Up to three more trocars are placed in a hemicycle at the lower abdomen. After mobilization of the colon, mesosigma and rectum, the mesosigma and mesorektum (depending where the resection borders are) is dissected from the colon. At the resection border the rectum is dissected with the endostapler. Trough a mini-laparotomy, the eventeration and dissection of the colon is performed. The colon is repositioned in the abdominal cavity and closed with the “Double-stapling”-technique. (13)

Single-port laparoscopic surgery (SPS), in which only one port instead of three is used during operation, is also feasible. However, laparoscopic colectomy with SPS requires a longer operation time, highly experienced surgeons, and advanced surgical techniques and

materials. Reduced-port laparoscopic surgery (RPS), which involves the insertion of an additional port in SPS, was introduced to overcome these challenges. (35)

A more thorough description of the laparoscopic procedure performed within this study can be found below in section *2. Materials and Methods.*

1.4. Diagnosis

1.4.1. Microscopic and culture-based diagnosis

Yeasts such as *Candida spp.* can be detected microscopically in the native specimen from a smear of the skin or mucosa. To make the fungal elements more visible the specimen can be stained with PAS, silver staining by Grocott-Gomori or optical brightening with Calcofluor. (8)

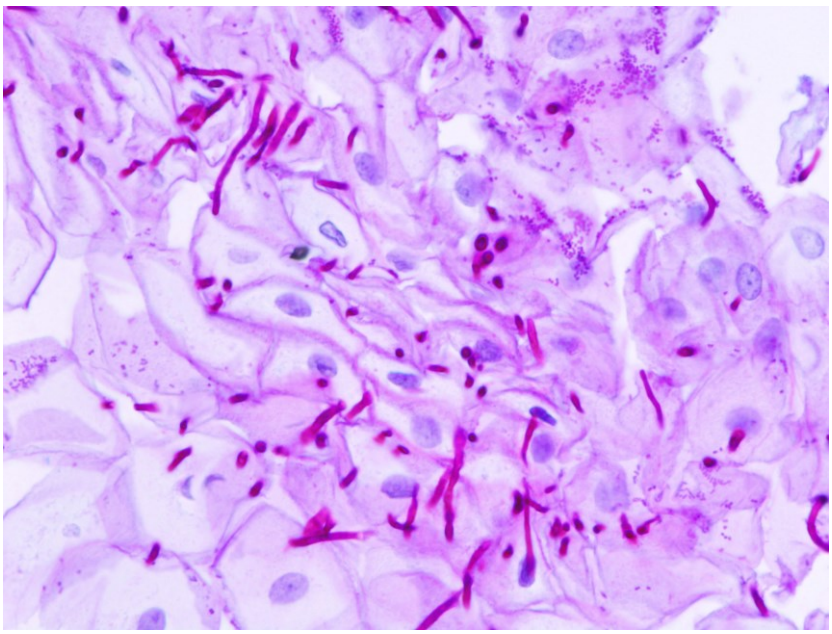

Figure 4: Micrograph of esophageal candidiasis showing hyphae, biopsy specimen, PAS stain (46)

Direct microscopy provides an important diagnostic benefit together with culture, due to its rapid availability of results, often within 1 to 4 hours of a specimen's arrival in the laboratory. Microscopy can distinguish whether an infection is caused by a septate mold (*Aspergillus spp.*) or non-septate mold (*Mucorales*). Typical staining methods used in routine laboratory include Gram stain, India ink, optical brighteners, Papanicolaou stain, methenamine silver and immunofluorescent stain. (2)

Cultures from different specimen such as blood, secretions, smears and biopsies, can be done on commonly used agar plates, such as blood or chocolate agar. However the proliferation speed is comparatively low. (8) Blood cultures usually are positive after 2 days of incubation, within a range of 0 to 10 days, in patients with fungemia. (23)
Differentiation between species is done by the morphology under the microscope, the testing of biochemical factors and mass spectrometry (MALDI-TOF). (8)

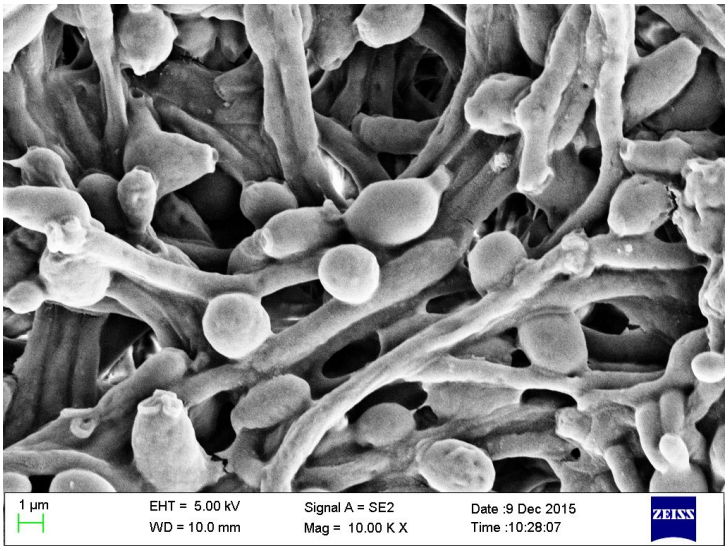

Figure 5: *C. albicans* visualized using scanning electron microscopy (21)

In patients suffering from candidemia most blood cultures are positive, however only around 40% of patients with a deep-seated infection, which can persist after candidemia, are positive in blood culture testing. Patients with a deep-seated infection without candidemia are negative in blood culture testing.
Taken together all different forms of invasive candidiasis (candidemia, intraabdominal candidiasis) the sensitivity of blood cultures is only around 50%. (14)

1.4.2. Non-culture-based diagnosis

Although blood cultures are still the gold standard for diagnosis of candidemia, the testing is very slow and can be inconclusive. It was shown that administration of antifungal therapy within 12h after drawing blood for cultures leads to reduced mortality within patients with multiple morbidities. Therefore many patients with clinical signs and symptoms of candidemia are treated empirically with antifungal therapy, which increases

the risk of antimicrobial resistances (AMR), the risk of adverse drug reactions and treatment costs. (23)

Due to the need for faster and more sensitive methods, non-culture-based methods were developed. Those methods include the detection of *Candida* DNA and circulating fungal antigens in the serum. Commercially available tests include 1,3-β-D-glucan (BDG), mannan antigen (Ag), Cand-Tec *Candida* antigen (CA) and *C. albicans* germ tube antibody assays (CAGTA; Vircell Kit and VirClia IgG Monotest, Grenada, Spain).
Candida antigen tests can be limited due to low serum concentrations and the rapid clearance from the bloodstream. (14, 15)

The Cand-Tec *Candida* antigen test uses uniform-sized latex particles coated with an Anti-*Candida* antibody to detect the presence of *Candida* antigens in the serum of patients using an agglutination reaction. (16) However, according to a study by Held et al., the Cand-Tec latex agglutination test (Ramco Laboratories, TX) that was used for CA measurement shows a poor sensitivity of only 13.0% whereas the specificity was 93.9%. (15)

Mannan is a polymer of mannose and builds as polysaccharide the outer cell wall layers of *Candida*. (17) A meta-analysis of 14 studies has shown a sensitivity and specificity of 58.9% and 97.5% for mannan antigen and 62.5% and 65.0% for anti-mannan antibody respectively. A better sensitivity and specificity of 89.3% and 63.0% was observed when combining mannan antigen and anti-mannan antibody, with best performances among patients with infections of *C. albicans, C. glabrata* and *C. tropicalis*. (14) However, only little clinical assessment has been done at present and the test might not detect some *Candida spp*. . Therefore it is not recommended to use testing for mannan and anti-mannan antibodies yet. (2)

C. albicans germ tube antibody assays (CATGA) detect responses against a hyphal protein (Hwp1), which is expressed during tissue invasion and biofilm formation. In different reports sensitivity and specificity widely differ from 42% - 96% and 54% - 100%, respectively. Also the sensitivity may be lower for infections caused by *C. tropicalis*.

Although the FDA did not clear PCR for detection of *Candida* DNA yet, there are test commercially available, which are mostly intended for whole blood and blood fractions.

Although the interpretation of the PCR data is very complicated, a meta-analysis of 54 studies that included almost 5000 patients tested with blood-based PCR, has shown a pooled sensitivity and specificity for proven or probable invasive candidiasis vs. at-risk controls of 95% (CI: 82-98%) and 92% (CI: 87-98%) respectively.

BDG testing will be further described in chapter *1.4.2.2. Diagnostic testing and 2. Materials and Methods*.

Major concerns with anti-*Candida* antibody include the need of time to mount detectable responses, and that positive results may not be able to distinguish acute from past infections. However, anti-*Candida* antibody testing has well performed in various studies. (14)

1.4.1. *Candida* Score

As the early diagnosis of IFIs still remains a challenge, some authors have developed clinical prediction rules to identify ICU patients at high risk of candidiasis, who could benefit from an early antifungal treatment.
A Spanish group identified in 2006 four predictors of proven invasive *Candida* infections, on which a score named "*Candida* Score" was built. The components of the "*Candida* Score" are severe sepsis (2 points), total parenteral nutrition (1 point), surgery (1 point), and multifocal *Candida* colonization (1 point).

With the "*Candida* Score" it is possible to differentiate amongst ICU patients with hospital-acquired severe sepsis or septic shock, those who would benefit from early antifungal treatment (score > 3) from those for whom invasive candidiasis is highly improbable (score ≤ 3). In the respective study the rates of invasive candidiasis were 0% in patients with a score of 2 or 3, 17.6% in patients with a score of 4, and 50% in patients with a score of 5. (27)

Another study, conducted by Leon et al., could show in a cohort of *Candida*-colonized patients staying more than 7 days in an intensive care unit and having a *Candida* score <3, a rate of invasive Candidiasis of <5% without antifungal treatment. Therefore invasive Candidiasis is highly improbable amongst *Candida*-colonized patients having a *Candida* score <3. (26)

1.4.2. 1,3-β-D-Glucan (BDG)

1.4.2.1. Structure

Glucanes are polysaccharides build of D-glucose monomers linked by glycoside bonds. They are naturally occurring in cell walls of e.g. cereals, bacteria and fungi. The physicochemical components depend on the different species. Usually, β-glucanes are arranged in six-sided D-glucose rings connected linearly to form a linear backbone with 1,3-β-gylcosidic bounds. However, this can vary with molecular mass, viscosity, branching structure and gelation properties.

Some β-glucan molecules can also form glucose side-chains, which can be attached to other types of molecules such as proteins. Yeast and other fungal β-glucans contain additionally β-1,6 side branches, while cereal β-glucans contain β-1,4 branches. The frequency, location, and length of those side-chains may play a role in the processes of immunomodulation. Differences in molecular weight, shape, and structure of β-glucans cause differences in biological activity. (19, 20)

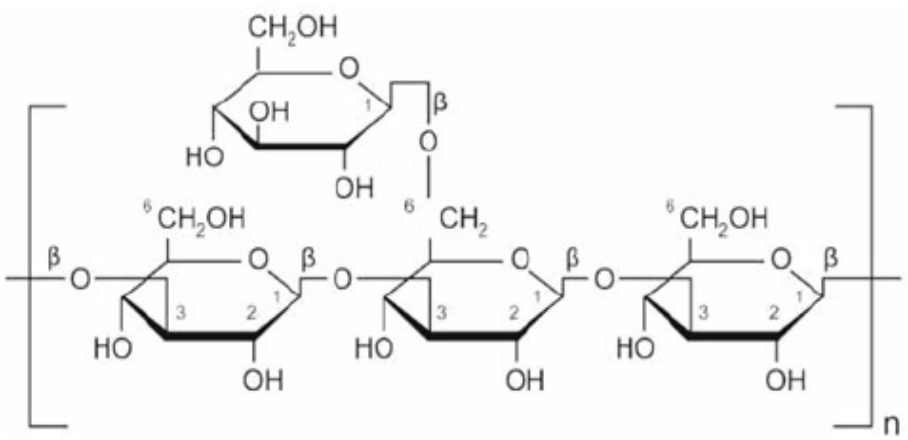

Figure 6: Structure of BDG (41)

1,3-β-D-Glucan is a glucose polymer and a major cell wall component of most pathogenic fungi, such as *Candida spp.* and *Aspergillus spp.*, expect *Mucorales* and *Cryptococcus neoformans.* (18)

1.4.2.2. Diagnostic testing

There are several assays that are commercially available for BDG testing, of which the *Fungitell*© test (Associates of Cape Cod, East Falmouth, MA) has been studied most comprehensively. (14)

Assays for BDG do not measure directly the BDG concentrations. They are based on the activation of the horseshoe crab coagulation cascade by binding BDG, using colorimetric or turbidimetric methods for quantification.

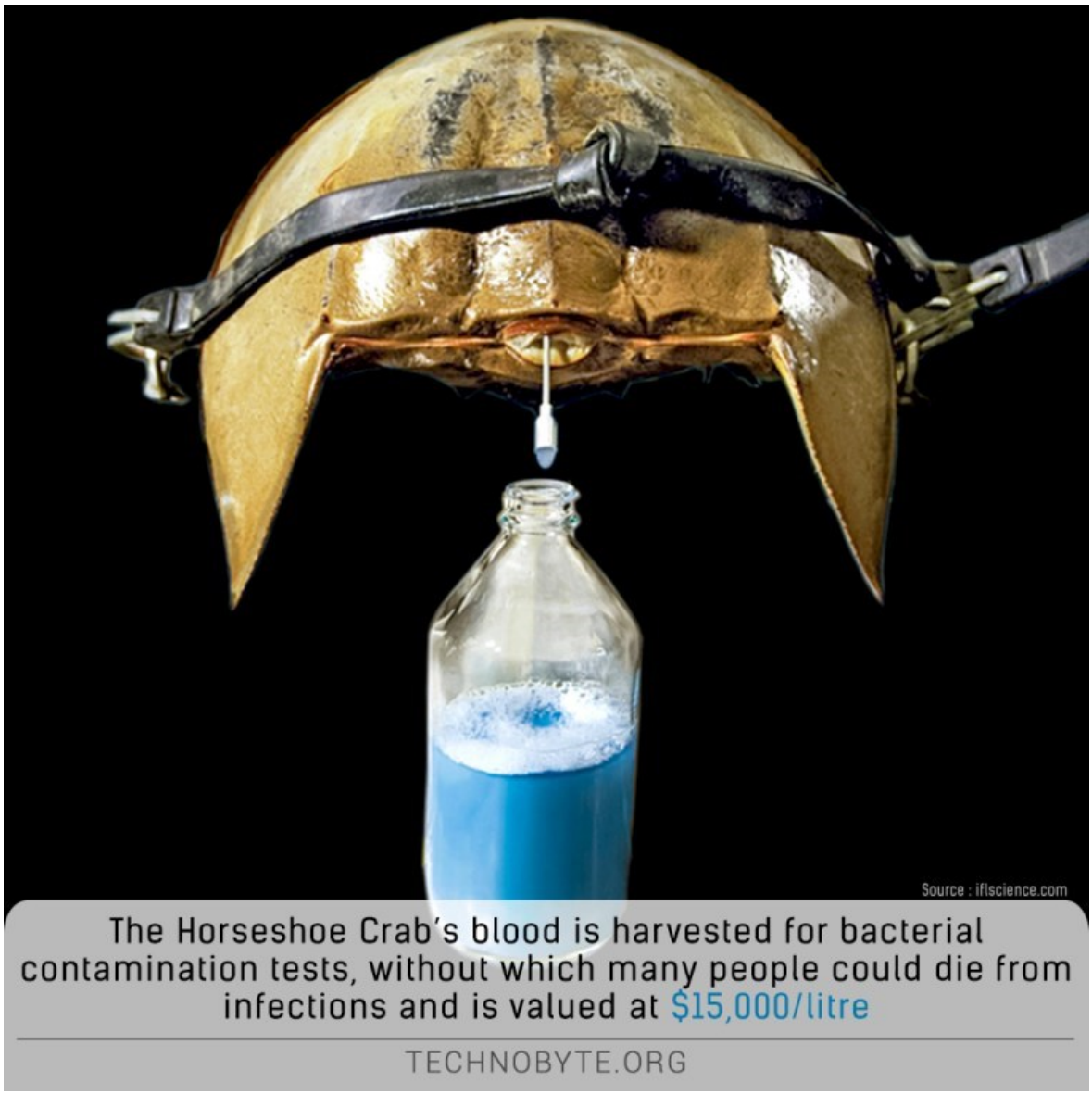

Figure 7: Harvesting Horseshoe Crab's blood (25)

Liposaccharides (LPS) and BDG initiate the coagulation cascade by activating different serine protease zymogens (factors C and G). Liposaccharide activates factor C, while BDG activates factor G. (14, 18)

BDG test, such as the *Fungitell*© test, cannot distinguish between *Candida* and other fungi.

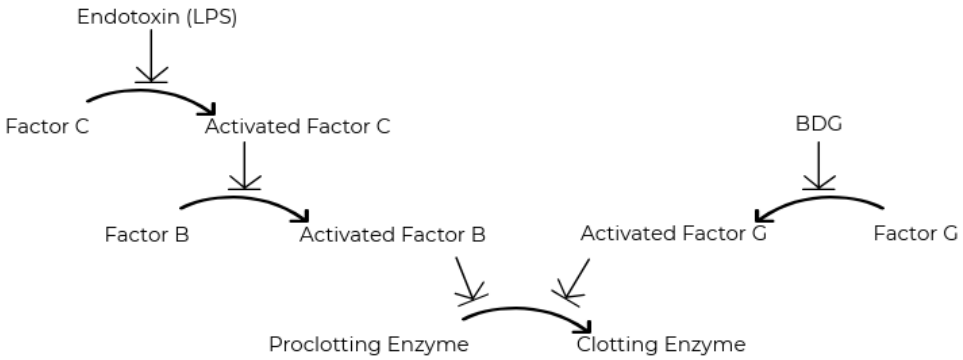

Figure 8: Enzyme Cascade of BDG-testing (18)

In the *Fungitell*© assay, in which BDG is detected in serum samples, 5 µl of serum is pretreated with 20 µl of an alkaline reagent (0.25 M potassium hydroxide and 1.2 M potassium chloride, mixed 1:1) for 10 minutes at 37°C in order to convert triple-helix glucan to single-helix glucan and inactivate serine proteases and serine protease inhibitors in the serum sample. After this step, 25 µl of each of the five standards with corresponding concentrations (500, 250, 125, 62.5, and 31.25 pg/ml) and 25 µl of the blank included in the assay are transferred to the microplate, followed by an addition of 100 µl *Fungitell*© reagent to each well. Then, the microplate is inserted into an incubating (37°C) plate reader. Every serum sample is tested in duplicate. The assay was monitored at 405 nm kinetically for 40 minutes.

The *Fungitell*© assay was adapted to be automated using a routine BCS XP coagulation analyser according to the manufacturer's instructions. After calibration and generation of a standard curve using six corresponding concentrations, linearity and precision were evaluated using the standard preparation included in the kit. (23)

In a study conducted by Held et al., the sensitivities and specificity were 87.5% and 85.5% for BDG testing with a cutoff of ≥80 pg/ml as positive test result in patients with culture-confirmed candidemia. (15) Another study by Ellis et al. showed a slightly lower sensitivity and specificity using the same cutoff in patients with persistent neutropenic fever of 86.8% and 76.2% and a PPV, NPV and overall accuracy of 76.7%, 86.5% and 81.3%. (24)

Moreover, a meta-analysis of 16 studies was conducted, including 2979 patients in the

main analysis. In four of the 16 studies the *Fungitell©* assay was used, 3 studies used the *Glucatell©* assay. Those assays are the commercial and the research version of the same assay marketed by *Associates of Cape Cod.* Four other studies used BDG assays developed by the *Seikagaku Corporation.*
The study showed a pooled sensitivity of BDG testing for diagnosis of IFIs of 76.8% (95% CI, 67.1% - 84.3%), and specificity of 85.3% (95% CI, 79.6% - 89.7%). Also, no considerable difference between the performance of BDG testing for the detection of systemic *Candida* or *Aspergillum* infections could be observed. (40)

Furthermore, a study by Prüller et al. from 2014 has shown an excellent precision for automated single-sample and also large-scale testing. In contrast to the manual standard protocol, single patient samples can be tested faster, more reliable and with reduced costs, which makes it clinically even more usable. (23)

1.4.2.3. Limitations of BGD testing

Interpretations of BDG studies are difficult due to heterogeneity in patient and control populations, different types of *Candida* or even other fungi, testing schedules, specific assays and different definitions of positive results. (18)
Furthermore, false-positive BDG results are reported to occur with bacteremia, hemodialysis, blood transfusions, human serum albumin, hemolysed specimen, contact with gauze and administration of antimicrobials intravenous. (3)

BDG results can be falsified due to cellulose, which is the 1-4-β-glycosidic isomer of BDG. Cellulose can be found in surgical gauzes and filters and is used in the manufacturing process of intravenous medication such as antibiotics and albumin. (6)
In a study conducted by Kanamori et al. six different types of commonly used surgical gauzes were immersed in sterile, purified water for up to 120 minutes. At scheduled time points, the BDG concentrations in the water was measured, whereas purified water without gauze was used as negative control. BDG levels after 120 minutes greatly varied between 11,7 pg/ml (lycoell) and 6612 pg/ml. (39)

Also, extracorporeal blood purification methods, such as hemodiafiltration or hemodialysis, where blamed for elevated BDG levels. However, in a study from Prattes et

al. no false positive BDG levels have been detected, due to synthetic dialyzer membranes, instead of cellulose ones, and ultra-pure production of dialysate. (6)

Furthermore transfusions of blood and different components, such as human albumin, can lead to false positive BDG serum levels. It is suspected that BDG is elevated due to filtration in the manufacturing process, where cellulose filters are used and therefore elute into the blood components. (29)

Moreover, it was demonstrated that the administration of immunoglobulin preparations (IVIG) also leads to false positive BDG levels in a vast majority of patients. BDG peak levels within 3 days after IVIG ranged from 21.47 to 660.38 (median 201.4) pg/ml. The elevated BDG levels were in some patients detectable for more than two weeks after IVIG administration, but normalized within 3 weeks in all patients. Therefore, BDG cannot be used to diagnose IFD within three weeks after IVIG administration. (30)

It was also shown that some antimicrobial agents were tested positive for BDG in their reconstituted-vial concentrations (RVC). In a study conducted by Marty et al., 7 out of 44 antimicrobial agents, have been tested positive for BDG, which are: colistin, ertapenem, cefazolin (in vials), trimethoprim-sulfamethoxazole, cefotaxime, cefepime, and ampicillin-sulbactam in decreasing order. (31)

Also bacterial infections have been identified to cause false positive BDG values. In a study conducted by Albert et al. 62 patients for whom invasive fungal infections were not suspected, were tested by the *Fungitell*© test: 19 control subjects and 43 patients with bacteremia. All 19 control subjects had negative 1,3-β-D-glucan tests; amongst the 39 bacteremic patients were 16 false-positive results. Within the 22 patients undergoing bacteremia due to Gram-negative bacilli, 13 false-positive results (59%) were observed. Among the 17 patients with infections involving Gram-positive cocci, three false-positive tests were recorded. None of the eight cases of *Streptococcus pneumoniae* bacteremia had false high BDG values. In summary, BDG levels were significantly higher in patients with Gram-negative bacilli bloodstream infection in comparison to those with bacteremia due to Gram-positive cocci. (32)

Candida spp. are part of the commensal flora of the gut. An overgrowth of *Candida spp*. in the GI tract can be caused by factors such as long-term antibiotic administration or immunosuppressive drugs, and is a well-known risk factor for systemic candidiasis. Another important pathogenic factor in invasive candidiasis is GI leakage, which may be caused by either direct, such as abdominal surgery, or indirect GI injury.
Recently it was shown that gut barrier impairment could also be induced by chronic colitis, such as Crohn disease, where increased serum BDG levels were observed. Furthermore, intestinal mucositis, caused by i.e. induction of chemotherapy, leads to a loss of integrity of the intestinal mucosal barrier, increasing the likelihood of translocation of bacterial and/or fungal commensals of the gastrointestinal tract, which also can lead to false positive BDG levels. (4, 5)

It is also well known that *Clostridium difficile*-associated diarrhea (CDAD) causes GI leakage. Another study demonstrates, that after inducing in the mouse model CDAD, the BDG serum levels are increasing parallel to the GI leakage. As there was no viable *Candida* or other fungi detected, the BDG elevation cannot be caused by an invasive fungal infection.
Due to all these reasons a high serum BDG is not only a biomarker of systemic fungal infection but could also be an indicator for GI leakage. (33)

2. Materials and Methods

2.1. Study population

This prospective study was performed at the Department of Internal Medicine, Section of Infectious Diseases and Tropical Medicine, Medical University Graz, Austria, together with the Department of Surgery, Hospital of St. John of God (Krankenhaus der Barmherzigen Brüder) Graz, Austria, with the approval of the local ethics committees.

All patients undergoing laparoscopic or open intestinal surgery involving the small bowel and/or colon and/or rectum at the Hospital of St. John of God (Barmherzige Brüder), Graz, between April and June 2018 were asked for participation in the study and included after signature of the informed consent. Open and laparoscopic surgical procedures were performed as applied in clinical routine and published previously. (35, 36) The surgeons performed the assignment to one of the groups according to the underlying disease and technical aspects.

Laparoscopic procedures were performed as reduced-port surgery using a multi-port system (OCTOtmPORT) at the umbilical incision site and one additional trocar (12mm diameter) in the right lower abdomen. An electric endoscopic stapling device (SIGNIA Stapling Device) was used for dividing/cutting the colon or rectum in the laparoscopic group. In open procedures a mechanical stapling device (Contour Curved Cutter Stapler) was used to cut/divide the colon or rectum. In both groups an electrothermal bipolar sealing device (LIGASUREtm) was used to dissect and seal tissue or vessels and to reduce blood loss. (37)

Patients were not eligible for the study in the case of a) ongoing antifungal therapy for treatment of active fungal infection or antifungal therapy within 4 weeks prior to inclusion, b) ongoing antibiotic therapy other than optional single shot surgical prophylaxis, c) ongoing *Enterococcus sp.* bacteremia or treatment of *Enterococcus sp.* bacteremia within 4 weeks prior to inclusion, d) clinical or radiological or laboratory evidence of a current infectious disease (i.e. temperature >38°C, elevated CRP >5mg/dl, leukocytosis >11400/µl, elevated neutrophils >78%) as assessed by the treating physician, e) administration of immunoglobulin, blood or blood products (i.e. thrombocytes, fresh frozen plasma) within 4

weeks prior to inclusion, f) abdominal surgery (laparoscopic or open) or other major surgeries (e.g. aortocoronary bypass) within 4 weeks prior to inclusion, g) subsequent invasive candidiasis (defined according to proposed *EORTC/MSG* definitions of fungal infections in ICU) (9) or h) other complicating infectious diseases after surgery within day 5 of the BDG observation time frame as described below. The complete observation period was 30 days after surgery for assessment of outcome and other parameters, e.g. intrahospital or extrahospital death, necessity of antibiotic or antimycotic therapy due to complicating infectious disease, occurrence of anastomotic leakage, and subsequent surgical procedures.

2.2. Hypothesis

The aim of this study was to identify if abdominal surgery is significantly elevating the BDG values due to cutting/dividing the small bowel and/or the colon and/or rectum or due to other parts/interventions of the surgical procedures (e.g. skin disruption or usage of gauze). Furthermore, the study is intended to examine a difference between the elevation levels of open and laparoscopic surgery.
A BDG level of ≥80 pg/ml was considered positive as recommended in previous literature and by the manufacturer.

The null hypothesis was, that there is no significant elevation of BDG values neither in patients with open nor with laparoscopic surgery.
The alternative hypothesis states that there is a significant elevation of BDG values due to the operation without the presence of a fungal infection.
The further study objective was the difference between the elevation level of open and laparoscopic surgery.

2.3. Study design

Blood samples used for determination of BDG values were obtained at 7 scheduled time points as outlined below through arterial and venous lines inserted just prior to sampling during routinely performed anesthesia: 1) one blood sample before intubation but prior to skin incision by the surgeon, 2) after skin incision but prior to dissection of intestinal mucosa (Both samples were used for determination of BDG values prior to dissection of intestinal mucosa. However, there were certain factors possibly influencing BDG values,

such as insertion of vascular lines, single shot antibiotic prophylaxis, damaged mucosa in cancer or surgical preparation of intestine/colon for resection of cancer.), 3) after completion of anastomosis, 4) after completion of skin sutures, 5) in the evening after surgery (approximately 5-8 hours after surgery), 6) in the morning of day 2 after surgery (approximately 18 hours after surgery), 7) in the morning of day 4-5 after surgery.
The day of surgery was designated as day 1. Thus, seven samples of five milliliters of whole blood were drawn in each patient. In case of signs and/or symptoms suggestive of candidemia, and/or bacteremia, and/or intraabdominal infection and/or other infectious complications, routine diagnostics were applied; including blood cultures and/or microbiological investigation of abdominal drainage fluids, and imaging studies as indicated by the treating physician.

Serum BDG test was performed according to recently described methods by Prüller et al. as already described in section *1.4.2.2. Diagnostic methods*. The standard protocol of the *Fungitell*© assay was adapted to be automated using a routine BCS XP coagulation analyser. The *Fungitell*© reagent was also reconstituted, aliquoted, and stored at −70°C, allowing single-sample testing if needed. (23)
According to the manufacturer, a BDG concentration of ≥80 pg/ml was considered to be positive, while a concentration of <60 pg/ml was considered to be negative. (6) The automated BD testing has been introduced in 2014 and subsequently used in clinical routine as well as in several studies.

2.4. Statistical analysis

Statistical analysis was performed using R 3.1.1 (www.r-project.org) and SPSS Statistics 23. To analyse changes in BDG levels between the different sample time-points the McNemar test and Wilcoxon signed rank test were used. Absolute differences in BDG values between sampling time points were calculated and compared between groups (laparoscopic versus open) with the Students T-test, respectively Mann-Whitney-U test. A p-value of <0.05 was considered to be statistically significant.

The study protocol was approved by the ethics committee of the Medical University of Graz (nr 30-043 ex 17/18) and the Hospital of St. John of God (Barmherzige Brüder), Graz, Austria. The study was registered at clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT03468803).

3. Results

3.1. Baseline Demographics and BDG levels

50 patients were included in the study, 24 had laparoscopic and 26 had open abdominal surgery. Demographic data, underlying diseases and peri-surgical interventions are shown below in *Table 5*.

Table 5: Demographic data, underlying diseases and peri-surgical interventions

	Laparoscopic surgery	**Open surgery**	**p-value**
n	24	26	
Sex (women)	7 (29.3 %)	13 (50 %)	0.133
Age, median (range)	68.32 (35.73-85.78)	75.8 (56.08-91.56)	0.008
BMI, median (range)	25.6 (19.26-32.39)	26.87 (19.93-37.28)	0.225
Underlying disease:			
Colon carcinoma	12 (50.0 %)	13 (52.0 %)	1.000
Rektum carcinoma	4 (16.7 %)	8 (30.8 %)	0.243
Diverticultis	5 (20.8 %)	3 (11.5 %)	0.370
Other	3 (12.5 %)	2 (7.7 %)	0.571
Endoscopic preoperative ink tattooing	13 (54.2 %)	7 (26.9 %)	0.490
Second intestinal surgery	1 (4.2 %)	3 (11.5 %)	0.337
Neoadjuvant chemotherapy	1 (4.2 %)	1 (3.8 %)	0.954
Leukocytes	7.110 (4.900 - 7.940, n = 11)	6.950 (5.215-9.975, n = 17)	0.404
CRP mg/dl	0.2 (0.1 - 0.3, n=10)	0.4 (0.13-2.47, n=16)	0.077
AB prophylaxis	24/24 (100%)	26/26 (100%)	1.000
AB treatment			
Day 1 (sample 6)	4/24 (16.7 %)	4/26 (15.4 %)	0.902
Day 4-5 (sample 7)	5/24 (20.8 %)	6/26 (23.1 %)	0.848

In total, 350 blood samples were collected. Four of 350 samples could not be assigned to scheduled sampling dates and were therefore not further analysed. Thus, BDG tests were performed with 346 samples from 50 patients. Each 2 samples from time point 5 and 2 samples from time point 7 were missing in 2 patients.

There was no statistical difference between patients with elevated and non-elevated BDG with regard to observed demographic or clinical parameters (e.g. sex, age, underlying diseases, type of antibiotic prophylaxis). None of the patients had renal replacement prior

or after surgery, received immunoglobulins, red blood cells or other blood products, developed invasive candidiasis or received systemic antimycotic treatment. All patients survived the observation period of 30 days.

In the initial serum sample prior to skin incision, 6 of 50 patients (12%) had elevated BDG levels (≥80pg/ml, indicating positive BDG values according to the manufacturer; each 3 patients undergoing laparoscopic and open surgery). Five of these 6 patients had carcinoma of the colon or rectum and one had a non-malignant tubulovilleous adenoma of the rectum.

Median peri-surgical BDG values in patients undergoing laparoscopic and open intestinal surgery are shown in *Table 5*. All 6 patients with initial BDG values ≥80pg/ml had single shot antibiotic prophylaxis (5 received cefuroxime plus metronidazole, 1 received ciprofloxacin plus metronidazole) prior to sampling for BDG determination, but none received immunoglobulins or blood products. Eleven of 50 (22%) patients had postoperative antibiotic therapy as indicated by the treating physician. Two of 7 patients with BDG values ≥80pg/ml at sampling time point 7 (4-5 days after surgery) had antibiotic therapy, but 9 patients with AB therapy on time point 7 had negative BDGs. All patients were afebrile after surgery during the sampling time frame (up to day 5).

Three patients underwent a second surgery due to clinically suspected anastomotic leakage, peritonitis, and post-surgical intestinal bleeding. After the second surgery BDG raised to values ≥80pg/ml in 2 of 3 patients.

3.2. Dynamics of BDG levels during and after surgery

Boxplots of BDG levels at all 7 time-points are displayed in *figures 9 to 11* (9: complete cohort; 10: open surgery; 11: laparoscopic surgery).

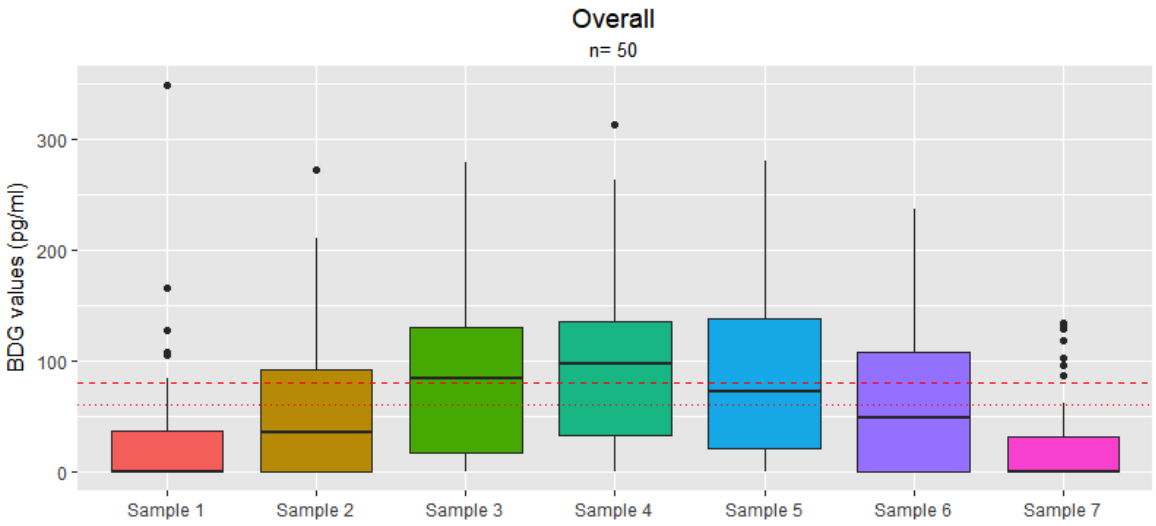

Figure 9: Complete cohort

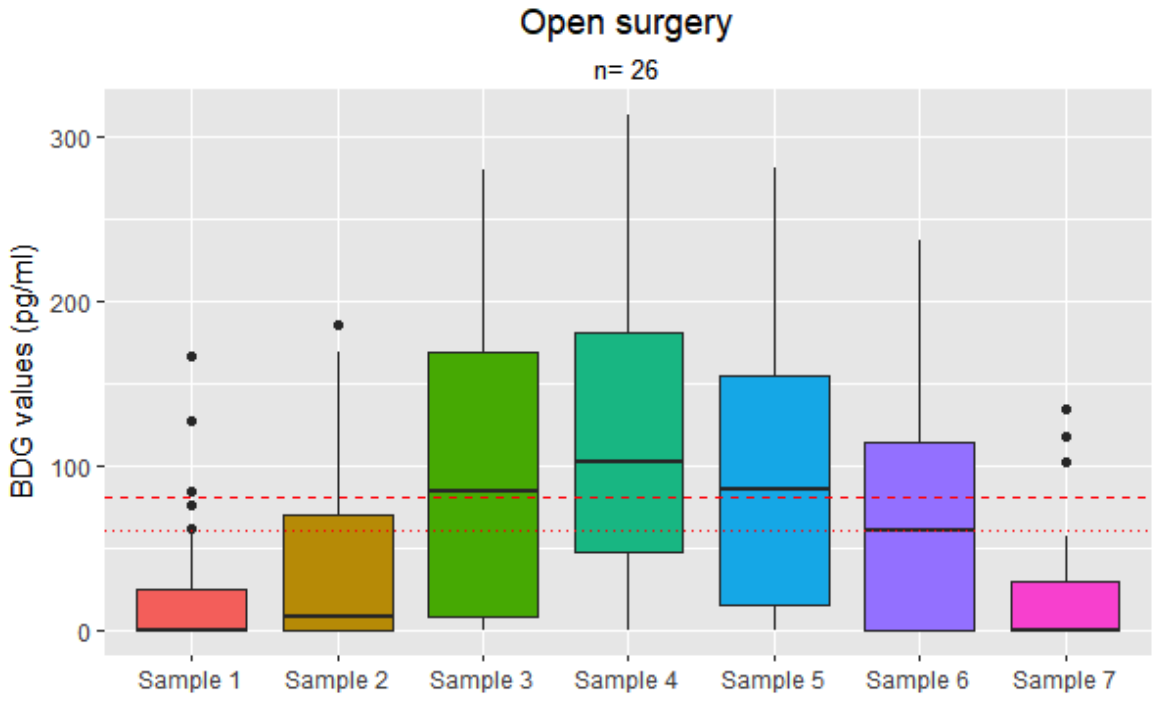

Figure 10: Open surgery

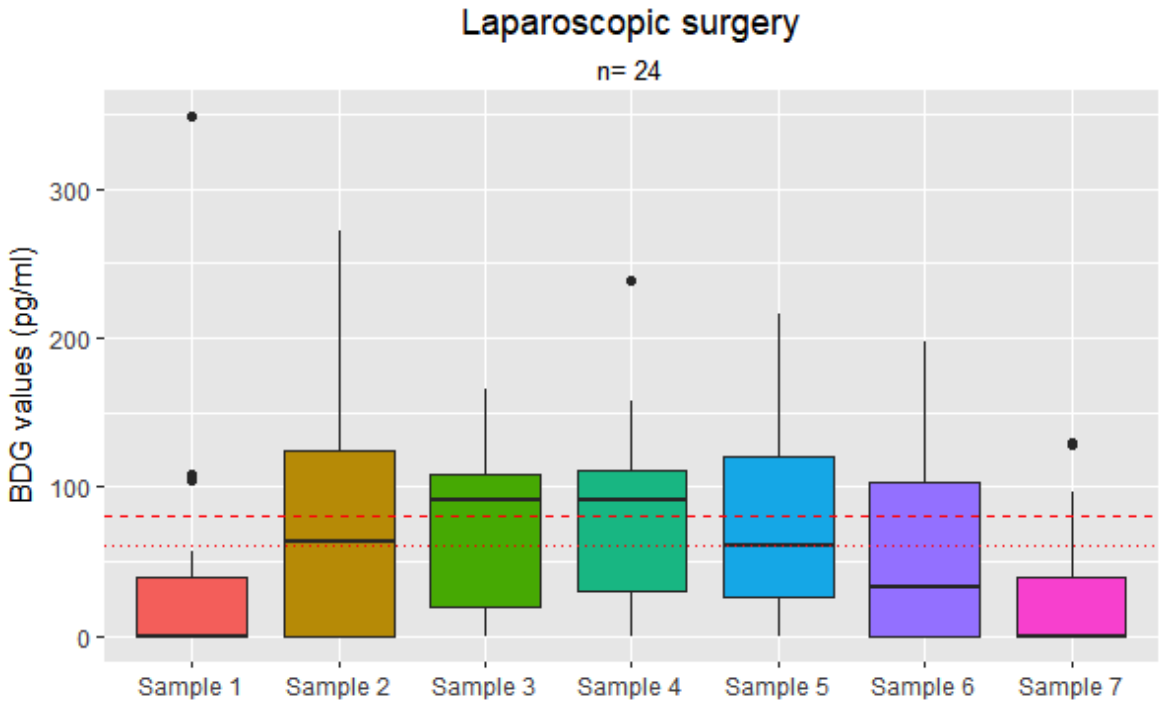

Figure 11: Laparoscopic surgery

Median levels of BDG at all seven sampling time points are shown in *figure 12*. The sample numbers refer to the following sampling time points (the day of surgery was designated as day 1): **sample 1**: after insertion of arterial lines and/or optional central venous access but before intubation and prior to skin incision by the surgeon; **sample 2**: after skin incision but prior to dissection of intestinal mucosa; **sample 3**: after completion of anastomosis; **sample 4**: after completion of skin sutures; **sample 5**: in the evening after surgery; **sample 6**: in the morning of day 2 after surgery; **sample 7**: in the morning of day 4-5 after surgery. The red dashed line indicates the 80pg/ml cut off for positive BDG values according to the instructions of the manufacturer. The dotted line indicates 60pg/ml under which BDG values are negative.

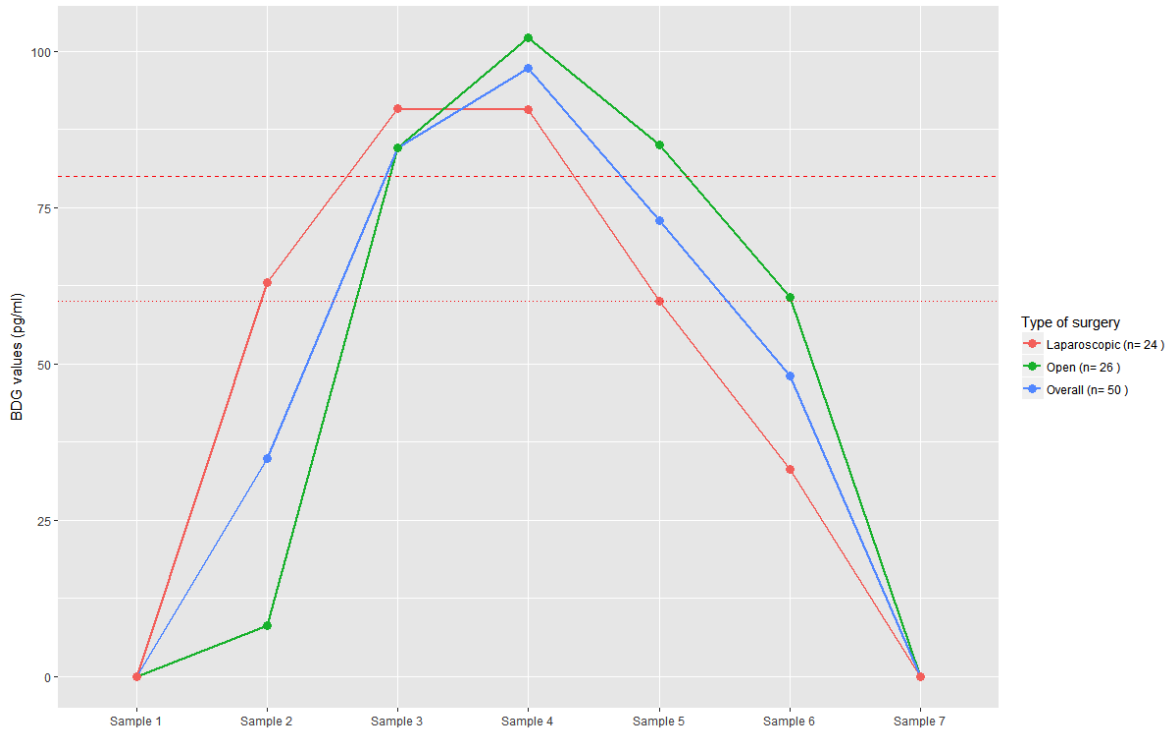

Figure 12: Median levels of BDG at all 7 time points

Figure 13 shows boxplots and medians of BDG levels at all seven sampling time points. The dark grey boxplots are representing the open surgery cases, the light grey boxplots the laparoscopic surgery cohort.

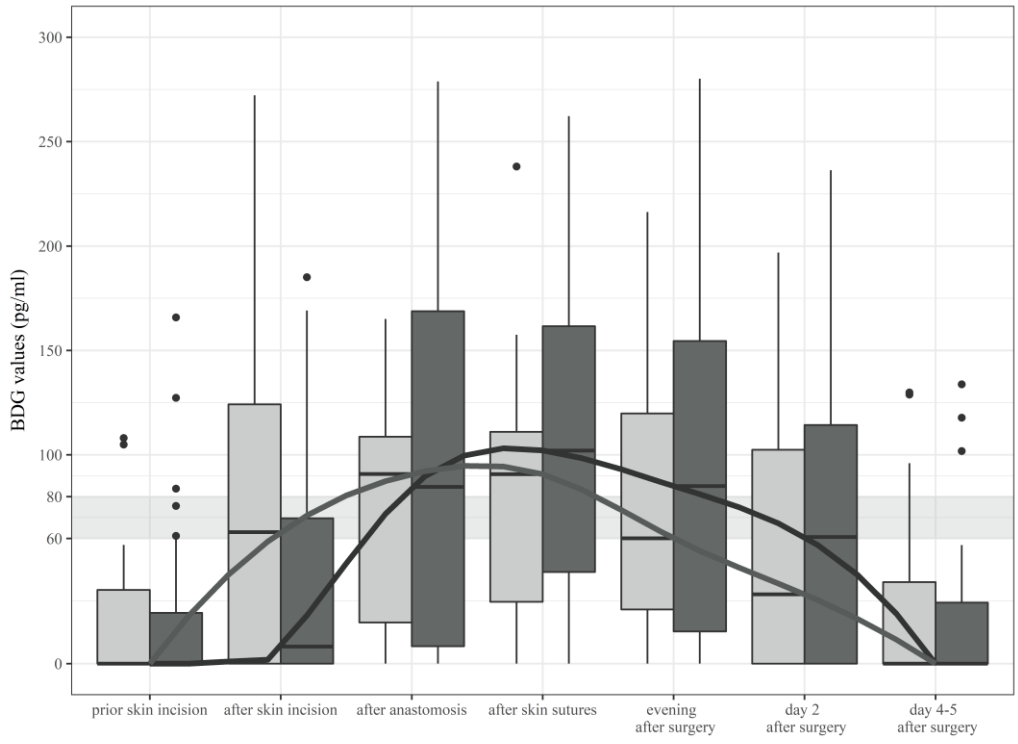

Figure 13: Boxplots and medians of BDG levels at all seven sampling time points. *Dark grey: open surgery; Light grey: laparoscopic surgery*

Figure 14 shows BDG levels of all patients (thin lines) and medians (thick lines) at all seven sampling time points. The dark grey lines are again representing the open surgery cases and the light grey ones the laparoscopic surgery cohort.

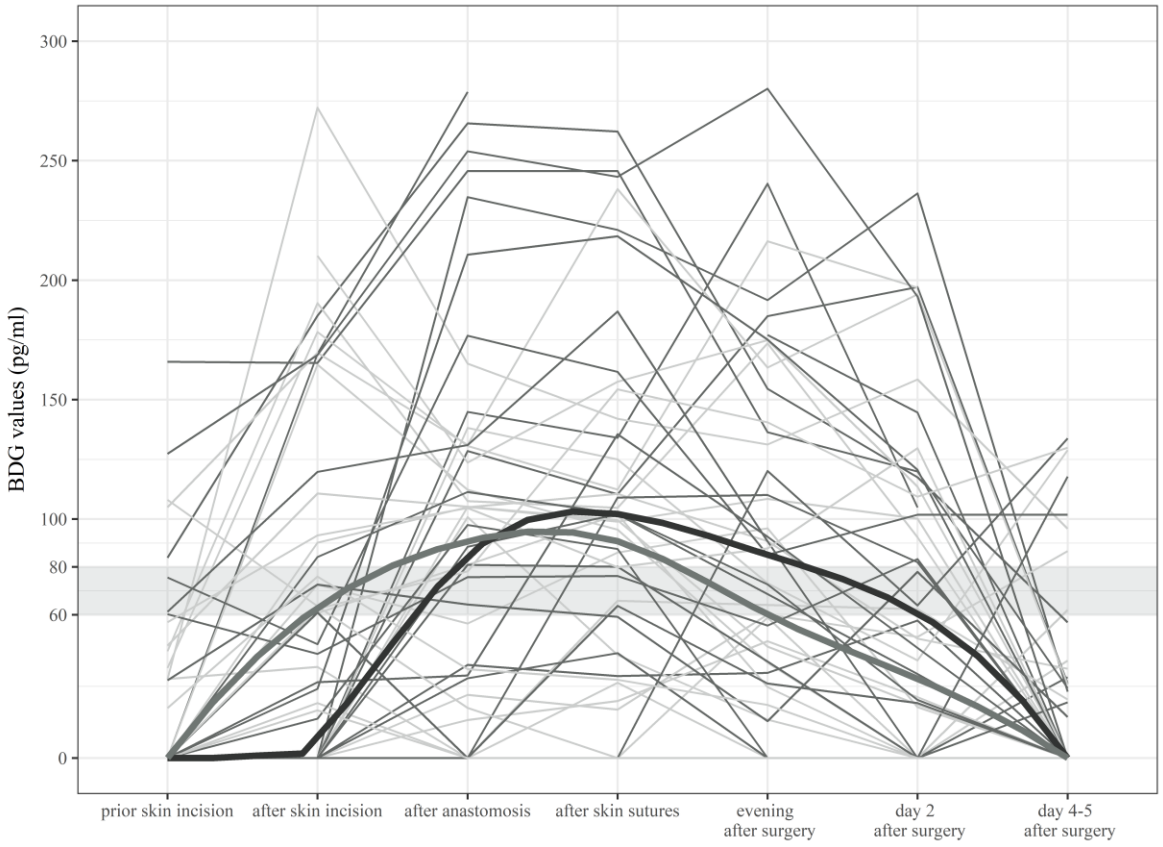

Figure 14: BDG levels of all patients (thin lines) and medians (thick lines) at all seven sampling time points. *Dark grey: open surgery; Light grey: laparoscopic surgery*

Figure 15 and 16 show boxplots of the differences of BDG values within the patients. "Diff Timept 2-1" means the difference of BDG values at sampling time point 2 compared to 1 for each individual patient. "Diff Timept 3-1" means the difference of BDG values at time point 3 compared to 1 and so on (*Figure 15*). "Diff Timept 3-2" means the difference of BDG values at time point 3 compared to 2 and so on (*Figure 16*).

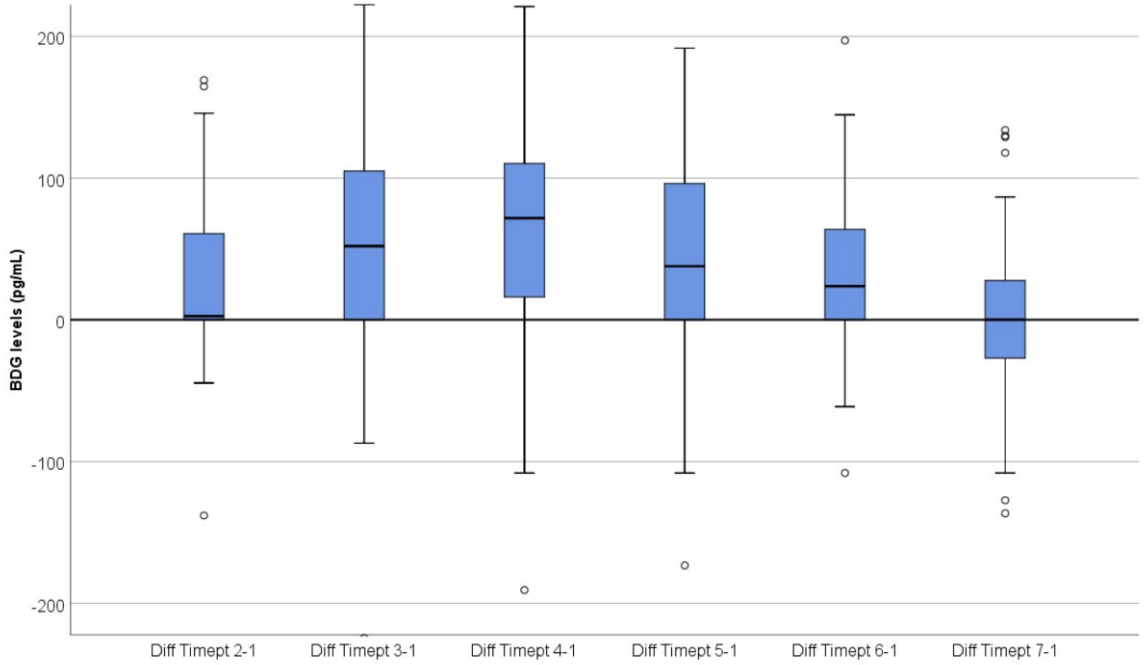

Figure 15: Differences of BDG values

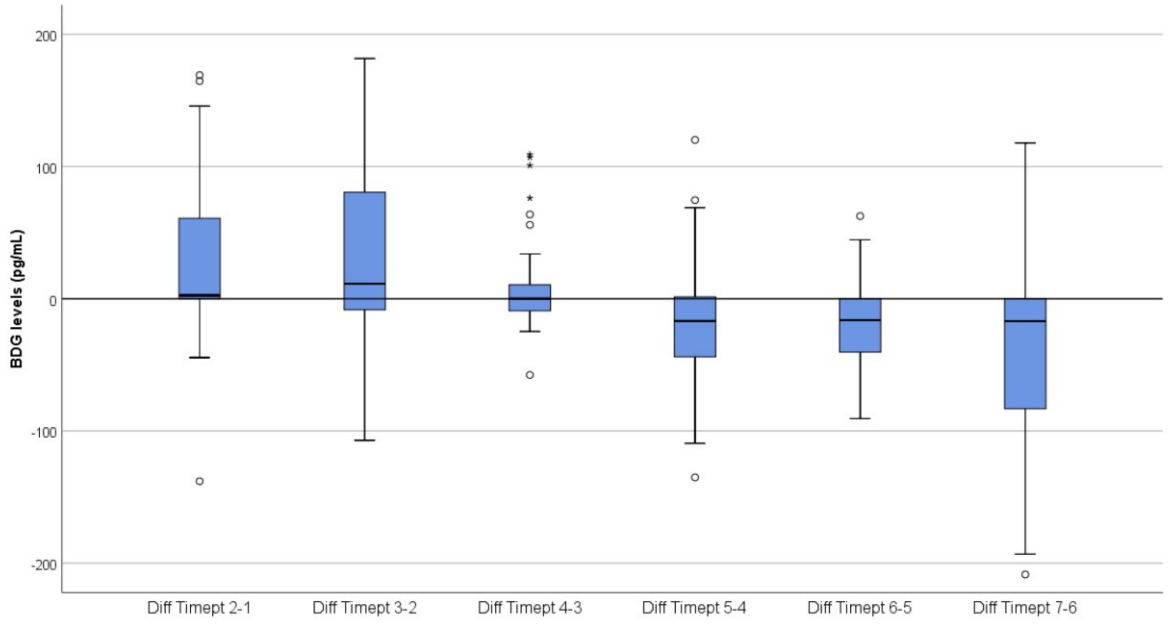

Figure 16: Differences of BDG values

Table 6 shows p-values calculated by the McNemar test for differences in proportions of patients with elevated serum BDG values ≥80pg/ml at given sampling time points respective to abdominal surgery time points. Overall proportion of patients with BDG

values ≥80pg/ml increased significantly after skin incision, reaching its peak after completion of skin sutures (time point 4). Thereafter proportions of patients with BDG values ≥80 pg/ml decreased, but – when compared to the baseline - remained elevated until (and including) the morning of day 2 after surgery. Significant p-values are bold.

Table 6: Proportions of patients with BDG values BDG ≥80 pg/ml at given sampling time points and absolute serum BDG levels in both patient groups

	Proportions of patients with BDG values ≥80 pg/ml*	*1, before intubation, prior to skin incision*	*2, just prior to dissection of intestinal mucosa*	*3, after completion of anastomosis*	*4, after completion of skin sutures*	*5, evening after surgery***	*6, morning of day 2 after surgery*	*7, morning of day 4-5 after surgery***
Proportions of patients with BDG values ≥80 pg/ml*	-	6/50 (12%)	15/50 (30%)	27/50 (54%)	29/50 (58%)	23/48 (48%)	19/50 (38%)	7/48 (15%)
1, before intubation, prior to skin incision	6/50 (12%)	-	**0.012**	**<0.001**	**<0.001**	**<0.001**	**0.001**	1.000
2, just prior to dissection of intestinal mucosa	15/50 (30%)	-	-	**<0.001**	**0.001**	0.057	0.424	0.167
3, after completion of anastomosis	27/50 (54%)	-	-	-	0.687	0.581	0.057	**0.001**
4, after completion of skin sutures	29/50 (58%)	-	-	-	-	0.180	**0.013**	**<0.001**
*5, evening after surgery***	23/48 (48%)	-	-	-	-	-	0.219	**0.003**
6, morning of day 2 after surgery	19/50 (38%)	-	-	-	-	-	-	**0.031**

** ≥80 pg/ml, i.e. positive BDG values according to the manufacturer.*
*** each 2 samples are missing at sample time point 5 time point 7*

Table 7 shows p-values calculated by the Wilcoxon signed-rank test for differences in serum BDG values between given sampling time points respective to intestinal surgery time points. Significant p-values are bold.

Table 7: Serum BDG levels at given sampling time points in patients undergoing laparoscopic or open abdominal surgery

	BDG levels (pg/mL); median (IQR)	*1, before intubation, prior to skin incision*	*2, just prior to dissection of intestinal mucosa*	*3, after completion of anastomosis*	*4, after completion of skin sutures*	*5, evening after surgery**	*6, morning of day 2 after surgery*	*7, morning of day 4-5 after surgery**
BDG levels (pg/mL); median (IQR)	-	0 (0-40)	35 (0-98)	85 (12-131)	97 (33-136)	73 (18-139)	48 (0-110)	0 (0-33)
1, before intubation, prior to skin incision	0 (0-40)	-	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1.000

2, just prior to dissection of intestinal mucosa	35 (0-98)	-	-	**0.027**	**0.001**	0.090	0.933	**0.006**
3, after completion of anastomosis	85 (12-131)	-	-	-	0.456	0.472	**0.010**	**<0.001**
4, after completion of skin sutures	97 (33-136)	-	-	-	-	**0.044**	**<0.001**	**<0.001**
*5, evening after surgery**	73 (18-139)	-	-	-	-	-	**0.001**	**<0.001**
6, morning of day 2 after surgery	48 (0-110)	-	-	-	-	-	-	**0.002**

**each 2 samples are missing at sample time point 5 time point 7*

3.3. Differences in Dynamics of BDG levels between Laparoscopic and Open surgery

The cross-sectional comparison of proportions of patients with BDG values ≥80pg/ml (Fishers exact Test) as well as BDG values as continuous variable (Mann-Whitney U Test) between patients with laparoscopic and open intestinal surgery is displayed in *Table 8*.

Table 8: Proportions of patients with BDG values BDG ≥80pg/ml at given sampling time points and absolute serum BDG levels in both patient groups

Proportions of patients with BDG values ≥80pg/ml at given sampling time points	Laparoscopic surgery	Open surgery	p-value
1, prior to intubation, prior to skin incision	3/24 (12.5%)	3/26 (11.5%)	1.000
2, just prior to dissection of intestinal mucosa	9/24 (37.5%)	6/26 (23.1%)	0.358
3, after completion of anastomosis	13/24 (54.2%)	14/26 (53.8%)	1.000
4, after completion of skin sutures	13/24 (54.2%)	16/26 (61.5%)	0.775
*5, evening after surgery**	10/23 (43.5%)	13/25 (52%)	0.578
6, morning of day 2 after surgery	8/24 (33.3%)	11/26 (42.3%)	0.570
*7, morning of day 4-5 after surgery**	4/23 (17.4%)	3/25 (12%)	0.696
Absolute serum BDG levels (pg/mL) Median, IQR	Laparoscopic surgery	Open surgery	p-value
1, prior to intubation,	0 pg/ml (0-43)	0 pg/mL (0-39)	0.434

prior to skin incision			
2, just prior to dissection of intestinal mucosa	63 pg/mL (0-151)	8 pg/mL (0-75)	0.083
3, after completion of anastomosis	91 pg/mL (17-111)	84 pg/mL (0-185)	0.475
4, after completion of skin sutures	91 pg/mL (26-112)	102 pg/mL (42-195)	0.243
*5, evening after surgery**	60 pg/mL (22-131)	85 pg/mL (7-165)	0.686
6, morning of day 2 after surgery	33 pg/mL (0-107)	61 pg/mL (0-118)	0.464
*7, morning of day 4-5 after surgery**	0 pg/mL (0-41)	0 pg/mL (0-30)	0.824

**each 2 samples are missing at sample time point 5 time point 7*

Boxplots of BDG levels at all 7 time-points for patients with laparoscopic versus open surgery are displayed in *Figure 9 to 11* and median levels of BDG values for both groups in *Figure 12*.

Overall the dynamics were mostly identical in both groups. Significant differences for changes of BDG values were found for comparing time point 3 (after preparation of the intestine and just prior to dissection of the intestinal mucosa), with significantly higher increases in the open surgery group (p=0.001), as well as time point 4 (after completion of skin sutures / at the end of surgery) vs. time point 1 (baseline), with significant higher increases in patients with open surgery vs. laparoscopic surgery (p=0.042). No differences between those groups were found when comparing other time points.

4. Discussion

The conducted study compared BDG values during and after open and laparoscopic abdominal surgery. In our study we were able to show that open and laparoscopic surgery led to elevated BDG values in a peri-surgical time frame. BDG reached concentrations ≥80pg/ml in 54% to 61% of patients during laparoscopic and open surgery. BDG was still positive 4-5 days after surgery in 12% (open) to 17% (laparoscopic) of patients, without any suspected or proven fungal infection or anastomotic leakage.

Previously, BDG values have been collected amongst patients after 48 hours of ICU treatment with an expected additional stay of at least 3 days and a medium stay of 16 days. As the samples where only taken after 48 hours of ICU treatment, no information about the time frame during or immediately after the surgery could be retrieved. However, the authors still found amongst nine out of 35 patients (26%) without any evidence of invasive candidiasis (proven, probable, possible) elevated BDG values early in the ICU admission and a subsequent BDG decrease. In eight of nine patients, BDG dropped without admission of antifungals. (34)

Within the surgical setting we found various circumstances applied during routine abdominal surgery that can cause falsely elevated BDG values. However, although we focused on the time frame of surgery starting with sampling after insertion of arterial lines for routine anesthesia until day 5 after surgery, we were not able to establish a direct causal relationship of certain underlying diseases, peri-surgical interventions/procedures or medications with the elevated BDG values.

As BDG values were significantly higher in patients undergoing open abdominal surgery compared to laparoscopic surgery after completion of intestinal anastomosis and after completion of skin sutures, we assume that specific interventions in open surgery, such as a greater use of cellulose based gauze and greater skin incision compared to laparoscopic surgery, might be partially responsible for the elevation of BDG. However, BDG increased very early after preparation of the intestine and just prior to the dissection of intestinal mucosa both in laparoscopic and open surgery indicating that cellulose based gauze and skin trauma could not solely be responsible for the elevation of BDG.

Also administration of antibiotic prophylaxis with colistin, ertapenem, cefazolin (in vials), trimethoprim-sulfamethoxazole, cefotaxime, cefepime, and ampicillin-sulbactam might cause false elevation of BDG values. (31) The antibiotics administered between sampling time point 1 and 2 (i.e. after skin incision but prior to dissection of intestinal mucosa), might therefore be responsible for the BDG increase detected at time point 2 together with skin incision and the usage of gauze in this procedure. However, although antibiotic prophylaxis was applied to all patients, BDG did not raise in all subjects. Additionally, 9 of 11 patients with antibiotic therapy in time point 7 (i.e. 4-5 days after surgery) had negative BDGs, indicating that administration of antibiotics is not associated with elevated BDG values in all patients.

As 12% of the patients had BDG values ≥80pg/ml prior to administration of perioperative antibiotic prophylaxis, intubation, abdominal skin disinfection, dressing and skin incision, the underlying disease might have contributed to elevated BDG. However, there was no causal relationship between the underlying disease, stage of underlying disease, or demographic factors and elevated BDG.
Whether placement of arterial lines (which was done prior to the first blood draw used for BDG testing) and the use of isopropanol or octenidin soaked gauze for skin disinfection might have contributed to the elevated BDG values remains unclear. However every patient received arterial lines, but not every patient showed elevated BDG. Therefore it is unlikely that the insertion of those lines led to positive BDG.

It was also shown that chronic inflammation of the gut such as colitis, i.e. Crohn disease, and intestinal mucositis, caused by i.e. induction of chemotherapy, leads to a loss of integrity of the intestinal mucosal barrier and to elevated BDG serum values. (4, 5) As 20,8% of laparoscopic patients and 11,5% of open patients had diverticulitis, an inflammation of abnormal pouches of the intestines, this circumstance might have contributed to the elevated BDG levels. To find a significant connection between those circumstances, further research has to be done.

Other reasons mentioned to cause elevated BDG values, such as administration of blood products or immunoglobulins, bacterial infections (e.g. *enterococcal* infections) prior to

surgery, invasive fungal infection or renal replacement therapy (RRT), where not found within the cohort of patients in our study.
Although intestinal surgery led to positive BDGs in 54% (laparoscopic) to 62% (open) of patients undergoing this procedure, no specific intervention could be blamed for elevated BDGs in the peri-surgical time frame.

4.1. Conclusion and Perspectives

In summary, among patients undergoing intestinal surgery, positive BDG values have to be interpreted with great caution as surgical procedures increase BDG values. BDG reached concentrations of ≥80pg/ml in 54% to 61% of patients during laparoscopic and open surgery. BDG was still positive 4-5 days after surgery in 12% (open) to 17% (laparoscopic) of patients without any suspected or proven fungal infection or anastomotic leakage. Previously, BDG testing showed a high negative predictive value (99%) but weak positive predictive value (72%) for detection of candidemia in the ICU setting including patients undergoing surgery. (22)

Our study adds data to the fact that BDG testing might be false positive due to a variety of underlying diseases and surgical interventions, especially during and in the first days after intestinal surgery. We suggest to primarily rely on negative BDG tests in clinical workup of patients with short history up to 5 days of intestinal surgery and suspicion of fungal infection. The high negative predictive value of BDG testing will support subsequent therapeutic decisions. The value of positive BDG tests in the perioperative setting up to 5 days after the surgery seems to be limited due to BDG elevations by surgical procedures and has therefore to be taken with precaution.
Further research is needed to identify exact reasons for elevated BDG serum values within a surgical setting.

5. References

(1) Eggimann P, Garbino J, Pittet D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. Lancet Infect Dis. 2003; 3(11): 685–702. PubMed PMID: 14592598.

(2) Schelenz S, Barnes R, Barton R. British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases. Lancet Infect Dis. 2015; 15(4): 461-474. PubMed PMID: 25771341.

(3) Schupp J, Petraitiene R, Jaskille A. Reactivity of (1-3)-BDG levels in patients with burn injury. Mycoses 2011; 55(3): 224-227. doi: 10.1111/j.1439-0507.2011.02068.x.

(4) Leelahavanichkul A, Worasilchai N, Wannalerdsakun S. Gastrointestinal leakage detected by serum (1-3)-b-D-Glucan in mouse models and a pilot study in patients with sepsis. Shock 2016; 46(5): 506–518. doi: 10.1097/SHK.0000000000000645.

(5) Prattes J, Raggam R, Vanstraelen K. Chemotherapy-Induced Intestinal Mucosal Barrier Damage: A Cause of Falsely Elevated Serum 1,3-B-D-Glucan Levels? J Clin Microbiol 2016; 54(3): 798-801. PubMed PMID: 26719433.

(6) Prattes J, Schneditz D, Prüller F. 1,3-ß-D-Glucan testing is highly specific in patients undergoing dialysis treatment. J Infect 2017; 74(1): 72-80. PubMed PMID: 27717781.

(7) Tissot F, Lamoth F, Hauser P. ß-Glucan Antigenemia Anticipates Diagnosis of Blood Culture–Negative Intraabdominal Candidiasis. Am J Respir Crit Care Med 2013; 188(9): 1100–1109. PubMed PMID: 23782027.

(8) Hof, H et al. Medizinische Mikrobiologie, 6. unveränderte Auflage. Thieme: Stuttgart 2017; p. 463-470

(9) De Pauw B, Walsh J, Donnelly P. Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008; 46(12): 1813-1821. PubMed PMID: 18462102.

(10) McManus B, Coleman C. Molecular epidemiology, phylogeny and evolution of *Candida albicans*. Infection, Genetics and Evolution 2014; 21: 166-178. [Retrieved from Internet, 2018 June, 17], Available from: https://doi.org/10.1016/j.meegid.2013.11.008.

(11) Larone D. Medically Important Fungi – A Guide to Identification, 3rd edition. ASM Press: Washington, D.C. 1995 [Retrieved from Internet, 2018 September, 11], Available from: http://www.mycosesstudygroup.org/thefungi/Candida_spp.htm

(12) Suerbaum S, Helmut H, Burchard G. Medizinische Mikrobiologie und Infektiologie, 7. überarbeitete Auflage. Springer: Berlin 2012; p. 604-606

(13) Schumpelieck V, Bleese N, Mommsen U. Kurzlehrbuch Chirurgie, 8. Auflage. Thieme: Stuttgart 2010. [Retrieved from Internet, 2018 June, 18] Available from: https://eref.thieme.de/ebooks/728659#/ebook_728659_SL22669077

(14) Clancy C, Nguyen M. Diagnosing invasive candidiasis. J Clin Microbiol 2018; 56(5): e01909-17. PubMed PMID: 29444828.

(15) Held J, Kohlberger I. Comparison of (1,3)-D-Glucan, Mannan/Anti-Mannan Antibodies, and Cand-Tec *Candida* Antigen as Serum Biomarkers for Candidemia. J Clin Microbiol 2013; 51(4): 1158-64. PubMed PMID: 23363830.

(16) Anderson T, Bryant H, Deirdre C. Evaluation of a *Candida* antigen detection method (Cand-Tec): Experience from a university teaching hospital. Can J Infect Dis. 1992; 3(4): 167–172. PubMed PMID: 22514365.

(17) Poissy J, Sendid B, Damiens S. Presence of *Candida* cell wall derived polysaccharides in the sera of intensive care unit patients: relation with candidaemia and *Candida* colonisation. Crit Care 2014; 18(3): R135. PubMed PMID: 24975380.

(18) Pickering J, Sant H, Bowles C. Evaluation of a (1-3)-β-D-glucan assay for diagnosis of invasive fungal infections. J Clin Microbiol 2005; 43(12): 5957-5962. PubMed PMID: 16333082.

(19) Zeković D, Kwiatkowski S, Vrvic M. Natural and Modified (1→3)-β-D-Glucans in Health Promotion and Disease Alleviation. Crit Rev Biotechnol 2005; 25(4): 205-230. PubMed PMID: 16419618.

(20) Sikora P, Tosh S, Brummer Y. Identification of high b-glucan oat lines and localization and chemical characterization of their seed kernel b-glucans. Food Chem 2013; 137(1–4): 83–91. PubMed PMID: 23199994.

(21) Vader1941. C albicans visualised using scanning electron microscopy. [Retrieved from Internet, 2018 September, 22] Available from: https://en.wikipedia.org/wiki/File:SEM_of_C_albicans.tif / https://creativecommons.org/licenses/by-sa/4.0/legalcode

(22) Posteraro B, De Pascale G, Tumbarello M. Early diagnosis of candidemia in intensive care unit patients with sepsis: a prospective comparison of (1-3)-β-D-glucan assay, *Candida* score, and colonization index. Crit Care 2011; 15(5): R249. PubMed PMID: 22018278.

(23) Prüller F, Wagner J, Raggam R. Automation of serum (1,3)-β-D-glucan testing allows reliable and rapid discrimination of patients with and without candidemia. Med Mycol 2014; 52(5): 455–461. PubMed PMID: 24906361.

(24) Ellis M, Al-Ramadi B, Finkelman M. Assessment of the clinical utility of serial β-D-glucan concentrations in patients with persistent neutropenic fever. J Med Microbiol 2008; 57(Pt 3): 287–295. PubMed PMID: 18287290.

(25) Harvesting horseshoe crabs. [Retrieved from Internet, 2018 October, 8] Available from: https://www.technobyte.org/interesting-facts/horseshoe-crabs-blood-blue-expensive/

(26) Leon C, Ruiz-Santana S, Saavedra P. Usefulness of the "*Candida* score" for discriminating between *Candida* colonization and invasive candidiasis in non-neutropenic critically ill patients: A prospective multicenter study. Crit Care Med. 2009; 37(5): 1624-1633. PubMed PMID: 19325481.

(27) Leroy G, Lambiotte F, Thévenin D. Evaluation of "*Candida* Score" in critically ill patients: a prospective, multicenter, observational, cohort study. Ann intensive care 2011; 1(1): 50. PubMed PMID: 22128895.

(28) Lüllmann H, Mohr K, Helm L. Pharmakologie und Toxikologie, 17. vollständig überarbeitete Auflage. Thieme: 2010, Stuttgart, p. 520-527

(29) Nagasawa K, Tano T, Kitbayahi G. Experimental proof of contamination of blood components by (1→3)-β-D-glucan caused by filtration with cellulose filters in the manufacturing process. J Artif Organs 2003; 6(1): 49-54. PubMed PMID: 14598125.

(30) Egger M, Prüller F, Raggam R. False positive serum levels of (1–3)-ß-D-Glucan after infusion of intravenous immunoglobulins and time to normalization. J Infect 2018; 76(2): 206-210. PubMed PMID: 29174967.

(31) Marty F, Lowry C, Lempitski S. Reactivity of (1-3)-β-d-glucan assay with commonly used intravenous antimicrobials. Antimicrob Agents Chemother 2006; 50(10): 3450–3453. PubMed PMID: 17005829.

(32) Albert O, Toubas D, Strady C. Reactivity of (1 → 3)-β-*d*-glucan assay in bacterial bloodstream infections. Eur J Clin Microbiol Infect Dis. 2011; 30(11): 1453–1460. PubMed PMID: 21479838.

(33) Leelahavanichkul A, Panpetch W, Worasilchai N. Evaluation of gastrointestinal leakage using serum (1→3)-β-D-glucan in a Clostridium difficile murine model. FEMS Microbiol Lett 2016; 363(18). PubMed PMID: 27573235.

(34) Mohr J, Sims C, Paetznick V. Prospective Survey of (1,3)- β-D-Glucan and its Relationship to Invasive Candidiasis in the Surgical Intensive Care Unit Setting. J Clin Microbiol 2011; 49(1): 58-61. PubMed PMID: 21048005.

(35) Kang J, Lee S, Kim C. Comparison of the short-term outcomes of reduced-port laparoscopic surgery and conventional multiport surgery in colon cancer: a propensity score matching analysis. Ann Surg Treat Res 2018; 94(3): 147–153. PubMed PMID: 29520349.

(36) Nakanishi M, Kuriu Y, Murayama Y. Usefulness of Reduced Port Surgery for Left Colon Cancer. Anticancer Res. 2016; 36(9): 4749–4752. PubMed PMID: 27630322.

(37) Gezen C, Kement M, Altuntas Y. Safety and effectiveness of 5-mm and 10-mm electrothermal bipolar vessel sealers (LigaSure) in laparoscopic resections for sigmoid colon and rectal cancers. J Laparoendosc Adv Surg Tech A 2012; 22(6): 572–577. PubMed PMID: 22458835.

(38) Pfaller M, Diekema D. Epidemiology of invasive candidiasis: a persistent public health problem. Clinical Microbiology Reviews 2007; 20(1): 133–163. PubMed PMID: 17223626.

(39) Kanamori H, Kanemitsu K, Miyasaka T. Measurement of (1-3)-beta-D-glucan derived from different gauze types. Tohoku J. Exp. Med. 2009; 217(2): 117–121. PubMed PMID: 19212104.

(40) Karageorgopoulos D, Vouloumanou E, Ntziora F. β-D-Glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis. Clin Infect Dis. 2011; 52(6): 750-770. PubMed PMID: 21367728.

(41) Akah P. Immunomodulatory and immunorestorative activities of β-D-glucan-rich extract and polysaccharide fraction of mushroom, Pleurutus tuberregium - Scientific Figure on ResearchGate. [Retrieved from Internet, 2018 October, 3] Available from: https://www.researchgate.net/Chemical-structure-of-b-1-3-1-6-D-glucan-Glucans-are-long_fig1_274724528

(42) Tambe Y. *Candida albicans*, Pseudohyphen und hefeartige Zellen. [Retrieved from Internet, 2018 October, 3] Available from: https://commons.wikimedia.org/wiki/File:C_albicans_en.jpg and https://creativecommons.org/licenses/by-sa/3.0/legalcode

(43) Garey K, Rege M, Pai M. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis. 2006; 43(1): 25-31. PubMed PMID: 16758414.
(44) Kandidose der Mundhöhle. [Retrieved from Internet, 2018 October 7] Available from: https://de.wikipedia.org/wiki/Kandidose#/media/File:Oral_thrush_Aphthae_Candida_albicans._PHIL_1217_lores.jpg
(45) Dupont H, Mahjoub Y, Chouaki T. Antifungal Prevention of Systemic Candidiasis in Immunocompetent ICU Adults: Systematic Review and Meta-Analysis of Clinical Trials. Crit Care Med 2017; 45(11): 1937-1945. PubMed PMID: 28857851.
(46) KGH. Micrograph of esophageal candidiasis showing hyphae, biopsy specimen, PAS. [Retrieved from Internet, 2018 October, 8] Available from: https://en.wikipedia.org/wiki/Candidiasis#/media/File:Esophageal_candidiasis_(2)_PAS_stain.jpg
(47) Magill S, Edwards J, Bamberg W. Multistate Point-Prevalence Survey of Health Care–Associated Infections. N Engl J Med. 2014; 370(13): 1198-1208. PubMed PMID: 24670166.