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Abkürzungsverzeichnis

AmpB	liposomales Amphotericin B
APACHE II	Acute Physiology and Chronic Health Evaluation II
ATP	Adenosintriphosphat
BDG	1,3-β-$_D$-Glucan
$[Ca^{2+}]_i$	intrazelluläre Kalziumkonzentration
CAF	Koffein
CFU	koloniebildende Einheiten (colony-forming units)
CI	Herzindex (cardiac index)
CO	Herzzeitvolumen (Cardiac output)
CYP	Cytochrome P
dHPLC	Denaturating High-Performance Liquid Chromatography
dL/L	Kontraktilität, als Verkürzung der Zellamplitude
EKG	Elektrokardiogramm
ER	Endoplasmatisches Retikulum
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ESICM	European Society of Intensive Care Medicine
FAERS	US Food and Drug Administration Adverse Events Reporting Systems
FDA	Food and Drug Agency
FC	Fold change
h	Stunden
HR	Hazard-Ratio
HF	Herzfrequenz
IC	invasive Candidiasis
IDSA	Infectious Disease Society of America
KG	Körpergewicht

LDH	Laktatdehydrogenase
LVEF	linksventrikuläre Ejektionsfraktion
LVEDV	linksventrikuläres enddiastolisches Volumen
MAP	arterieller Mitteldruck (mean arterial pressure)
min	Minuten
PCR	Polymerase Kettenreaktion (polymerase-chain-reaction)
RYN	Ryanodin
RyR	Ryanodin-Rezeptor
sp.	Spezies (Singular)
spp.	Spezies (Plural)
SD	Standardabweichung (standard deviation)
SR	Sarkoplasmatisches Retikulum
sABP	systolic Arterial blood pressure, systolischer arterieller Blutdruck
THF	Zeit bis zum hämodynamischen Versagen (time to hemodynamic failure)
TLR4	Toll-like-Rezeptor 4
ZVD	zentraler Venendruck
ZVK	zentralvenöser Katheter

1 Einleitung

1.1 Invasive Pilzinfektionen in der operativen Intensivmedizin

Lebensbedrohliche Pilzinfektionen bei intensivmedizinisch behandelten Patienten haben innerhalb der letzten Jahrzehnte dramatisch an Bedeutung gewonnen. Bedingt durch enorme Fortschritte der modernen Medizin werden perioperativ zunehmend stark immungeschwächte- oder schwerstkranke Patienten behandelt, welche in hohem Maße durch invasive Pilzinfektion gefährdet sind[1–3]. Aktuelle Studien belegen, dass in Europa Pilzspezies in bis zu 20% der Fälle an der Entwicklung einer Sepsis auf einer Intensivstation beteiligt sind[4,5]. Dabei zeigten sich *Candida spp.* mit einer Letalität von ca. 25% in über 90% der Erkrankungen als ursächliche Erreger[4,6]. Insgesamt verursachten *Candida spp*. über die letzten Jahre in Deutschland etwa 6,5% aller nosokomialen Blutstrominfektionen[6]. Neben der invasiven Candidiasis (IC) verursachen Schimmelpilze wie Aspergillen und noch seltener Fadenpilze ebenfalls lebensbedrohliche Infektionen[7]. Hochrisikopatienten für invasive Pilzerkrankungen stellen dabei insbesondere Patienten mit prädisponierenden Grunderkrankungen (z.B. hämatologisch-/onkologische Patienten) und Patienten nach solider Organtransplantation unter Immunsuppression, sowie hochbetagte Patienten und Patienten nach medizinischen Maßnahmen (zentrale Venenkatheter, antibiotische Therapie, mechanische Beatmung, Hämodialyse, viszeralchirurgische Eingriffe, parenterale Ernährung) dar[8–11]. Auf Grund der beschriebenen Risikofaktoren sind daher operative Intensivpatienten in besonderem Maße von invasiven Pilzinfektionen bedroht.

1.2 Invasive Candidiasis

1.2.1 Epidemiologie

Die IC ist in den Industrieländern die häufigste bei hospitalisierten Patienten vorkommende Pilzerkrankung. Sie umfasst Infektionen durch *Candida Spezies (spp.)*, die sich durch Candidämie sowie fokale Infektionen multipler Lokalisationen manifestieren. Dabei liegt die jährliche Inzidenz der Erkrankung bei etwa 2-14 pro 100.000 Einwohnern[12,13] und die Mortalität der IC bei bis zu 40%[12,14]. IC sind häufig endogene Erkrankungen, ausgehend von der physiologischen Kolonisation des

Verdauungstraktes. Klinische Symptome einer Pilzinfektion sind meist unspezifisch und nicht von einer bakteriellen Infektion zu unterscheiden. Die Candidämie zeigt eine altersabhängige Inzidenz mit einem Maximum im höheren Lebensalter[1,2,13,15]. Tabelle 1 führt die häufigsten Risikofaktoren für die Entstehung einer IC auf.

Tabelle 1: Risikofaktoren für die Entstehung einer invasiven Candidiasis[1,2,13,15]

Kritisch kranke Patienten mit prolongierter intensivmedizinischer Behandlung
Viszeralchirurgische Eingriffe mit rezidivierenden Anastomoseninsuffizienzen oder wiederholten Laparotomien
Akute nekrotisierende Pankreatitis
Maligne hämatologische Erkrankungen
Organtransplantation
Solide Tumorerkrankung
Neonaten mit niedrigem Geburtsgewicht
Breitbandantibiotikatherapie
Einliegende zentralvenöse Katheter
Totale parenterale Ernährung
Hämodialyse
Therapie mit Glukokortikoiden oder Chemotherapeutika
Kolonisierung mit *Candida* an mindestens 2 Körperstellen

Neben der als häufigsten Form der IC beschriebenen Candidämie sind weitere IC mit fehlendem Blutkulturnachweis zu unterscheiden. Dazu zählen chronisch disseminierte hepatorenale Formen der IC bei Patienten mit hämatologischen Tumorerkrankungen und Infektionen anderer Organsysteme sowie die invasive abdominelle Candidiasis. Organmanifestationen der IC können jedoch durch Erregeraussaat ebenfalls zu einer sekundären Candidämie führen[15–17].

1.2.2 Pathogenese der invasiven Candidiasis

Die IC entsteht zumeist auf dem Boden einer Erregertranslokation aus bereits kolonisierten Arealen. *Candida spp.* der physiologischen Darmflora dringen durch Translokation oder durch eine Anastomoseninsuffizienz nach Laparotomie in die vormals sterile Bauchhöhle ein und verursachen entweder eine lokalisierte abdominelle Infektion (z. B. Peritonitis) oder auch nachfolgend eine Candidämie[15]. Bei Patienten mit zentralvenösen Kathetern führt eine aus dem Darm oder der Haut stammende *Candida*-Translokation zur Kolonisierung des Katheters. Dabei bilden einige *Candida spp.* einen Biofilm, welcher dazu in Lage ist, die Erregerevasion zu verhindern[11]. Diese infizierten Katheter dienen in der Folge als Erregerreservoir für eine anhaltendende Candidämie. Bei bestehender Candidämie, sei es durch kolonisierte intravaskuläre Katheter oder auf andere Weise, können sekundäre metastatische Infektionen in Lunge, Leber, Milz, Nieren, Knochen oder dem Auge auftreten[10]. Diese tiefsitzenden Infektionen können lokalisiert bleiben oder zu sekundären Candidämien führen. Während der Candidämie können die Pilze über den Blutstrom in den Urin gelangen (Candidurie). Seltener kann eine tiefsitzende Candidiasis als Folge einer aufsteigenden Pyelonephritis auftreten und entweder lokalisiert bleiben oder zu sekundärer Candidämie führen[15]. Häufig handelt es sich jedoch insbesondere bei abdominellen Infektionen um Mischbilder aus sowohl bakteriellen- als auch Pilzinfektionen[18]. Die adäquate und zeitnahe Erregerdiagnostik bildet dabei ein Kernelement der nachfolgenden antiinfektiven Therapie[19–23].

1.2.3 Erregerspektrum der invasiven Candidiasis

Candida albicans ist der weltweit häufigste Erreger der IC[12,13]. Etwa 30-50% der gesunden Erwachsenen weisen eine Kolonisation mit *C. albicans* im Oropharynx oder im Intestinaltrakt auf[24]. Diese Kolonisation kann bei prädisponierten Risikopatienten und/oder im Rahmen von medizinischen Eingriffen zu invasiven Infektionen führen. Allerdings beobachten aktuelle Studien eine Verschiebung des Erregerspektrums der IC hin zu non-albicans Stämmen[12,13,25]. *C. glabrata* ist der zweithäufigste Erreger der IC bei Erwachsenen und stellt gerade in Nordeuropa und den Vereinigten Staaten eine zunehmende Bedrohung für operative Intensivpatienten dar. *C. glabrata* birgt insbesondere durch seine häufig vorbestehende Kolonisation von Harnwegen und des Darms eine Gefahr für Risikopatienten nach viszeralchirugischen- und urologischen

Eingriffen[26]. In der pädiatrischen Intensivmedizin stellt *C. parapsilosis* einen wichtigen Krankheitserreger dar. Er zeichnet sich dabei durch die Fähigkeit zur Biofilm-Produktion auf Fremdmaterial und Epithelzellen, sowie durch ein vermindertes Ansprechen auf eine antimykotische Therapie, bei gleichzeitig geringer Virulenz aus[10,27]. Demgegenüber gilt *C. tropicalis* als Erreger mit der höchsten Virulenz aller *Candida*-Stämme. Candidämien durch *C. tropicalis* zeigen sich häufiger bei hämatologischen Patienten als im perioperativen Umfeld und weisen eine höhere Letalität im Vergleich zu Infektionen mit anderen *Candida spp.* auf[28]. Zusätzlich ist für *C. tropicalis*, im Gegensatz zu anderen *Candida spp.*, eine exogene Übertragung (z.B. durch medizinisches Personal) auf einen vorher nicht kolonisierten Patienten möglich[10,29,30]. Infektionen mit *C. krusei* sind selten in der perioperativen Intensivmedizin, sie treten meist bei hämatologischen Risikopatienten mit Neutropenie auf[26].

1.2.4 Diagnoseverfahren der invasiven Candidiasis bei kritisch kranken Patienten

Der Goldstandard für die Diagnose einer IC besteht aktuell weiterhin im kulturellen Erregernachweis aus Blut, anderen sterilen Flüssigkeiten (z.B. Liquor, Aszites) oder dem histopathologischen Nachweis aus erkranktem Gewebe. Die hohe Mortalität von Patienten mit IC ist dabei mutmaßlich zumindest in Teilen durch die mangelnde Sensitivität der aktuell verwandten diagnostischen Verfahren begründet[31]. Der positive Blutkulturnachweis einer Candidämie gelingt im Rahmen einer *Candida*-Endokarditis bei der Verwendung von drei unabhängigen Proben in etwa 80-90% der Fälle[32]. Dagegen detektieren Blutkultur-basierte Verfahren, bei Fällen von nachgewiesener oder wahrscheinlicher disseminierter IC, initial in mehr als 50% der Proben keinen ursächlichen Erreger. Positive Erregernachweise sind zusätzlich erst nach einer zeitlichen Latenz von 2-3 Tagen nach der Probenentnahme verfügbar[17,33]. Ebenso finden sich häufig bei Patienten mit Candidämie nur geringe Zahlen an *Candida* koloniebildenden Einheiten (colony-forming units, CFU). Weiterhin zeigt sich die Anzahl der im Blut nachgewiesenen CFUs erreger- und altersabhängig. So besteht die höchste Erregerlast bei Infektionen mit C. parapsilosis, sowie häufig bei pädiatrischen Patienten[34]. Mehrere Studien konnten belegen, dass die frühzeitige Initiierung einer adäquaten antimykotischen Therapie ein Schlüsselfaktor für das Überleben bei Patienten mit Candidämie darstellt[20,31,35,36]. Dazu folgern Kullberg et al., dass die

mangelnde Sensitivität der kulturabhängigen Nachweisverfahren häufig die Begründung für eine präventive und empirische antimykotische Anwendung lieferte. Allerdings führe diese Erklärung dadurch auch in Teilen zu unnötigen Behandlungen, welche zum einen mit hohen Therapiekosten, zum anderen mit der Entwicklung von Arzneimittelresistenz und dem Auftreten von unerwünschten Arzneimittelwirkungen verbunden seien[15]. Der Einsatz einer präemptiven antimykotischen Therapie bei nicht-neutropenen ICU-Patienten wird derzeit kontrovers diskutiert. Gerade in den letzten Jahren konnten mehrere Studien in diesem Patientenkollektiv keinen positiven Einfluss einer frühen empirischen Antimykotikatherapie auf die Mortalität dieser Patienten belegen[37–39].

Die klassischen kulturbasierten Nachweisverfahren sind aktuell die einzigen verwendeten Methoden, welche ebenso eine nachfolgende komplette Resistenztestung ermöglichen. Demgegenüber wurde in den letzten Jahren eine zunehmende Zahl von unterschiedlichen indirekten Diagnoseverfahren zur Erregeridentifikation entwickelt. Indirekten Diagnoseverfahren verwenden Surrogat-Marker und die Polymerase Kettenreaktion (PCR) zur Erregeridentifikation. Aktuell ergänzen sie die klassische Erregerdiagnostik, sind aber noch nicht in der Lage, diese gänzlich zu ersetzen. *Candida*-Mannan-Antigen, Antimannan-Antikörper und 1,3-β-$_D$-Glucan (BDG) sind die bevorzugten Surrogatmarker für IC[40–42]. Vergleichsstudien konnten belegen, dass sowohl PCR- als auch BDG-basierte Tests eine höhere Sensitivität im Vergleich zu Blutkulturverfahren bei der Diagnose von IC zeigen[43]. Allerdings können gerade bei Risikopatienten falsch positive BDG-Nachweise als Folge von *Candida*-Kolonisation, Hämodialyse, Antibiotikatherapie und Verunreinigungen auftreten, wodurch die Wertigkeit des Tests limitiert wird[44,45]. BDG kann jedoch aufgrund seines hohen negativen prädiktiven Wertes für IC bei Patienten mit niedrigem- bis moderaten Risiko zum Ausschluss einer IC eingesetzt werden[15,21]. Aktuell sind eine Reihe von in-house PCR Tests für die Diagnose einer IC klinisch verfügbar. Allerdings wird deren Verwendung durch eine teils mangelnde Validierung und Standardisierung eingeschränkt[15,33]. Die neueste Generation vollautomatischer PCR-basierter Nachweissysteme (SeptiFast, T2Candida Panel) konnte in ersten Studien vielversprechende Ergebnisse zeigen[46–48]. Deren Verwendung könnte die Identifikation therapiebedürftiger Patienten im perioperativen Umfeld zukünftig

erleichtern. Allerdings fehlen hierzu aktuell noch Daten aus großen multizentrischen Studien.

Neben den beschriebenen Verfahren zum direkten und indirekten Erregernachweis wurden in den letzten Jahren verschiedene klinische Scores bezüglich ihrer Vorhersagewahrscheinlichkeit für IC evaluiert. Eine spanische Arbeitsgruppe um León entwickelte den "Candida Score", welcher einen signifikanten Zusammenhang zwischen steigenden Score-Werten und der Rate an IC zeigen konnte[49–51]. Weitere positiv evaluierte klinische Scores stellen der „Colonization index“, „Corrected colonization index“ und der „Ostrovsky clinical prediction rule-Index“ dar[52].

1.3 Medikamentöse Therapie der invasiven Candidiasis

Auf Grund der systemimmanenten Einschränkungen bei der Diagnostik lebensbedrohlicher IC erfolgt die Therapie häufig neben der bewiesenen IC auch bei Risikopatienten mit hochgradigem Verdacht auf das Vorliegen einer IC. Zur Therapie der IC stehen aktuell insgesamt drei Substanzklassen zur Verfügung. Echinocandine (Anidulafungin, Caspofungin, Micafungin), Azole (z.B. Voriconazol, Fluconazol, Itraconazol) oder Polyene (Amphotericin B (AmpB)) werden zur Therapie der IC eingesetzt. Die Auswahl des verwendeten Antimykotikums ergibt sich dabei aus dem betroffenen Körperkompartiment, dem Immunstatus des betroffenen Patienten, der aktuellen Krankheitsschwere, sowie der Resistenzlage des Erregers (siehe Tabelle 2). Zur primären Therapie der Candidämie wird beispielsweise generell die Gabe eines Echinocandins empfohlen[21–23]. Tiefsitzende IC (z. B. des Auges, der Herzklappen, des zentralen Nervensystems oder die hepatolienale Candidiasis) werden hingegen präferiert durch Azole oder liposomales AmpB, aufgrund ihrer besseren Gewebegängigkeit, therapiert[21,22,53]. Ebenfalls müssen intrinsische Resistenzen der unterschiedlichen *Candida spp.* in die therapeutischen Überlegungen mit einbezogen werden. Ein weiteres Kriterium bei der Wahl des adäquaten Antimykotikums bilden erworbene Resistenzen. Neben den erworbenen Resistenzen unter laufender Azoltherapie[54,55] wurden in aktuellen Studien zur Wirksamkeit von Echinocandinen ebenfalls in zunehmendem Maße Resistenzen beschrieben[56–58]. Dabei wurden insbesondere Punktmutationen in den Genen FKS1 und FKS2 als Ursache einer Echinocandinresistenz von *Candida spp.* identifiziert[53,59]. Die Therapie der IC richtet sich im Speziellen nach den aktuellen Empfehlungen der „Infectious Disease Society

of America (IDSA)“, der „European Society of Clinical Microbiology and Infectious Diseases (ESCMID)” und der „European Society of Intensive Care Medicine” (ESICM). Die aktuellen Therapieempfehlungen sind in Tabelle 2 zusammengefasst[10,15,21–23].

Tabelle 2: Empfehlungen für die Therapie der invasiven Candidiasis[10,21–23]

Mykose	**Standardtherapie**	**Alternative**
Candidämie, *nicht-neutropene Patienten*	Echinocandin	Fluconazol[a] liposomales Amphotericin B[b]
Candidämie, *Neutropenie*	Echinocandin	Fluconazol liposomales Amphotericin B
Verdacht auf invasive *Candida*-Infektion, *nicht-neutropene Patienten*	Echinocandin[c] Fluconazol	liposomales Amphotericin B
Verdacht auf invasive *Candida*-Infektion, *Neutropenie*	Echinocandin liposomales Amphotericin B Voriconazol	Fluconazol Itraconazol[d]

[a] Akzeptable Alternative zu einem Echinocandin bei nicht-kritisch kranken Patienten und bei Patienten mit niedriger Wahrscheinlichkeit für eine Infektion durch eine Fluconazol-resistente *Candida spp.*

[b] Angemessene Alternative im Falle von Unverträglichkeit, mangelnder Verfügbarkeit oder Resistenz gegen andere Antimykotika

[c] bei hoher Krankheitsschwere, nach Azol-Exposition, oder Risiko von *C. glabrata / C. krusei*

[d] ohne Azol-Anamnese

Kritisch kranke Intensivpatienten werden häufig parallel mit mehreren Arzneimitteln verschiedener Stoffklassen therapiert. Aus diesem Grund gilt es, mögliche Arzneimittelinteraktionen bei der klinischen Anwendung von Antimykotika in der Intensivmedizin zu beachten. Azole zeigen auf Grund ihrer Beeinflussung des

Cytochrome P (CYP) 450-Systems multiple Interaktionen[60]. Polyene zeigen dagegen pharmakodynamische Interaktionen durch die Beeinflussung von Nierenfunktion[61] und Elektrolythaushalt und zeigen im Allgemeinen ein unvorteilhaftes Nebenwirkungsprofil, was ihren Einsatz als Initialtherapie nach der aktuellen Datenlage nicht rechtfertigt[21,22]. Echinocandine zeigten in Studien im Vergleich zu AmpB Deoxycholat signifikant geringere toxische Effekte bei gleicher therapeutischer Effektivität[62]. Auf Grund seiner geringeren Toxizität werden ausschließlich liposomale AmpB Formulierungen als Alternative bei Therapieversagen zur Therapie der IC empfohlen[21–23].

1.3.1 Echinocandine zur Behandlung der invasiven Candidiasis

Die Echinocandine (Anidulafungin, Caspofungin und Micafungin) gehören zur neuesten Substanzklasse der klinisch angewendeten Antimykotika. Im Jahre 2001 war Caspofungin das erste Antimykotikum dieser Substanzklasse, welches durch die amerikanischen Food and Drug Agency (FDA) die Zulassung für die Behandlung der invasiven Aspergillose und nachfolgend zur Therapie der IC erhielt[63,64]. Echinocandine sind semisynthetische Lipopeptide und besitzen eine zentrale zyklische Hexapeptidstruktur (Abbildung 1)[59]. Die einzelnen Echinocandine zeigen strukturelle Unterschiede in ihren lipophilen N-Acyl-Seitenketten. Diese bedingen spezifische Unterschiede in der Pharmakokinetik und Pharmakodynamik der einzelnen Substanzen. Basierend auf diesen Unterschieden zeigen die jeweiligen Substanzen unterschiedliche Löslichkeiten. Anidulafungin und Caspofungin sind lipophiler im Vergleich zum hydrophilen Micafungin, was zu Unterschieden in der Gewebeverteilung beitragen könnte[65]. Sie inhibieren selektiv die große intramembranäre Untereinheit des Enzyms β-(1,3)-$_D$-Glucansynthase und hemmen somit die β-(1,3)-$_D$-Glucan Synthese. Etwa 60% der Pilzzellwand bestehen aus β-(1,3)-$_D$-Glucan, welches sich dadurch für ihre Integrität und Abgrenzung gegenüber äußeren Einflüssen verantwortlich zeigt[66–68]. Die Störung der β-(1,3)-$_D$-Glucan-Synthese führt zu einer erhöhten Zellwandpermeabilität mit einem daraus resultierenden Ungleichgewicht im osmotischen Druckgefälle. Die Folge aus dieser Synthesestörung ist eine nachfolgende Lyse der betroffenen Pilzzelle[69]. Dieser fungizide Effekt ist für alle drei klinisch verwendeten Echinocandine bei *Candida spp.* und *Saccharomyces spp.* belegt[70]. Im Gegensatz zu den Azolen zeigen die Echinocandine ebenfalls eine

potente Wirkung gegen biofilmständige *Candida spp.*[71]. Demgegenüber wirken Echinocandine statisch auf das Wachstum von *Aspergillus spp.*

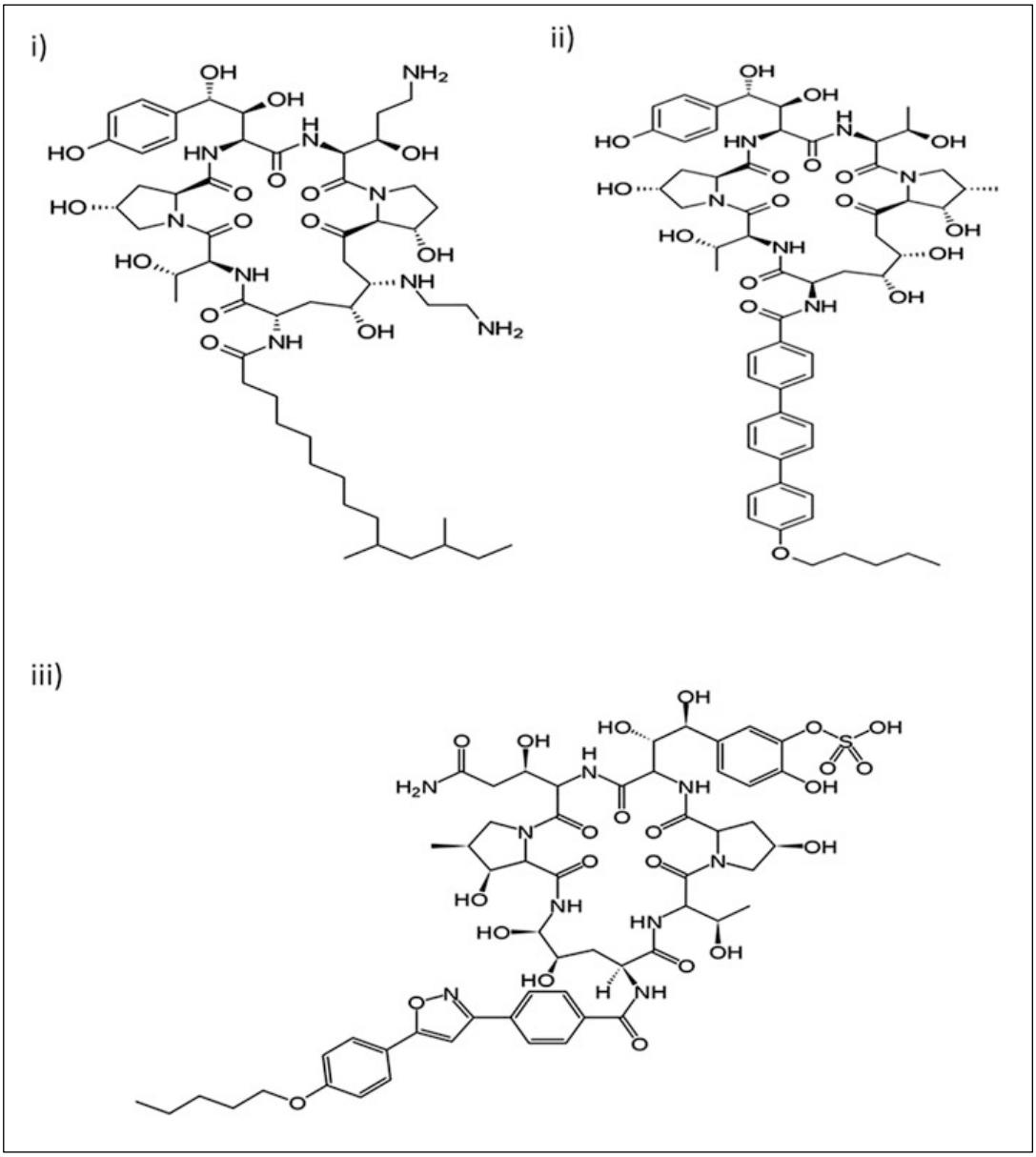

Abbildung 1: Strukturformel der klinisch verwendeten Echinocandine. (i) Caspofungin (ii) Anidulafungin und (iii) Micafungin[72]*.*

1.4 Unerwünschte Arzneimittelwirkungen antimykotischer Therapie

Pilze sind ebenso wie humane Zellen Eukaryonten. Auf Grund dessen verfügen Pilze nur über wenige Angriffsziele für selektiv wirksame Medikamente, welche in humanen Zellen nicht vorhanden sind[10]. Dadurch ist das Risiko für unerwünschte Arzneimittelwirkungen unter antimykotischer Therapie im Vergleich zur Antibiotikatherapie, die sich gegen die primitiveren prokaryontischen Bakterienzellen richtet, deutlich erhöht[59]. Studien belegen beispielsweise eine erhöhte Nephrotoxizität sowie Fieber, Schüttelfrost, Erbrechen, Kopfschmerzen und Thrombophlebitis unter der Therapie mit AmpB Deoxycholat, was in der Vergangenheit den therapeutischen Einsatz häufig limitierte[73,74]. Lipidformulierte AmpB Präparate zeigten in einer aktuellen Metanalyse, bei vergleichbarer therapeutischer Effizienz gegenüber *Candida spp.* und *Aspergillus fumigatus*, eine signifikant geringere Nephrotoxizität[74]. Azolantimykotika wiesen in verschiedenen Studien und Meta-Analysen ebenfalls eine erhöhte Inzidenz an Nephro-, Neuro- und Hepatotoxizität auf[75]. Dagegen konnten Echinocandine seit ihrer Markteinführung in unterschiedlichen Patientenkohorten ihr vorteilhaftes Nebenwirkungsprofil unter Beweis stellen[76–80]. Allerdings sind spezielle Patientengruppen beschrieben, welche ein erhöhtes Risiko für unerwünschte Nebenwirkungen unter Echinocandin-Therapie oder auch veränderte Wirkspiegel zeigen[81–83]. Caspofungin führte bei Patienten mit moderater (Child-Pugh-score: 7–9) und schwerer (Child–Pugh-score: 10–12) Leberinsuffizienz zu Leberenzymerhöhungen bis hin zum akuten Leberversagen[84,85]. Auch für Anidulafungin und Micafungin sind Fälle von akuten hepatischen Nebenwirkungen beschrieben[86,87]. Seit 2013 werden zunehmend auch Daten zu hämodynamischen Einschränkungen unter Echinocandin-Therapie publiziert. Fink et al. berichteten über einen 41-jährigen männlichen intensivpflichtigen Patienten, bei welchem es nach Infusion von Anidulafungin zu einer akuten hämodynamischen Instabilität mit Hypotonie, Bradykardie und nachfolgender kardiopulmonaler Reanimation kam[88]. Hindahl und Wilson präsentierten einen Fall eines schnell aufgetretenen pulmonalen Ödems infolge einer Anidulafungin-Applikation, welches zu hämodynamischen Einschränkungen führte[89]. Lichtenstern et al. veröffentlichten 2013 eine Fallserie von 3 intensivpflichtigen Patienten, bei denen es jeweils während einer Therapie mit Anidulafungin oder Caspofungin zu einem Abfall des Herzindex (cardiac index, CI) oder Abfall des mittleren arteriellen Blutdrucks (mean arterial pressure, MAP)

gekommen war[90]. Eine Recherche innerhalb des US Food and Drug Administration Adverse Events Reporting Systems (FAERS) für den Zeitraum von 2004-2012 ergab 11.761 Einträge im Zusammenhang mit einer Echinocandin-Therapie. Im Mittel waren darunter jährlich 3,5 Fälle von Herzversagen unter Caspofungin-Therapie und jeweils 1 Fall unter Anidulafungin- und Micafungin-Therapie[91]. Gleichzeitig konnte in einem *ex vivo* Modell der Ratte mit isolierter Langendorff-Perfusion gezeigt werden, dass sowohl Anidulafungin als auch Caspofungin die kardiale Kontraktilität signifikant reduzieren[92]. Die Autoren vermuteten als pathophysiologische Ursache eine toxische Beeinflussung der mitochondrialen Funktion, welche insbesondere bei Patienten mit bereits vorbestehenden kardialen Funktionseinschränkungen und bei zentralvenöser Applikation auftreten könnte[92]. Nachfolgend wiesen Shirey et. al. in einem *in vitro* Modell nach, dass Caspofungin und Micafungin den Elektronentransfer der Atmungskette beeinflussen[93]. In der Folge veröffentlichten Stover und Cleary ein Review zum Thema der hämodynamischen Nebenwirkungen der Echinocandine, in dem sie den Begriff der „Antimykotikaassoziierten, medikamenteninduzierten Herzerkrankung“ (Antifungal-Associated Drug-Induced Cardiac Disease) prägten[91].

2 Fragestellungen und Zielsetzungen der Arbeit

Die Mortalität der IC bei operativen Intensivpatienten liegt trotz moderner diagnostischer- und therapeutischer Maßnahmen weiterhin bei bis zu 40%[14]. Die aktuellen Leitlinien der IDSA und der ESICM/ESCMID empfehlen Echinocandine als Standardtherapie der IC[21–23]. Aktuelle Studien belegen dabei die Notwendigkeit einer frühzeitigen Initiierung einer zielgerichteten antimykotischen Therapie[36,94]. Allerdings zeigen Fallberichte und tierexperimentelle Daten, dass insbesondere kritisch kranke Intensivpatienten durch potenzielle hämodynamische Nebenwirkungen der Echinocandine gefährdet sein könnten[65,88–90]. Das Ziel dieser Arbeit war es daher, die Auswirkungen hämodynamischer Nebenwirkungen der Echinocandine auf die Therapie und den Krankheitsverlauf kritisch kranker Intensivpatienten näher zu beleuchten.

Zu diesem Zweck wurde in einem ersten Schritt die Wertigkeit des kultur-unabhängigen Denaturating High-Performance Liquid Chromatography- (dHPLC) Verfahrens im Vergleich zur klassischen kulturbasierten mikrobiomischen Analyse bei Patienten mit abdomineller Sepsis verglichen. Dabei sollte die Frage beantwortet werden, ob es Unterschiede bei der Erregeridentifikation zwischen beiden Verfahren gibt und ob diese Verfahren eine klinische Relevanz bei der Wahl einer antiinfektiven Therapie haben.

Um nachfolgend den Einfluss unterschiedlicher antimykotischer Therapieregime bei vermuteter oder bestätigter *Candida*-Peritonitis auf das Outcome operativer Intensivpatienten zu evaluieren und den möglichen Einfluss hämodynamischer Nebenwirkungen der Echinocandine zu untersuchen, führten wir eine retrospektive Datenanalyse an insgesamt 137 Patienten mit *Candida*-Peritonitis durch. Die Fragestellung dabei war, ob die gewählte Behandlungsstrategie (keine Therapie versus empirische oder spezifische Therapie) einen Einfluss auf das Outcome der Patienten hatte.

Gleichzeitig sollten die Echinocandin-assoziierten Nebenwirkungen *ex vivo* und *in vivo* quantifiziert und ihre pathophysiologischen Grundlagen identifiziert werden. Dazu bedienten wir uns dreier verschiedener aufeinander folgender Modelle. In einem ersten Schritt wurden dazu Kardiomyozyten aus adulten Ratten isoliert. Nach deren

Inkubation entweder mit Echinocandinen oder Fluconazol wurden in diesem *ex vivo* Modell die Auswirkungen auf die Kontraktilität der Kardiomyozyten bestimmt. Die Fragestellungen dabei waren: Beeinflussen Echinocandine das Kontraktionsverhalten isolierter Kardiomyozyten der Ratte in einem ex vivo Modell? Sind die Veränderungen der Kontraktilität dosisabhängig? Gibt es toxische Effekte der Echinocandine auf isolierte Kardiomyozyten der Ratte?

Im zweiten Schritt wurden die Auswirkungen supranormaler Echinocandinkonzentrationen auf die hämodynamische Funktion männlicher Lewis-Ratten untersucht. Dazu bedienten wir uns einem modifizierten *in vivo* Hämodynamikmodell[95,96]. Es wurden adulte Ratten anästhesiert, intubiert, maschinell beatmet und mit zentralvenös applizierten Echinocandinen in klinisch angewandten- sowie supranormalen Dosierungen behandelt. Über einen einliegenden Linksherzkatheter konnten die hämodynamischen Zielwerte erfasst werden. Weiterhin wurde die Überlebenszeit der Tiere erhoben. Ebenso wurde untersucht, ob die Therapie mit Echinocandinen einen Einfluss auf die mitochondriale Enzymaktivität oder die mitochondrialen Transkripte der linksventrikulären Kardiomyozyten hat. Die untersuchten Fragestellungen dabei waren: Unterscheiden sich die Überlebenszeiten von Ratten nach supranormaler Echinocandintherapie im Vergleich zur empfohlenen (humanen) Dosierung? Induziert die Echinocandintherapie Veränderungen der linksventrikulären Funktion? Entstehen durch die Echinocandintherapie Veränderungen der mitochondrialen Enzymaktivität oder der mitochondrialen Transkripte?

Auf Grund unserer Hypothese, wonach kritisch kranke Intensivpatienten in besonderem Maße von den hämodynamischen Nebenwirkungen der Echinocandine betroffen sein könnten, erweiterten wir den Versuchsaufbau unseres Hämodynamik-Modells der Ratte im nächsten Schritt um einen Endotoxin-induzierten Schock. Dieser sollte die vorbestehende Kreislaufdepression während der Sepsis imitieren. Dabei untersuchten wir die folgende Fragestellung: Sind Ratten im Endotoxinschock im besonderen Maße von hämodynamischen Veränderungen während einer Echinocandin-Therapie betroffen?

In einem dritten Schritt sollten die pathophysiologischen Ursachen der Echinocandin-induzierten Kreislaufveränderungen untersucht werden. Der Hypothese einer

Beeinflussung des intrazellulären Kalziumhaushaltes folgend, untersuchten wir, ob Echinocandine die intrazelluläre Kalziumhomöostase in einem *ex vivo* Modell mit humanen Kardiomyozyten beeinflussen.

Abschließend sollten die gewonnen laborexperimentellen Daten mit klinischen Daten kritisch kranker chirurgischer Patienten mit IC verglichen werden. Dazu führten wir eine retrospektive Datenanalyse operativer ICU Patienten durch. Dabei untersuchten wir die folgende Frage: Kommt es bei kritisch kranken ICU Patienten mit vermuteter oder bestätigter IC zu relevanten hämodynamischen Veränderungen unter Echinocandin-Therapie?

3 Zusammenfassung der Ergebnisse eigener Arbeiten

3.1 Mikrobiomische Analyse von intraabdominellen Infektionen durch den Einsatz von Denaturing High-Performance Liquid Chromatography: Eine prospektive Observationsstudie (Anlage 1)

Wie in der Einleitung erläutert, bilden die frühzeitige Identifikation des auslösenden Erregers einer Infektion sowie die unverzügliche antimikrobielle Therapie die Kernelemente zur erfolgreichen Behandlung einer Sepsis[19]. Die aktuellen Guidelines der „Surviving Sepsis Campaign“ fordern in diesem Zusammenhang eine umgehende Abnahme von Blutkulturen mit nachfolgender mikrobiologischer Untersuchung und Resistenztestung[97,98]. Die aktualisierte Fassung der Leitlinien weist in diesem Zusammenanhang darauf hin, dass kulturunabhängige Verfahren die Diagnose der Sepsis in Zukunft potentiell ergänzen könnten.

In Anlage 1 wurde basierend auf diesen Überlegungen die klassische kulturbasierte mikrobiomische Analyse zur Erregeridentifikation bei Patienten mit abdomineller Sepsis mit dem kulturunabhängigen Verfahren der Denaturating High-Performance Liquid Chromatography (dHPLC) verglichen[98]. Nach Genehmigung der lokalen Ethikkommission des Fachbereichs Medizin der Justus-Liebig-Universität Gießen (AZ: 111/09) wurden hierzu 42 Proben aus intraoperativ entnommenem Aszites verwendet. Dabei konnte in 38,1% der untersuchten Proben kein Erregernachweis mittels der klassischen kulturbasierten Verfahren erfolgen, während im Vergleich dazu mit dem dHPLC basierten WAVE®-System (ADS Biotec, Omaha, USA) nur 31% der Erregernachweise negativ waren. Studien zu PCR-basierten Nachweisverfahren bei Patienten mit Blutstrominfektionen oder Pneumonie zeigten dabei vergleichbare Ergebnisse[99–103]. In unseren Untersuchungen konnte bei ca. 40% der Patienten mittels beider Verfahren ein Erreger identifiziert werden. Zwei mikrobiologische Isolate fanden sich bei 23,8% der Proben in der kulturbasierten Methode und 19% in der HPLC basierten Methode. Dagegen zeigte die HPLC Diagnostik eine signifikant höhere Detektionsrate für *Enterobacteriacae* und *Enterococcus faecium*. Auch gelang der Nachweis von seltenen Erregern wie *Haemophilus, Lactobacillus, Clostridia, Methylobacterium, Colinsella aerofaciens* und *Solobacterium moorei* ausschließlich mittels der kulturunabhängigen Methode. Im Gegensatz dazu war das WAVE®-System

aus methodenimmanenten Gründen nicht in der Lage, Pilznachweise zu erbringen. In der kulturbasierten Diagnostik zeigten sich jedoch in 26,2% der Isolate Pilze (davon C. albicans: 54,5%, C. glabrata: 27,3%, C. parapsilosis 9,1%, andere: 9.1%). Neben der höheren Detektionsrate für mikrobielle Isolate bestand ein weiterer Vorteil des PCR basierten Systems in der deutlich kürzeren Untersuchungsdauer. Allerdings besteht die Domäne der klassischen kulturabhängigen Diagnostik weiterhin in der Empfindlichkeitstestung, da die dHPLC-Methode nur den Nachweis bereits bekannter Resistenzgene erbringen kann.

In der kulturbasierten Diagnostik zeigten sich in 26,2% der Isolate Pilze (davon C. albicans: 54,5%, C. glabrata: 27,3%, C. parapsilosis 9,1%, andere: 9.1%). Das kulturunabhängige dHPLC WAVE®-System lieferte bei Patienten mit intraabdomineller Sepsis zusätzliche Informationen, insbesondere bei seltenen und anspruchsvollen bakteriellen Erregern. Ebenfalls zeigte es eine höhere Detektionsrate bei polymikrobiellen Infektionen und lieferte im Vergleich zu kulturbasierten Verfahren schnellere Ergebnisse, war jedoch nicht in der Lage Pilzinfektionen zu identifizieren.

3.2 Der Einfluss von realen Behandlungsstrategien für *Candida*-Peritonitis - Eine retrospektive Analyse (Anlage 2)

Die nosokomiale *Candida*-Peritonitis wurde in einer klinischen Studie als unabhängiger Risikofaktor für Mortalität bei Intensivpatienten identifiziert[104]. Sowohl die Candidämie als auch die *Candida*-Peritonitis sind bei Intensivpatienten mit einem Anstieg der 30-Tage-Mortalität auf bis zu 31% assoziiert[105]. Jedoch sind *Candida*-Isolate aus abdominellen Infektionsfoci bei operativen Intensivpatienten häufig nachzuweisen, ohne dass diese einen Einfluss auf die Mortalität haben. Auf Grund dessen empfehlen die aktuellen therapeutischen Leitlinien eine antimykotische Therapie nur für Hochrisikopatienten mit wiederkehrenden gastrointestinalen Perforationen oder bei Patienten mit chirurgisch therapierter nekrotisierender Pankreatitis[21,106]. Für Patienten im septischen Schock, sowohl mit bewiesener Candidämie und frühzeitiger antimykotischer Therapie konnte ein Überlebensvorteil nachgewiesen werden[20,94].

In der Studie der Anlage 2 untersuchten wir aus diesem Grunde die Frage: Haben unterschiedliche antimykotische Therapieregime (A: keine antimykotische Therapie, B: empirische Therapie, C: spezifische Therapie) bei nachfolgend bestätigter *Candida*-Peritonitis einen Einfluss auf das Überleben operativer Intensivpatienten?

Zur Beantwortung dieser Fragen führten wir eine retrospektive monozentrische Datenauswertung von 137 Patienten mit mikrobiologischem *Candida*-Nachweis durch, welche auf der operativen Intensivstation des Universitätsklinikums Gießen und Marburg, Standort Gießen, behandelt wurden (Ethikvotum AZ: 152/11). In dieser Gruppe von Patienten mit intraabdominellem *Candida*-Nachweis erhielten 56 (40,9%) Patienten primär keine, 29 (21,2%) Patienten eine empirische und 52 (38%) Patienten eine spezifische antimykotische Therapie, letztere basierend auf den Ergebnissen der mikrobiologischen Untersuchungen. In der Gruppe der Patienten, welche eine antimykotische Therapie erhielten, befanden sich mehr Patienten mit im Trend höherem medianen Lebensalter (A: 62.18 ± 15.63 vs. B: 63.66 ± 14.20 vs. C: 68.08 ± 11.93; p = 0,08) und höherer Krankheitsschwere, dargestellt durch einen erhöhten APACHE II-Score (A: 15.71 ± 5.82, B: 17.62 ± 4.67, C: 18.12 ± 5.84; p = 0,06). Der häufigste nachgewiesene ursächliche Erreger war in diesem Kollektiv *C. albicans*, gefolgt von *C. glabrata* und *C. tropicalis*. C. krusei. *C. parapsilosis* wurde nur vereinzelt nachgewiesen. In der multivariaten Analyse konnten das Alter (p = 0,012; Hazard-

Ratio (HR) [95%-Konfidenzintervall] 1,038 [1,008-1,069]), die Leukozytenzahl (p = 0,02; HR = 1,043 [1,007-1,081]), der APACHE II Score (p < 0,001; HR = 1,116 [1,055-1,181]) und ein akutes Leberversagen (p < 0,001; HR = 4,443 [2,328-8,480]) als unabhängige Prädiktoren einer erhöhten 30-Tage Sterblichkeit identifiziert werden. In der Gruppe der Patienten ohne antimykotische Therapie zeigte sich die geringste Gesamtsterblichkeit (n = 21; 33,9%). Im Vergleich dazu verstarben in der Gruppe der Patienten mit empirischer Therapie 14 Patienten (48,3%), sowie in der Gruppe der Patienten mit spezifischer antimykotischer Therapie 23 Patienten (44,2%). Dieser Unterschied zwischen antimykotisch behandelten- und unbehandelten Patienten (p = 0,043) war zum einen der höheren Krankheitsschwere dieser Patientengruppe geschuldet, zum anderen zeigte dieser Unterschied aber gleichzeitig, dass eine frühe empirische antimykotische Therapie keinen Überlebensvorteil bei intraabdominellen *Candida*-Infektionen bewirkte. In der Zusammenschau der hier erhobenen Daten und im Vergleich mit der aktuellen Literatur kann aus diesem Grunde weder eine frühzeitige empirische noch eine spezifische antimykotische Therapie bei *Candida*-Peritonitis empfohlen werden. Es sollten dabei zusätzlich auch die potenziellen schwerwiegenden Nebenwirkungen mit in die therapeutische Entscheidung einbezogen werden.

Alter, Leukozytenzahl, APACHE II Score und akutes Leberversagen sind bei kritisch kranken Patienten mit *Candida*-Peritonitis unabhängige Prädiktoren für eine erhöhte 30-Tage-Sterblichkeit. Eine frühe empirische antimykotische Therapie führte in diesem Patientenkollektiv zu keinem Überlebensvorteil.

3.3 Auswirkung von Echinocandinpräparaten auf die Funktion adulter ventrikulären Kardiomyozyten der Ratte - Ergebnisse einer *in-vitro*-Studie (Anlage 3)

IC stellen ein hohes Risiko für intensivpflichtige Patienten dar und sind mit einer erhöhten perioperativen Sterblichkeit vergesellschaftet[107–109]. Dementsprechend ist eine adäquate antimykotische Therapie unerlässlich für die Reduktion der Erregerlast bei chirurgischen Patienten, um eine systemische Inflammation und Organversagen zu therapieren[110]. Aktuelle Leitlinien empfehlen Echinocandine als Medikamente der ersten Wahl zur Behandlung von IC[21–23]. Wie bereits in der Einleitung ausgeführt, stehen Echinocandine aber auf Grund von Fallberichten und tierexperimentellen Daten im Verdacht, hämodynamische Veränderungen zu verursachen[65,88,90]. Ziel der in Anlage 3 beigefügten experimentellen Arbeit war es aus diesem Grunde, die Auswirkungen der Echinocandine (Anidulafungin, Caspofungin und Micafungin) im Vergleich zu Fluconazol auf die kontraktile Funktion isolierter ventrikulärer Kardiomyozyten der adulten Ratte zu untersuchen. Zu diesem Zweck wurden Kardiomyozyten adulter Lewis Ratten nach einem bereits zuvor publizierten Protokoll isoliert[111]. Nachfolgend präinkubierten wir diese Zellen in jeweils separaten Experimenten mit unterschiedlichen Konzentrationen (0; 0,1; 3,3; 10; 33; 100 µg/ml) der einzelnen oben angegebenen Antimykotika, jeweils über 90 Minuten (min). Nach anschließender biphasischer elektrischer Stimulation wurden einerseits die Kontraktilität, als Verkürzung der Amplitude der Zellen- (dL/L ± Standardabweichung [standard deviation, SD]), sowie andererseits das Verhältnis von elongierten vitalen zu runden Zellen (als Surrogat für Zelltod, % rounded ± SD) gemessen und mit dem Ausgangszustand verglichen. Resultierend konnte ein Anstieg der mittleren Kontraktilität bei Anidulafunginkonzentrationen von 3,3 µg/ml (9,479 ± 2,646 dL/L; $p \leq 0,001$) und 10 µg/ml (9,627 ± 2,8 dL/L; $p \leq 0,001$) beobachtet werden. Caspofunginexposition (10 µg/ml) bewirkte im Gegensatz dazu eine signifikante Reduktion der mittleren Kontraktilität (4,834 ± 2,079 dL/L; $p \leq 0,0001$). Micafungin-Konzentrationen von 3,3-33 µg/ml bewirkten einen signifikanten Anstieg der Zellverkürzung (3,3 µg/ml: 9,436 ± 2,35 dL/L; $p \leq 0,05$; 10 µg/ml: 10,01 ± 3,156 dL/L; $p \leq 0,0001$; 33,3 µg/ml: 10,71 ± 3,196 dL/L; $p \leq 0,0001$). Konzentrationen ab 33 µg/ml führten bei Anidulafungin (33,3 µg/ml: 86,6 ± 9% rounded; 100 µg/ml: 99,8 ± 1% rounded) und Caspofungin (33,3 µg/ml: 97,2 ± 3,3% rounded; 100 µg/ml: 100%

rounded) zu annähernd vollständigem Abrunden aller untersuchten Zellen. Selbiges konnte für eine Konzentration von 100 µg/ml für Micafungin (95,3 ± 3,2% rounded) beobachtet werden. In diesem Versuchsaufbau hatte Fluconazol keinerlei Einfluss, auf die Kontraktion der Zellen oder auf die Zellform, respektive die Rate an kontraktilen Zellen. Eine Vorinkubation der Echinocandine mit 10 mg/ml Albumin führte in unserem Versuchsaufbau zur Inhibition aller gemessenen Veränderungen der Kontraktilität. Diese Ergebnisse zeigen, dass alle untersuchten Echinocandine einen dosisabhängigen Einfluss auf die *in vitro*-Kontraktilität isolierter Kardiomyozyten der adulten Ratte haben. Jedoch sind weitere *in vivo* Untersuchungen nötig, um mögliche Implikationen für die antimykotische Therapie genauer zu evaluieren und eventuelle Schwellendosen für den Einritt toxischer Wirkungen zu ermitteln.

Die Echinocandine Anidulafungin, Caspofungin und Micafungin zeigen einen dosisabhängigen Einfluss auf die *in vitro*-Kontraktilität isolierter Kardiomyozyten der Ratte.

3.4 Kardiale Nebenwirkungen von Echinocandinen nach zentralvenöser Applikation in adulten Ratten (Anlage 4)

Ausgehend von den Ergebnisse aus Anlage 3, welche den direkten Einfluss von Anidulafungin, Caspofungin und Micafungin auf die *in vitro*-Kontraktilität isolierter Kardiomyozyten der Ratte zeigten, wurden die potenziellen kardialen Nebenwirkungen der Echinocandine weitergehend in einem modifizierten *in vivo* Hämodynamikmodell der Ratte[95,96] untersucht (Anlage 4).

Das Ziel der Untersuchungen war es, die hämodynamischen Nebenwirkungen von zentralvenös verabreichten, klinisch verwendeten Dosierungen der Echinocandine zu untersuchen. Dazu wurden Anidulafungin (2,5 mg/kg Körpergewicht (KG)), Caspofungin (0,875 mg/kg KG) und Micafungin (3 mg/kg KG) im Vergleich zu zehnfach erhöhten Dosierungen dieser Medikamente (Anidulafungin 25 mg/kg KG, Caspofungin 8,75 mg/kg KG, Micafungin 30 mg/kg KG) appliziert.

Als Zielgrößen dienten hierbei die Zeit bis zum hämodynamischen Versagen (time to hemodynamic failure, THF), kardiale Funktion (systolischer arterieller Blutdruck (systolic arterial blood pressure, sABP), Herzzeitvolumen (cardiac output, CO), linksventrikuläre Ejektionsfraktion (LVEF) linksventrikuläres enddiastolisches Volumen (LVEDV)), mitochondriale Enzymaktivität (Komplexe I bis III, Cytochrome C Oxidase, Succinate Dehydrogenase) und –Genexpression.

Nach Genehmigung durch das zuständige Regierungspräsidium in Gießen (GI20/26 Nr. 3/2012) wurden insgesamt 42 männliche Lewis Ratten (n = 6 pro Gruppe) in einem Hämodynamikmodell untersucht. Nach inhalativer Narkoseinduktion mittels Isofluran (Baxter, Unterschleissheim, Deutschland), erfolgte die endotracheale Intubation der Tiere mit nachfolgender volumenkontrollierter-, gewichtsadaptierter Beatmung (Harvard Inspira, MA, USA). Es erfolgte anschließend die kontinuierliche Ableitung eines Elektrokardiogramms (EKG) und eine rektale kontinuierliche Temperaturmessung in Verbindung mit einer aktiven Wärmematte zum Temperaturerhalt. Nach Anlage einer Venenverweilkanüle in die laterale Schwanzvene wurden über eine Spritzenpumpe (Braun, Melsungen, Deutschland) kontinuierlich Ringer Lösung (10 ml/kg/h; Braun, Melsungen, Deutschland) und Fentanyl (10 μg/kg/h; Ratiopharm, Ulm, Deutschland) infundiert. Es erfolgten im Weiteren die Anlage eines Druckkatheters (SPR-1000; Millar Instruments, Houston,

TX, USA) zur Ableitung des ABP in die Schwanzarterie, die Anlage eines ZVK in die V. jugularis int. dextra, sowie die Anlage eines linksventrikulären Druck-Volumen-Katheters (SPR-838, Millar, Houston, TX, USA) in die rechte A. carotis. Die Applikation der verwendeten Echinocandine erfolgte über den einliegenden ZVK.

Die Tiere der Hochdosis-Anidulafungingruppe (25 mg/kg KG) und Hochdosis–Caspofungingruppe (8,75 mg/kg KG) zeigten alle einen unmittelbaren Abfall der hämodynamischen Funktion im Sinne einer signifikanten Reduktion des CO, LVEDV und sABP. Die Tiere der Hochdosis-Anidulafungingruppe zeigten zusätzlich einen signifikanten Abfall der LVEF. Alle Tiere dieser Anidulafungin- und Caspofungin Hochdosisgruppen verstarben innerhalb des 6-stündigen Untersuchungszeitraumes (Anidulafungin: 175 min versus 360 min [Kontrollgruppe]; $p < 0,001$; Caspofungin: 94 min versus 360 min [Kontrollgruppe]; $P < 0,001$).

Demgegenüber zeigten sich keinerlei hämodynamische Veränderungen in der Kontrollgruppe (n = 6; keine Medikamentengabe) und den Niedrigdosisgruppen für Anidulafungin (n = 6), sowie Caspofungin (n = 6). Tiere, welche eine Therapie mit Micafungin erhielten, zeigten weder in der Hoch- (n = 6) noch in der Niedrigdosisgruppe (n = 6) hämodynamische Veränderungen oder reduzierte Überlebenszeiten.

Weiterhin zeigte sich bei der Untersuchung der mitochondrialen Enzymaktivität (Komplexe I bis III, Cytochrome C Oxidase, Succinate Dehydrogenase) eine signifikante Reduktion der Aktivität der Komplex III-Aktivität in der Hochdosis-Micafungingruppe im Vergleich zur Kontrollgruppe ($p < 0,05$), sowie eine signifikante Reduktion der Cytochrom C Oxidase-Aktivität in der Niedrigdosis-Micafungin Gruppe ($p < 0,05$). Alle weiteren Gruppen zeigten keinerlei signifikante Veränderungen der Enzymaktivität des linksventrikulären Myokards. Die Untersuchung der mitochondrialen Transkripte zeigte ebenfalls keinerlei signifikante Unterschiede zwischen den einzelnen Gruppen.

Die Echinocandine Anidulafungin und Caspofungin induzierten bei Hochdosis-Applikation im Hämodynamikmodell der Ratte ein Kreislaufversagen mit signifikant reduziertem CO, ABP und LVEDV. Die Beobachtungen waren nicht mit Änderungen der mitochondrialen Enzymaktivität oder der mitochondrialen Transkripte assoziiert. Die Applikation von Micafungin führte in diesem Modell zu keinerlei hämodynamischen Veränderungen.

Kardiale Nebenwirkungen von Echinocandinen in endotoxämischen Ratten (Anlage 5)

Die in Anlage 4 beschriebenen hämodynamischen Veränderungen nach Caspofungin- und Anidulafungin-Applikation traten jeweils ausschließlich in den Hochdosisgruppen auf. Abgeleitet von den bereits beschriebenen klinischen Fallberichten[88–90] über hämodynamische Beeinträchtigungen unter Echinocandin-Therapie und unter der Annahme, dass möglicherweise eine bereits vorbestehende hämodynamische Depression die potenziellen Nebenwirkungen der Echinocandine verstärken könnte, untersuchten wir in Anlage 5 die hämodynamischen Nebenwirkungen einer Echinocandin-Applikation in einem Endotoxinschock-Modell der Ratte (GI 20/26 Nr. 3/2012, JLU-Nr. 540_M).

Dazu wurde das in Anlage 4 beschriebene Hämodynamik-Modell[95,96] der Ratte um eine intravenöse Gabe von bakteriellem Lipopolysaccharid (LPS; 1 mg/kg KG) mit konsekutivem Endotoxinschock ergänzt. Als Zielparameter dienten dabei, neben den hämodynamischen Messwerten (CO, ABP, LVEF und LVEDV), die THF.

Die Tiere der Kontrollgruppe zeigten nach LPS Gabe dezente Änderungen der hämodynamischen Parameter [CO (t_0 versus t_1, +6,34% ± 11,07%), EF (t_0 versus t_1, -8% ± 4,62%), ABP (t_0 versus t_1, -10.94% ± 4.34%), EDV (t_0 versus t_1 +19,16% ± 6,14%)]. Zusätzlich verstarb keines der Tiere der Kontrollgruppe während des Versuchszeitraums (t = 180 min). Die Gabe von Caspofungin in laut Fachinformation empfohlener humaner Dosierung (0,875 mg/kg KG) führte zu einer im Vergleich zur Kontrollgruppe (LPS + NaCl 0,9%) signifikant reduzierten THF ($p < 0,05$). Ebenfalls zeigte sich dabei eine Reduktion von CO, LVEF, und ABP. Tiere nach Anidulafungin-Gabe (2,5 mg/kg KG) zeigten ebenfalls einen Trend zu reduzierter THF ($p = 0,0578$) im Vergleich zur Kontrolle mit gleichzeitiger Reduktion von CO, LVEF und ABP. Alle Tiere der Micafungin-Gruppe (3 mg/kg KG) zeigten im Vergleich zur Kontrollgruppe keine signifikante Veränderung der Hämodynamik über die Dauer der dreistündigen Versuchsperiode. Messungen der Laktatdehydrogenase (LDH) am Ende des Observationszeitraumes zeigten im Vergleich zur Kontrollgruppe erhöhte Werte für die mit Anidulafungin behandelten Tiere. Micafungin und Caspofungin induzierten keinerlei signifikante Erhöhung der LDH im Vergleich zur Kontrollgruppe.

Anidulafungin- und Caspofungin-Applikation führten in klinisch empfohlenen Dosierungen im Endotoxinschock-Modell der Ratte im Vergleich zur Kontrolle zu signifikant reduziertem CO, ABP und LVEDV. Die Tiere der Caspofungin-Gruppe zeigten zusätzlich eine signifikant verkürzte Zeit bis zum Kreislaufversagen. Die Applikation von Micafungin führte in diesem Modell zu keinerlei hämodynamischen Veränderungen.

3.5 Caspofungin moduliert die Ryanodin-Rezeptor-vermittelte Kalziumfreisetzung in humanen Kardiomyozyten (Anlage 6)

Ausgehend von den in Anlage 3-5 beschriebenen Veränderungen war das Ziel der in Anlage 6 beschriebenen Arbeit, die pathophysiologischen Grundlagen der beobachteten hämodynamischen Veränderungen zu identifizieren. Zu diesem Zweck wurden zunächst Kardiomyozyten adulter Lewis-Ratten nach einem bereits beschriebenen Protokoll isoliert[111] und für 30 min mit unterschiedlichen Dosierungen von Caspofungin (0 bis 200 µg/ml) inkubiert. Die intrazelluläre Kalziumkonzentration ($[Ca^{2+}]_i$) wurde nachfolgend mittels Fura-2-AM in der Fluoreszenzmikroskopie quantifiziert.

Dabei zeigten sich ab einer Caspofungin-Konzentration von 50 µg/ml signifikante Erhöhungen der spontanen $[Ca^{2+}]_i$ Oszillationen (12,5 µg/ml, Änderung der max. % Oszillationsfrequenz in s^{-1}, fold change (FC) = 1,13; p = 0,2; 25 µg/ml, FC = 1,11; p = 0,5; 50 µg/ml, FC = 1,60; $p < 0,001$; 100 µg/ml, FC = 2,28; $p < 0,01$; 200 µg/ml, FC = 2,74; $p < 0,001$) im Vergleich zur unbehandelten Kontrolle.

Um weiterführend die Caspofungin-induzierten Veränderungen der $[Ca^{2+}]_i$ Homöostase humaner Zellen zu untersuchen, bedienten wir uns in den folgenden Versuchsreihen humaner Kardiomyozyten aus adulten Spenderherzen (HCM, C-12810, PromoCell GmbH Heidelberg, Germany).

Auch hierbei zeigte sich eine dosisabhängige Erhöhung der $[Ca^{2+}]_i$ Konzentration durch Caspofungin (75 – 200 µg/ml). Diese Effekte waren bei Caspofunginkonzentrationen von > 130 µg/ml sowohl in Ca^{2+}-haltigem (2,5 mM Ca^{2+}, $p < 0,01$) als auch in Ca^{2+}-freiem (1 mM EGTA, $p < 0,01$) Puffermedium nachweisbar, was eine Ca^{2+}-Freisetzung aus intrazellulären Ca^{2+}-Speichern implizierte, da es in diesem Fall nachweislich nicht zu einem Ca^{2+}-Einstrom aus dem Extrazellularraum kommen konnte. Ausgehend von diesen Daten generierten wir eine Dosis-Wirkungskurve für die Caspofungin-induzierte intrazelluläre Ca^{2+}-Freisetzung. Diese zeigte eine effektive Wirkdosis (ED_{50}) von 148,1 µg/ml in Ca^{2+}-haltigen Puffermedium und eine ED_{50} von 116,9 µg/ml in Ca^{2+}-freien Puffermedium.

Um den genauen Ursprung der Caspofungin-induzierten intrazellulären Ca^{2+}-Freisetzung zu identifizieren, wurden weiterführend bekannte intrazelluläre Ca^{2+} Speicher des Endoplasmatischen Retikulums (ER) vor Caspofungingabe

pharmakologisch entleert, oder eine Ca^{2+}-Freisetzung aus diesen Speichern pharmakologisch inhibiert. Dabei wurden durch die vorherige Applikation von Koffein (CAF, 30 mM) intrazelluläre, Koffein-sensitive ER Ca^{2+}-Speicher entleert. In einer anderen Versuchsanordnung wurde durch vorherige Applikation von Ryanodin (RYN, 40 µM) die Ca^{2+}-Freisetzung aus intrazellulären ER Ca^{2+}-Speichern inhibiert. Hier zeigte sich für CAF eine signifikante Reduktion der Caspofungin-induzierten Ca^{2+}-Freisetzung (CAF+ versus CAF-; FC 1,04; $p < 0{,}001$).

Die Applikation von RYN zur Inhibition des Ryanodin-Rezeptors am ER führte ebenfalls zu einer signifikanten Reduktion der Caspofungin-induzierten Erhöhung der $[Ca^{2+}]_i$ und somit einer verminderten Freisetzung aus dem ER (RYN+ versus RYN-; FC 1,15; $p < 0{,}01$). Die Ergebnisse belegen in der Zusammenschau, dass Caspofungin in isolierten HCM eine Erhöhung der $[Ca^{2+}]_i$ hauptsächlich durch Freisetzung von Ca^{2+}-Ionen aus dem ER induziert.

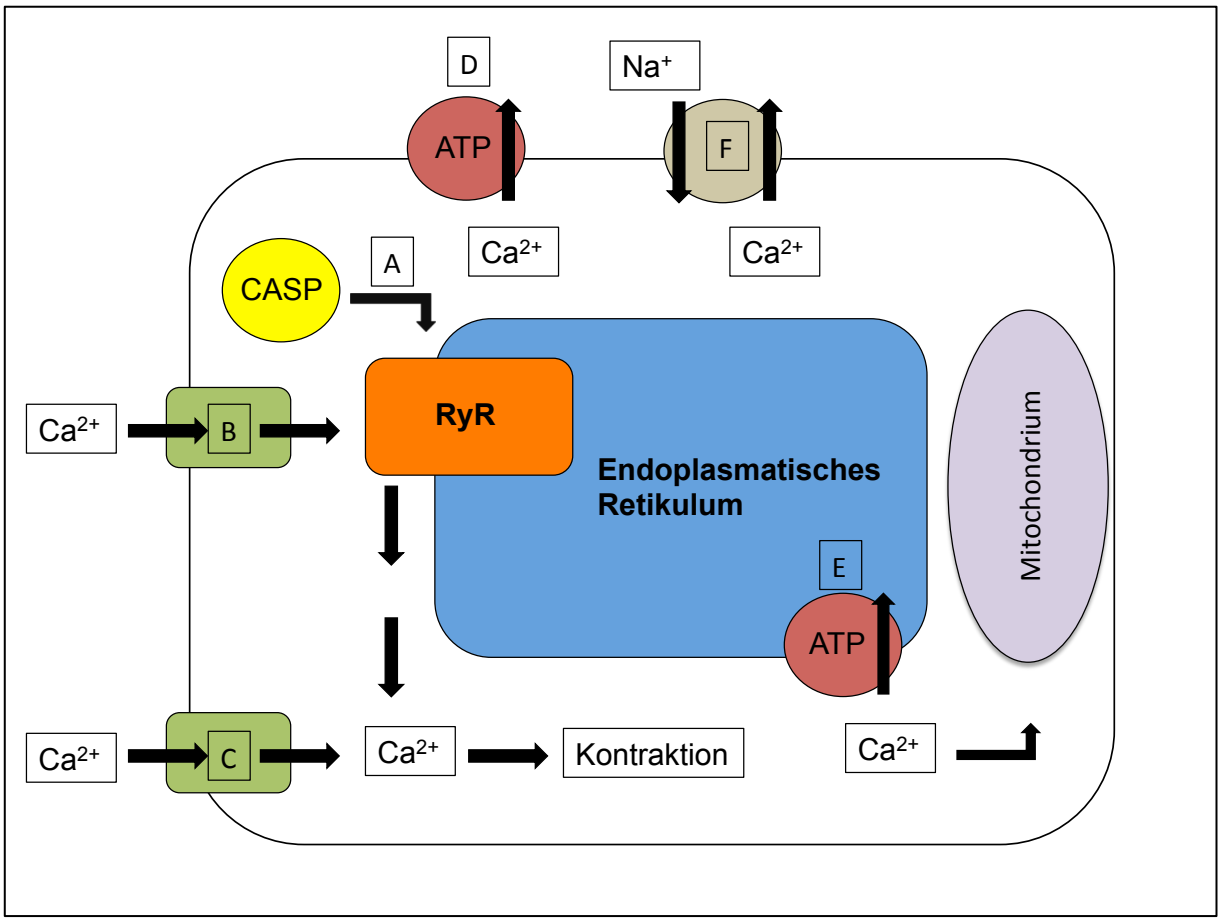

Abbildung 2: Schematische Darstellung des Mechanismus der Caspofungin-induzierten intrazellulären Kalziumfreisetzung; CASP: Caspofungin, RyR: Ryanodinrezeptor, ATP: Adenosintriphosphat, A: Caspofungin induzierte Aktivierung des Ryanodinrezeptors, B/C: Ca^{2+}-Einstrom durch L-Typ Kalziumkanal, D: ATP abhängiger Ca^{2+}-Export, E: ATP abhängige Ca^{2+}-Aufnahme in das ER, F: $Na^{+}/Ca2^{+}$-Austauscher

Caspofungin-Applikation bewirkt in HCM eine dosisabhängige Erhöhung der $[Ca^{2+}]_i$. Die Ca^{2+}-Freisetzung erfolgt dabei aus intrazellulären, Coffein-sensitiven Speichern des ER, sehr wahrscheinlich durch Aktivierung des Ryanodin-Rezeptors.

3.6 Hämodynamische Veränderungen bei chirurgischen Intensivpatienten unter Therapie mit Echinocandinen (Anlage 7)

Nach Identifikation des in Anlage 6 beschriebenen pathophysiologischen Mechanismus einer Caspofungin bedingten Ca^{2+}-Freisetzung aus dem ER, wurden in Anlage 7 die Auswirkungen dieses Effektes auf ein Kollektiv kritisch kranker Intensivpatienten untersucht.

Dazu wurde eine monozentrische, retrospektive Kohortenanalyse unter operativen Intensivpatienten durchgeführt, welche während ihres Stationsaufenthaltes eine intravenöse antimykotische Therapie erhielten (Ethikvotum AZ 117/13). Das Ziel der Untersuchung war es, dabei die hämodynamischen Parameter, CI, Herzfrequenz (HF), Blutdruck und zentraler Venendruck (ZVD), sowie die Notwendigkeit und Dosis einer kreislaufunterstützenden Therapie mit Noradrenalin, Suprarenin oder Dobutamin jeweils 2 Stunden (h) vor- und 2 h nach Therapie mit einem Antimykotikum zu vergleichen. Neben den Echinocandinen (Anidulafungin, Caspofungin und Micafungin) wurden hierbei auch Therapien mit Azolantimykotika (Fluconazol und Voriconazol) mit in die Auswertung einbezogen.

Es wurden insgesamt 342 primäre antimykotische Therapieepisoden (Anidulafungin n = 33 (9,6%); Caspofungin n = 116 (33,9%); Fluconazol n = 132 (38,6%); Micafungin n = 17 (5%), Voriconazol n = 44 (12,9%)) ausgewertet. Dabei zeigten sich keinerlei signifikante Änderungen im Hinblick auf die untersuchten isolierten hämodynamischen Parameter (CI, HF, Blutdruck, ZVD). Ebenso ergaben sich, bis auf eine geringe aber signifikante Reduktion der Noradrenalindosis in der Fluconazol-Gruppe ($p \leq 0.001$), keinerlei signifikante Unterschiede bei der Analyse der kreislaufunterstützenden Therapien 2 h vor- und 2 h nach Gabe der initialen antimykotischen Medikation. Die Auswertung des kombinierten Endpunktes (Abfall des mittleren arteriellen Blutdrucks ≥ 10 mmHg und/oder neue- oder gesteigerte kontinuierliche Dosis von Noradrenalin, Adrenalin oder Dobutamin) zeigte bei Betrachtung der einzelnen Medikamente in 50% der Therapieepisoden für Caspofungin (n/n: 58/116) und für 48,5% der Anidulafungin-Therapien (n/n: 16/33) eine hämodynamische Einschränkung. Im Gegensatz dazu zeigten sich nach Micafungin-Therapie in 23,5% (n/n: 4/17), nach Fluconazol-Therapie in 17,4% (n/n: 23/132) und nach Voriconazol-Therapie in 34,1% (n/n: 15/44) hämodynamische Einschränkungen im Sinne eines positiven kombinierten

Endpunktes. Weiterhin erfolgte ein Vergleich zwischen der Gruppe der Patienten, welche mit den Echinocandinen Anidulafungin oder Caspofungin behandelt wurden und Patienten, welche mit einem Azolantimykotikum (Fluconazol, Voriconazol) behandelt wurden.

Dabei zeigte sich in der Gruppe der Echinocandine (Anidulafungin/Caspofungin) eine signifikante Steigerung der Anzahl von Patienten mit einem Abfall des MAP ≥ 10 mmHg (n = 37 [25%] vs. n = 27 [15%], OR = 1.8, p = 0.04) sowie eine signifikant erhöhte Verwendung von Noradrenalin (n = 38 [26%] vs. n = 12 [7%], OR = 4.7, p ≤ 0.001) und Dobutamin (n = 12 [8%] vs. n = 4 [2%], OR = 3.8, p = 0.02). Ein Gruppenvergleich zwischen den Echinocandinen Anidulafungin und Caspofungin mit der Gruppe der Azolantimykotika zeigte zusätzlich für die Gruppe der Echinocandine signifikant erhöhte Werte für den kombinierten Endpunkt (n = 74 [50%] vs. n = 38 [21%], OR = 3.6, p ≤ 0.001).

Die Echinocandine Caspofungin und Anidulafungin führten in einer retrospektiven Analyse bei operativen Intensivpatienten im Vergleich zu den Azolantimykotika (Fluconazol, Voriconazol) zu einer signifikant höheren Rate an hämodynamische Beeinträchtigungen im Sinne eines MAP Abfalls ≥ 10 mmHg. Weiterhin zeigten sich in dieser Gruppe im Vergleich zu Therapien mit Azolantimykotika signifikant erhöhte Steigerungen/Neuansetzungen einer kreislaufunterstützenden Therapie mittels Noradrenalin sowie Dobutamin.

4 Diskussion

Die Sepsis stellt heutzutage die Haupttodesursache auf nicht-kardiologischen Intensivstationen dar. Trotz aller Fortschritte auf dem Gebiet der Intensivmedizin liegt die Mortalität des septischen Schocks in den Industrieländern aktuell unverändert hoch bei ca. 35%[112]. Neben bakteriellen Infektionen zeigen sich invasive Pilzinfektionen in zunehmendem Maße als ursächliche Erreger septischer Erkrankungen[12,13]. Zusätzlich zur Fokussanierung stellt die frühzeitige zielgerichtete antiinfektive Therapie das Kernelement einer erfolgreichen Therapie septischer Erkrankungen dar[19]. Savage et al. konnten zeigen, dass analog zu septischen Erkrankungen bakterieller Entität, Patienten mit einer Candidämie bei inadäquater primärer antimykotischer Therapie eine dreifach erhöhte Mortalität aufweisen[113]. Auf Grund der Fortschritte in der perioperativen Medizin sowie der sich verändernden Demographie unterliegt eine große Zahl an Patienten einem erhöhten Risiko für die Entwicklung einer invasiven Pilzinfektion. Hochrisikopatienten für invasive Pilzerkrankungen stellen dabei, neben Patienten mit prädisponierenden Grunderkrankungen (z.B. hämatologisch-/onkologische Patienten) und Patienten nach solider Organtransplantation unter Immunsuppression, insbesondere hochbetagte Patienten und Patienten nach medizinischen Maßnahmen (zentrale Venenkatheter, antibiotische Therapie, mechanische Beatmung, Hämodialyse, viszeralchirurgische Eingriffe, parenterale Ernährung) dar[8–11]. *Candida spp.* zeigen sich dabei in > 90% der Fälle als ursächliche Erreger invasiver Mykosen[4,6].

Die Echinocandine (Anidulafungin, Caspofungin und Micafungin) sind zur primären Therapie der IC bei kritisch kranken Intensivpatienten empfohlen[22,23,40]. Neben ihrer guten Wirksamkeit gegen die meisten *Candida spp.* besitzen die Echinocandine im Vergleich zu anderen Antimykotika (Azole, Polyene) ein vorteilhaftes Nebenwirkungsspektrum. Allerdings wurden in den letzten Jahren mehrere Fallberichte zu hämodynamischen Nebenwirkungen der Echinocandine bei kritisch kranken Intensivpatienten[88–90] sowie tierexperimentelle Daten zu potenziellen kardialen Nebenwirkungen veröffentlicht[65]. Das Ziel der vorliegenden Arbeit war es daher, potenzielle hämodynamische Nebenwirkungen der Echinocandine zu identifizieren, ihre Pathogenese aufzudecken und Implikation für die klinische Therapie kritisch kranker Patienten mit IC abzuleiten.

Die Identifikation des auslösenden Erregers bildet einen der wichtigsten Schritte zur erfolgreichen Therapie von septischen Erkrankungen. Somit bedingt diese auch die Wahl des passenden Antiinfektivums und dessen potenziellen Nebenwirkungen.

Aus diesem Grunde überprüften wir initial ein neuartiges diagnostisches Verfahren zur PCR-basierten Erregeridentifikation (dHPLC WAVE®-System) im Vergleich zur klassischen kulturbasierten Diagnostik. Das kulturunabhängige dHPLC WAVE®-System lieferte bei Patienten mit intraabdomineller Sepsis zusätzliche Informationen, insbesondere bei seltenen und anspruchsvollen bakteriellen Erregern. Ebenfalls zeigte es eine höhere Detektionsrate bei polymikrobiellen Infektionen und lieferte im Vergleich zu kulturbasierten Verfahren schnellere Ergebnisse. Es war dabei jedoch nicht in der Lage Pilzinfektionen zu identifizieren. In der klassischen Kultur zeigten sich jedoch in 26,2% der Isolate Pilze.

Trotz der hier gezeigten methodenimmanenten Limitationen des untersuchten Systems sind kulturunabhängige Verfahren in hohem Maße dazu in der Lage, die bestehende Diagnostik zu ergänzen und die Therapie von IC zu optimieren. BDG beispielweise kann aufgrund seines hohen negativen prädiktiven Wertes für IC bei Patienten mit niedrigem bis moderaten Risiko zum Ausschluss einer IC eingesetzt werden[15,21]. Dies könnte zukünftig dazu genutzt werden, überflüssige antimykotische Therapien einzusparen und potenzielle unerwünschte Arzneimittelwirkungen zu reduzieren. Ebenfalls zeigte die neueste Generation vollautomatischer PCR-basierter Nachweissysteme (SeptiFast, T2Candida Panel) in ersten Studien vielversprechende Ergebnisse[46–48]. Jedoch sind bisher alle PCR-basierten Systeme nur in der Lage, bereits bekannte Erreger und Resistenzgene zu erkennen. Weiterführend folgerten Kullberg et. al., dass die mangelnde Sensitivität von Blutkulturen häufig die Begründung für eine präventive und empirische antimykotische Therapie liefert, welche zum einen mit hohen Therapiekosten, zum anderen mit der Entwicklung von Arzneimittelresistenz und dem Auftreten von unerwünschten Arzneimittelwirkungen verbunden ist[15]. Auch aus diesem Grund werden präventive und empirische antimykotische Therapiestrategien derzeit kontrovers diskutiert. So konnten mehrere Studien an nicht-neutropenen ICU-Patienten keinen positiven Einfluss einer frühen empirischen antimykotischen Therapie einer IC auf die Mortalität der Patienten belegen[37–39]. Demgegenüber stehen jedoch eine größere Anzahl von Studien, welche

die positiven Auswirkungen einer prophylaktischen Antimykotikatherapie auf das Auftreten von invasiven *Candida*-Infektionen und nachfolgender Mortalität, bei Patienten mit hämatoonkologischen Malignomen unter Chemotherapie und bei Patienten nach solider Organtransplantation belegen[114,115].

Neben den diagnostischen Verfahren verglichen wir daher nachfolgend retrospektiv verschiedene Strategien zur Therapie der IC bezüglich ihrer Auswirkungen auf die Mortalität kritisch kranker Intensivpatienten. Es zeigte sich, dass in unserem Kollektiv von Patienten mit *Candida*-Peritonitis das Patientenalter, die Leukozytenzahl, der APACHE II Score und ein akutes Leberversagen unabhängige Prädiktoren für eine erhöhte 30-Tage-Sterblichkeit darstellten. Eine frühe empirische antimykotische Therapie führte in diesem Kollektiv zu keinem Überlebensvorteil. Die Daten sind dabei analog zu einer prospektiven multizentrischen Studie von Montravers et al.. Hierbei konnte bei 93 Patienten mit *Candida-P*eritonitis kein Zusammenhang zwischen der Zeit bis zum Beginn einer adäquaten antimykotischen Therapie und der Sterblichkeit der Patienten nachgewiesen werden[116]. Eine im Jahre 2017 veröffentlichte prospektive Kohortenstudie an 279 Patienten zeigte jedoch eine schlechtere Prognose für Patienten, welche an einer *Candida*-Peritonitis mit niedriger Krankheitsschwere litten und erst spät im Krankheitsverlauf eine systemische antimykotische Therapie erhielten[117]. Analog zu unserem Studienkollektiv konnte ebenso in einer prospektiven, multizentrischen Observationsstudie an 835 Intensivpatienten mit vermuteter *Candida*-Infektion kein Überlebensvorteil hinsichtlich einer frühen primären antimykotischen Therapie mit Echinocandinen gezeigt werden[118].

In der Zusammenschau der erhobenen Daten und im Vergleich mit der aktuellen Literatur kann aus diesem Grunde eine frühzeitige empirische antimykotische Therapie bei Verdacht auf das Vorliegen einer *Candida*-Peritonitis, im Gegensatz zur Therapie der bakteriellen Peritonitis, nicht empfohlen werden. Es sollten dabei zusätzlich auch die potenziell schwerwiegenden Nebenwirkungen mit in die therapeutische Entscheidung einbezogen werden. Beispielsweise kann die Therapie mit systemischen Antimykotika mit erhöhten Raten an Lebertoxizität oder hämodynamischen Einschränkungen assoziiert sein[65,78,85,90,119].

Ausgehend von diesen Ergebnissen wurden die Auswirkungen einer Echinocandin-Therapie auf die kardiale Funktion näher untersucht. Dazu wurde ein *in vitro* Modell

der Ratte zur Bestimmung der kontraktilen Funktion isolierter adulter Kardiomyozyten verwendet. Die Echinocandine Anidulafungin, Caspofungin und Micafungin zeigten hier einen dosisabhängigen Einfluss auf die Kontraktilität der Kardiomyozyten.

Es stellte sich dabei ein Anstieg der mittleren Kontraktilität für Anidulafungin-Konzentrationen von 3,3 µg/ml und 10 µg/ml dar. Caspofungin-Stimulation (10 µg/ml) bewirkte im Gegensatz dazu eine signifikante Reduktion der mittleren Kontraktilität. Micafungin-Konzentrationen von 3,3-33 µg/ml bewirkten einen signifikanten Anstieg der Zellverkürzung. Konzentration ab 33 µg/ml führten bei Anidulafungin und Caspofungin zu annähernd vollständigem Abrunden der Zellen. Gleiches konnte für eine Konzentration von 100 µg/ml für Micafungin beobachtet werden.

Die Beobachtung eines dosisabhängigen Effektes, welcher in niedrigen Konzentrationen positive Auswirkungen auf die Kontraktilität zeigte, in hohen Dosen jedoch zum Zelltod führte, bildete den Ausgang für die Hypothese, dass Echinocandine Alterationen der zellulären Kalziumhomöostase bedingen könnten. Kalziumionen übernehmen eine Schlüsselrolle während der kardialen Kontraktion. Dabei sind Störungen der Kalziumhomöostase während verschiedenster Erkrankungen und durch exogene Einflüsse beschrieben worden[120]. Nach Depolarisation der Zellmembran wird Kalzium aus dem sarkoplasmatischen Retikulum (SR) freigesetzt, welches für die Kopplung von Erregung und myokardialer Kontraktion von entscheidender Bedeutung ist. Die Mitochondrien stehen dabei als wichtigster Energielieferant für die kontraktile Funktion der Kardiomyozyten in engem Zusammenspiel mit dem SR.

Ca^{2+} ist für die optimale Funktion dieser Organellen unerlässlich. Unterschiedliche Faktoren sind dazu in der Lage die Ca^{2+}-Freisetzung aus dem SR ins Zytosol zu modulieren. So aktivieren beispielsweise zytosolisches Ca^{2+} und Koffein die sarkoplasmatische Ca^{2+}-Freisetzung. Zusätzlich sind diverse Pharmaka bekannt, welche dazu in der Lage sind mit dem RYN-Rezeptor zu interagieren. Beispielsweise ist das Inhalationsnarkotikum Halothan ein Aktivator des RYN-Rezeptors. Bei genetischer Disposition kann dies zu dem gefürchteten Krankheitsbild der malignen Hyperthermie führen[121,122]. Demgegenüber hemmen Magnesium, Ruthenium Rot, Ryanodin und Azidose den RYN-Rezeptor[123]. Bei isolierten, terminal insuffizienten Kardiomyozyten konnte beispielsweise ein verlängertes Aktionspotential und ein

verminderter Anstieg der intrazellulären Calcium-Konzentration nachgewiesen werden[124]. Somit könnten auch die in unseren Versuchen beschriebenen Steigerungen der Kontraktilität, bei niedrigen Dosierungen von Anidulafungin und Micafungin, durch eine Steigerung der intrazellulären Ca^{2+}-Konzentration bedingt sein. Demgegenüber kann eine intrazelluläre Ca^{2+}-Akkumulation jedoch ebenso die mitochondriale Funktion beeinträchtigen, was zu einer verringerten ATP-Produktion führt[125] und somit letztendlich die kontraktilen Einschränkungen bei höheren Konzentrationen von Caspofungin erklären könnte.

Daneben ergaben unsere Versuche jedoch, dass eine Vorinkubation der Echinocandine mit 10 mg/ml Albumin dazu in der Lage war, alle gemessenen Veränderungen der Kontraktilität zu inhibieren. Echinocandine sind hochgradig proteingebundene Medikamente, welche ihre Wirkung nur durch die freie Fraktion im Blut vermitteln[59]. Shirey et al. beschreiben beispielsweise Echinocandin-induzierte Affektionen der mitochondrialen Funktion, welche ebenfalls durch Zugabe von Albumin vollständig reversibel waren[126]. Kritisch kranke Patienten im septischen Schock leiden häufig an massiven Störungen des Flüssigkeitshaushaltes. Bedingt durch die Zytokinfreisetzung kommt es in der Folge zum sogenannten „capillary leak“ mit akuter Schrankenstörung des Blutgefäßbettes, mit konsekutiver interstitieller Flüssigkeitsansammlung und intravasaler Hypovolämie sowie Hypoalbuminämie[127,128]. Diese Hypoalbuminämie mit daraus bedingten erhöhten freien Echinocandinspiegeln im Plasma könnte nachfolgend eine erhöhte Gefahr für das Auftreten unerwünschter hämodynamischer Nebenwirkungen während der Therapie mit Echinocandinen bedeuten.

Um die beschriebenen *in vitro* Ergebnisse aus den Versuchen mit isolierten Kardiomyozyten in einem komplexen Organismus zu untersuchen, wurde nachfolgend ein adaptiertes Hämodynamik-Modell der Ratte eingesetzt[95,96]. Die zentralvenöse Hochdosisapplikation der Echinocandine Anidulafungin (25 mg/kg KG) oder Caspofungin (8,75 mg/kg KG) über eine Infusionsdauer von 30 min induzierte dabei im Hämodynamikmodell der Ratte ein akutes Kreislaufversagen mit signifikant reduziertem CO, ABP und LVEDV. Stover et al. konnten ebenfalls einen signifikanten Abfall des CO bei Ratten, welche eine zentralvenöse Applikationen von Caspofungin (3-6 mg/kg) oder Anidulafungin (11,5 mg/kg KG) über jeweils 10 min erhielten, mittels

einer transthorakalen Ultraschallmessung belegen[129]. Sie leiteten daraus die Theorie des „Antifungal-Associated Drug-Induced Cardiac Disease“ ab und vermuteten als pathophysiologischen Mechanismus eine direkte Interaktion der Echinocandine mit der Mitochondrienmembran und einem daraus resultierenden konsekutiven intrazellulärem ATP-Mangel[91,126]. Allerdings konnten wir in unserem Modell keine Echinocandin-assoziierten Änderungen der mitochondrialen Enzymaktivität oder der mitochondrialen Transkripte detektieren.

Die Applikation von Micafungin führte in diesem Hämodynamik Modell in keiner der verwendeten Dosierungen zu signifikanten hämodynamischen Veränderungen. Analog dazu detektierten Stover und Cleary bei Ratten nach Micafunginapplikation (1-50 mg/kg KG) mittels Messung des CO keine signifikanten Veränderungen[130]. Wie bereits in der Einleitung beschrieben, zeigen die einzelnen Echinocandine strukturelle Unterschiede in ihren lipophilen N-Acyl-Seitenketten[65,72]. Basierend auf diesen Unterschieden zeigen die jeweiligen Substanzen unterschiedliche Löslichkeiten. Anidulafungin und Caspofungin sind deutlich lipophiler im Vergleich zum eher hydrophilen Micafungin, was zu Unterschieden in der Gewebeverteilung, Albuminbindung und nachfolgend einem niedrigeren Potenzial für hämodynamische Nebenwirkungen beitragen könnte[65]. Daneben belegten Studien, dass kritisch kranke Intensivpatienten im Vergleich zu gesunden Probanden signifikant erhöhte Caspofungintalspiegel aufwiesen[131]. Des Weiteren beschrieben Stone et al. bereits für gesunde Probanden Caspofunginplasmaspiegel von bis zu 20 µg/ml[132]. Analog dazu wurden für Anidulafungin Plasmakonzentration von bis 14 µg/ml [133,134] sowie 8,8 µg/ml für Micafungin beschrieben [135].

Da es sich in der überwiegenden Zahl der Fallberichte über hämodynamische Nebenwirkungen der Echinocandine um kritisch kranke Intensivpatienten handelte, wurden nachfolgend deren hämodynamische Nebenwirkungen in einem experimentellen Endotoxinschock-Modell der Ratte untersucht. Dazu wurde das in Anlage 4 beschriebene Hämodynamikmodell[95,96] der Ratte um eine intravenöse Gabe von bakteriellem Lipopolysaccharid (LPS; 1 mg/kg KG) mit konsekutivem Endotoxinschock ergänzt. Es zeigte sich, dass die Applikation von klinisch empfohlenen Dosen von Anidulafungin (2,5 mg/kg KG) und Caspofungin (0,875 mg/kg KG) im Vergleich zur unbehandelten Kontrolle zu einem signifikant reduzierten CO und

dadurch konsekutiv zu einer signifikant verkürzten THF führten. Die Applikation von Micafungin führte auch in diesem Modell zu keinerlei hämodynamischen Veränderungen. Diese Ergebnisse untermauerten den hochgradigen Verdacht auf eine Aggravation der kardialen Nebenwirkungen im Rahmen einer vorbestehenden kardialen Funktionseinschränkung.

Der septische Schock, als maximale Ausprägung einer septischen Erkrankung ist gekennzeichnet durch eine Hypotonie und einen erhöhten Laktatserumspiegel von > 2 mmol/l trotz ausreichender Volumentherapie[136]. Unter dem Einfluss proinflammatorischer Mediatoren kommt es in der Frühphase der Sepsis zu einer peripheren Vasodilatation und dem Auftreten eines capillary leak, mit einer nachfolgenden Flüssigkeitsverschiebung aus dem Gefäßsystem ins Interstitium. Daraus resultiert eine verminderte kardiale Vorlast, welche in einer verminderten Auswurfleistung und einer meist kompensatorisch erhöhten Herzfrequenz mündet. Neben der Einschränkung der systolischen Pumpleistung ist die septische Kardiomyopathie ebenfalls durch eine Störung von Herzfrequenzregulation und Herzfrequenzvariabilität sowie der kontraktilen Funktion charakterisiert[128]. In der Pathophysiologie der septischen Kardiomyopathie bildet das Zusammenspiel aus intrazellulärer Kalziumhomöostase und SR ebenfalls eine wichtige Rolle. Yang et al. konnten 2018 zeigen, dass durch Toll-like-Rezeptor 4 (TLR4)-Aktivierung mitochondriale reaktive Sauerstoffspezies entstehen, welche nachfolgend zu einem sarkoplasmatischen Ca^{2+} Leck führten[137]. Dieser Pathomechanismus könnte eine Erklärung für die Aggravation des Effektes der Echinocandine Anidulafungin und Caspofungin in unserem Endotoxinschockmodell darstellen.

Zur weitergehenden Identifikation des ursächlichen Pathomechanismus wurden verschiedene pathophysiologische Überlegungen anhand der bisher erhobenen Studienergebnisse abgeleitet. Die Ergebnisse der Kontraktilitätsmessungen in isolierten Kardiomyozyten ergaben für niedrige Dosierungen von Anidulafungin, Caspofungin sowie für Micafungin eine gesteigerte Kontraktilität.

Die intrazelluläre Kalziumhomöostase besetzt eine Schlüsselrolle bei der kontraktilen Funktion des Herzens. Um die Auswirkungen einer Echinocandinapplikation auf die Veränderungen der $[Ca^{2+}]_i$ zu untersuchen, erfolgten fluoreszenzmikroskopische Untersuchungen in isolierten Kardiomyozyten der Ratte und HCM. Dabei zeigte sich,

dass Caspofungin eine dosisabhängige Erhöhung der $[Ca^{2+}]_i$ auslöst. Die Ca^{2+}-Freisetzung erfolgt dabei aus einem intrazellulären, Coffein-sensitiven Speicher, höchst wahrscheinlich durch Aktivierung des Ryanodin-Rezeptors. Davon abgeleitet erscheint es denkbar, dass gerade kritisch kranke Patienten mit bestehender septischer Kardiomyopathie im besonderen Maße von Dysregulationen der intrazellulären Kalziumhomöostase betroffen sein könnten.

Durch die Sepsis-induzierte mitochondriale Dysfunktion und den dadurch bedingten intrazellulären ATP-Mangel wäre die Zelle nicht in der Lage, eine ATP-abhängigen Kalziumaufnahme ins SR zu generieren. Andererseits führt eine Störung der $[Ca^{2+}]_i$-Homöostase zu einer konsekutiven Ca^{2+}-Überladung der Mitochondrien, wenn die $[Ca^{2+}]_i$ 500 nM übersteigt. In der Folge kommt es zur Öffnung eines hypersensitiven Kanals in der Mitochondrienmembran (mitochondrial permeability transition pore, mPTP), welcher zur mitochondrialen- und kontraktilen Dysfunktion beiträgt[138,139]. Zusätzlich können innerhalb der Mitochondrien Komplexe aus Ca^{2+} und Phosphat entstehen. Dieses führt weiterhin zu einem Mangel an Phosphat als Substrat der ATP-Synthese[140]. Hassoun et al. zeigten in einem LPS-induzierten Endotoxinämiemodell der Ratte eine verminderte Ca^{2+}-Aufnahme in das SR und ein erhöhtes Ca^{2+}-Leck aus dieser Organelle, welches mit einem erhöhten mitochondrialen Ca^{2+}-Gehalt assoziiert war. Die Verabreichung des RYN-Rezeptor-Antagonisten Dantrolen verhinderte in diesem Modell die mitochondriale Ca^{2+}-Überlastung, verbesserte die mitochondriale Dysfunktion sowie die kardiale Kontraktilität[141].

Die Ergebnisse implizieren, dass die mitochondriale Ca^{2+}-Überladung einen wichtigen Mechanismus in der mitochondrialen Dysfunktion des Myokards während der Sepsis darstellen könnte[141]. Abgeleitet davon würde eine Echinocandin-Applikation einen negativen synergistischen Effekt bedingen und somit über diesen Pathomechanismus eine zusätzliche Gefahr für bereits hämodynamisch kompromittierte Patienten bedeuten.

In der Folge untersuchten wir, auf der Basis der laborexperimentellen Ergebnisse, in einer retrospektiven Datenanalyse hämodynamische Veränderungen bei kritisch kranken Intensivpatienten 2 h vor- und 2 h nach antimykotischer Therapie. Bei der Auswertung dieser Ergebnisse zeigte sich, dass bis auf eine geringe aber signifikante Reduktion der Noradrenalindosis in der Fluconazol-Gruppe, keiner der isoliert

betrachteten hämodynamischen Parameter eine signifikante Änderung während des Untersuchungszeitraumes aufwies. Allerdings ergab die Analyse eines kombinierten hämodynamischen Endpunktes, bestehend aus Abfall des mittleren arteriellen Blutdrucks ≥ 10 mmHg **und/oder** neue- oder gesteigerte kontinuierliche Dosis von Noradrenalin, Adrenalin oder Dobutamin, dass Caspofungin und Anidulafungin in 50% bzw. 48% der Behandlungsepisoden zu hämodynamischen Beeinträchtigungen führten. Diese beiden Ergebnisse erscheinen in der retrospektiven Betrachtung sinnvoll, da hämodynamische Veränderungen bei kritisch kranken Patienten durch das kontinuierliche hämodynamische Monitoring engmaschig kontrolliert werden und sich daraus zumeist sofortige Therapiemaßnahmen ableiten. Dabei trifft man bei der Behandlung hämodynamischer Veränderungen im perioperativen Umfeld auf eine extreme Heterogenität in der Wahl der eingesetzten Therapien. Beispielsweise können Hypotensionen unterschiedlicher Ätiologie durch die Gabe verschiedener vasoaktiver- oder positiv inotrop wirkender Substanzen (z.B. Cafedrin-Theodrenalin, Adrenalin, Milrinon, Dobutamin, Ephedrin, Levosimendan, Noradrenalin, Vasopressin) behandelt werden. Gleichzeitig herrscht eine große Heterogenität bei der perioperativen Volumentherapie. Dabei variieren sowohl die Art (Kristalloide, Kolloide, Blutprodukte) als auch die Dosierung der unterschiedlichen Therapien[142].

Ein nachfolgender Vergleich zwischen den Echinocandinen Anidulafungin und Caspofungin mit den Azolantimykotika Fluconazol und Voriconazol ergab, dass unter Therapie mit den Echinocandinen eine signifikante Steigerung der Anzahl von Patienten mit einem Abfall des MAP ≥ 10 mmHg auftrat. Weiterhin zeigte sich in dieser Gruppe eine signifikant erhöhte Verwendung der kreislaufunterstützenden Medikamente (Noradrenalin, Dobutamin). Zusätzlich zeigten sich für die Gruppe der Echinocandine signifikant erhöhte Werte für den kombinierten Endpunkt. Aus diesen Ergebnissen folgerten wir, dass gerade hämodynamisch instabile Patienten unter einer Therapie mit den Echinocandinen Anidulafungin und Caspofungin gefährdet sein könnten.

Gleichzeitig zeigt sich jedoch die Aussagekraft retrospektiver Analysen auf Grund ihrer Datenqualität häufig eingeschränkt. So konnten Lahmer et al. in einem Kollektiv von 15 septischen internistischen Intensivpatienten mit Neutropenie oder Candidämie keine negativen hämodynamischen Veränderungen oder Steigerungen der

kontinuierlich applizierten Katecholamindosen nach Applikation von Anidulafungin oder Caspofungin zeigen. Die dabei gewählte geringe Infusionsgeschwindigkeit von jeweils 1,1 mg/min und die positive Flüssigkeitsbilanz (Tag 1: +2450 ± 460 ml) könnten die hämodynamische Funktion dabei günstig beeinflusst haben[143]. Allerdings sind auf Grund der sehr geringen Fallzahl generalisierte Aussagen nur schwierig zu treffen. Darüber hinaus sind hämodynamische Nebenwirkungen der Echinocandine im klinischen Alltag, nach Auswertungen der FDA FEARS-Datenbank, sehr seltene Ereignisse[91]. Jedoch könnte eine Echinocandin-assoziierte hämodynamische Nebenwirkung bei Intensivpatienten fälschlicherweise als Aggravation des Krankheitsbildes im Rahmen der Sepsis bewertet werden. Deshalb ist eine Limitation der vorliegenden Ergebnisse das Fehlen einer prospektiven multizentrischen Studie, welche in einer großen Kohorte an Patienten mit septischem Schock die vorliegenden Ergebnisse bestätigt. Weiterhin gilt es, in zusätzlichen experimentellen Untersuchungen den Einfluss von Anidulafungin und Micafungin auf den Ca^{2+}-Stoffwechsel zu untersuchen, sowie die Auswirkungen der intrazellulären Caspofungin-induzierten Ca^{2+}-Freisetzung auf die ATP-Synthese der Mitochondrien näher zu analysieren. Erst nachfolgend wäre man in der Lage, Therapieschemata zu entwickeln, welche hämodynamische Nebenwirkungen bei der antimykotischen Therapie kritisch kranker Patienten sicher verhindern könnten. Die Therapie der IC sollte daher weiterhin in Übereinstimmung mit den aktuellen Empfehlungen der IDSA und ESICM/ESCMID erfolgen[21–23]. Zusätzlich sollte dabei jedoch in besonderem Maße auf die Einhaltung der empfohlenen Infusionsdauer geachtet werden, um potenziell toxische Spitzenspiegel gerade bei der Therapie kritisch kranker Patienten zu vermeiden. Weiterhin sollten moderne Diagnoseverfahren eingesetzt werden, um Therapien nur bei bestätigter IC oder hochgradigem Verdacht auf deren Vorliegen einsetzen zu müssen. Des Weiteren sollte die antimykotische Therapie kritisch kranker Patienten nur unter kontinuierlicher hämodynamischer Überwachung erfolgen, um potenzielle hämodynamische Veränderungen frühzeitig zu detektieren und nachfolgend erfolgreich therapieren zu können.

5 Zusammenfassung

Invasive *Candida*-Infektionen gefährden zunehmend Patienten im perioperativen Umfeld. Neben bakteriellen Infektionen zeigen sich invasive Pilzinfektionen in zunehmendem Maße als ursächlich für die Entstehung septischer Erkrankungen. Die chirurgische Sanierung des auslösenden Infektionsfokus sowie die frühzeitige zielgerichtete antiinfektive Therapie stellen dabei die Kernelemente einer erfolgreichen Behandlung septischer Erkrankungen dar.

Durch die Fortschritte der perioperativen Medizin und eine veränderte Demographie zeigt eine große Zahl der perioperativ behandelten Patienten Risikofaktoren für die Entwicklung invasiver Pilzinfektionen. Echinocandine (Anidulafungin, Caspofungin und Micafungin) werden zur primären Therapie von invasiven *Candida*-Infektionen bei kritisch kranken intensivmedizinisch betreuten Patienten empfohlen. In den letzten Jahren wurden mehrere Fallberichte zu hämodynamischen Nebenwirkungen der Echinocandine bei Intensivpatienten sowie tierexperimentelle Daten zu kardialen Nebenwirkungen veröffentlicht.

Das Ziel der vorliegenden Arbeit war es daher potenzielle hämodynamische Nebenwirkungen der Echinocandine zu identifizieren, ihre Pathogenese aufzuklären und mögliche Implikation für die klinische Therapie kritisch kranker Patienten mit invasiven *Candida*-Infektionen abzuleiten.

Die durchgeführten klinischen Studien zeigten, dass bei Patienten mit intraabdomineller Sepsis in der klassischen kulturbasierten Diagnostik in 26,2% der Fälle Pilzisolate nachweisbar waren. PCR-basierte Nachweisverfahren könnten zukünftig in der Lage sein, die Diagnostik sinnvoll zu ergänzen. Weitergehend stellten sich bei Patienten mit *Candida*-Peritonitis das Alter, die Leukozytenzahl, der APACHE II Score und akutes Leberversagen als unabhängige Prädiktoren für eine erhöhte 30-Tage-Sterblichkeit dar. Eine frühe empirische antimykotische Therapie führte in diesem Kollektiv zu keinem Überlebensvorteil. Gründe dafür könnten unter anderem auch unerwünschte Nebenwirkungen der eingesetzten Antimykotika (z.B. Hepatotoxizität oder hämodynamische Einschränkungen) darstellen.

In einem *in vitro* Modell isolierter Kardiomyozyten der Ratte konnten wir einen dosisabhängigen Einfluss der Echinocandine Anidulafungin, Caspofungin und

Micafungin auf die Kontraktilität der Zellen nachweisen. Nachfolgend zeigte sich, dass Anidulafungin und Caspofungin nach Hochdosisapplikation im Hämodynamikmodell der Ratte eine akute hämodynamische Depression mit konsekutivem Kreislaufversagen induzieren. Die Beobachtungen waren dabei nicht mit Änderungen der mitochondrialen Enzymaktivität oder der mitochondrialen Transkripte assoziiert. Die Applikation von Micafungin führte in diesem Modell zu keinerlei hämodynamischen Veränderungen.

Weiterfolgend zeigten sich nach Applikation von Anidulafungin und Caspofungin in klinisch empfohlenen Dosen in einem Endotoxinschock-Modell der Ratte im Vergleich zur Kontrolle ebenfalls eine signifikante Reduktion der kardialen Auswurfleistung sowie eine signifikant verkürzte Zeit bis zum Herz-Kreislaufversagen.

In den weiterführenden Untersuchungen zu den pathophysiologischen Ursachen der beschriebenen hämodynamischen Nebenwirkungen konnten wir zeigen, dass Caspofungin in humanen Kardiomyozyten eine dosisabhängige Erhöhung des intrazellulären Kalziumspiegels bewirkt. Die Ca^{2+}-Freisetzung erfolgt dabei aus intrazellulären, Koffein-sensitiven Speichern sehr wahrscheinlich durch Aktivierung des Ryanodin-Rezeptors.

Nachfolgend zeigte eine retrospektive Analyse operativer Intensivpatienten unter primärer antimykotischer Therapie, dass Therapien mit Caspofungin und Anidulafungin im Vergleich zu Therapien mit Azolantimykotika signifikant häufiger zu hämodynamischen Beeinträchtigungen führten.

Zusammenfassend sollte, abgeleitet aus unseren Ergebnissen, während der Therapie mit Echinocandin-Antimykotika bei kritisch kranken Intensivpatienten ein wichtiger Fokus auf deren hämodynamische Nebenwirkungen gelegt werden. In besonderem Maße sollte dabei die empfohlene Infusionsdauer der einzelnen Medikamente beachtet werden, um potenziell toxische Spitzenspiegel, gerade bei der Therapie kritisch kranker Patienten, zu vermeiden.

Weiterhin sollten moderne Diagnoseverfahren eingesetzt werden, um Echinocandine nur bei bestätigter IC oder bei hochgradigem Verdacht auf deren Vorliegen zu verabreichen. Zusätzlich sollte die antimykotische Therapie kritisch kranker Patienten nur unter kontinuierlicher hämodynamischer Überwachung erfolgen, um potenzielle

hämodynamische Veränderungen frühzeitig zu detektieren und nachfolgend erfolgreich therapieren zu können.

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8 Anlagen

8.1 Anlage 1

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Microbiomic Analysis of Intra-Abdominal Infections by Using Denaturing High-Performance Liquid Chromatography: A Prospective Observational Study

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Abstract

Background: Intra-abdominal infections represent a subgroup of septic syndromes with high death rates and the need for prompt and appropriate antimicrobial therapy. Conventional culture-based microbial identification has notable shortcomings in the diagnostics of polymicrobial infections. Modern culture-independent molecular methods may represent a new diagnostic approach. The current study aimed to compare the results obtained from the denaturing high-performance liquid chromatography WAVE® system as a culture-independent diagnostic tool with those obtained from standard culture-based microbiologic testing in the clinical setting of severe intra-abdominal sepsis.
Patients and Methods: The study included 42 samples of pathologic intra-abdominal fluids, collected from 37 patients with intra-abdominal sepsis. Micro-organisms grown in culture and detected by the WAVE system were compared. Further, we recorded clinical data including baseline characteristics and the use of antibiotic agents.
Results: In 38.1% of the analyzed samples, the classic, culture-based methods showed no bacterial growth on agar plates, in comparison with the microbiomic analysis in which the proportion of samples with negative signal was 31%. In about 40% of the patients, both methods detected one microbiologic agent, whereas in approximately one quarter of the samples, two or more agents were identified. The detection rate of certain bacteria such as Enterobacteriacae or *Enterococcus faecium* was significantly higher using the microbiomic analysis. Bacteria such as *Haemophilus*, *Lactobacillus*, *Clostridium*, *Methylobacterium*, *Collinsella aerofaciens,* and *Solobacterium moorei* were detected exclusively using microbiomic analysis.
Conclusion: The culture independent molecular WAVE system provided additional information, especially concerning unusual, fastidious bacteria in patients with intra-abdominal infections. Further, it has a higher detection rate for polymicrobial infection and delivers results much sooner than conventional microbiologic methods.

Keywords: abdominal infection; antimicrobial management; diagnosis; intra-abdominal infection; sepsis

Early and appropriate anti-infective therapy is crucial for the outcome of patients with sepsis and peritonitis [1]. For de-escalating an initial broad-spectrum antibiotic agent, the microbiologic identification of the causative isolate is of critical importance. Conventional culture-based microbial identification has notable shortcomings in the diagnostics of polymicrobial infections. Besides poor sensitivity, especially in the presence of previous antimicrobial therapy and because of the presence of obligate anaerobes, incubation periods of 72 hours are needed to

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obtain reliable results. Further, rapid results are needed to keep up with the clinical dynamics of patients with sepsis. To overcome the impediment imposed by the time factor—namely, the prolonged incubation time implied by a culture-dependent diagnostic pathway—considerable progress has been made in the development of culture-independent techniques. Polymerase chain reaction followed by denaturing high-performance liquid chromatography (dHPLC) represents a fast approach to profile microbial diversity in a specific sample [2–4].

Initially developed from the premises that large groups of micro-organisms in many environments cannot be cultured, the technique focuses on detecting 16S ribosomal ribonucleic acid (rRNA) encoding genomic sequences, which are conserved and specific within bacterial species. Early studies provided data that in natural environments only 1% of bacterial species are cultivable [1]. Applied to human microbial communities, microbiomic sequencing has so far provided valuable results in phylogenetic studies [5,6]. As a diagnostic tool, dHPLC has proven to reveal microbial diversity that often eludes conventional diagnostic methods [7]. Results can be obtained in approximately 6–24 hours, whereas traditional culture-based diagnosis often takes at least 48–72 hours to provide a result [2,3,8–10]. Therefore, this temporal advantage is of high clinical relevance when dealing with patients with sepsis by allowing an early adjustment of the antibiotic treatment [11].

Adequate antimicrobial therapy leads to significant reduction in deaths and is an essential part of sepsis management [12–15]. A delay in initiating adequate anti-infective treatment in patients with sepsis leads to an increase in death of up to 30% [16,17]. In cases of sepsis-induced hypotension, the survival rate of patients drops about 5% to 10% within every hour of delayed administration of an adequate anti-infective treatment [17]. Therefore, current international guidelines recommend that empiric intravenous antibiotic agents should be administered within the first hour after the clinical diagnosis of severe sepsis and septic shock, although studies revealed that this goal is difficult to achieve in everyday medicine [18,19].

When choosing an empiric antibiotic scheme, the site of infection as well as individual risk factors of the patient with sepsis are important variables. In any case, the initial anti-infective therapy will imply a broad-spectrum antibiotic or in the case of life-threatening infections, a combination of broad-spectrum antibiotic agents [20,21].

Another aspect to be considered is obtaining appropriate microbiologic samples before the first antibiotic dose is administered, because this could lead to rapid sterilization of the specimens and therefore limit conventional microbiologic diagnostic [18]. In case systemic spread of the causative organism is suspected, at least two sets of blood cultures have to be attained [18,19]. Once the source of infection has been localized by radiographic imaging, obtaining samples from these sites should also be sought (e.g., abscesses, fluid collections, ascites). Nevertheless, obtaining microbiologic samples should not delay the initiation of antibiotic therapy [18].

When the source of sepsis is situated in the abdominal cavity, microbial identification is based mostly on the specimen collected from the infection site during initial operation for source control [22,23]. According to current international guidelines for the diagnosis and management of complicated intra-abdominal infections, blood cultures do not provide additional clinically relevant information, except in determining the duration of antibiotic therapy [18]. Therefore, microbiologic evaluation should rely on cultures from the site of infection.

Further microbiologic diagnostic evaluation can be performed on drainage fluids or on samples collected during revision surgical procedures. This allows the detection of changes in the bacterial spectrum or bacterial antibiotic resistance with consequent adjustment of antibiotic therapy. Evaluating the relevance of microbial detection from these intra-abdominal fluid samples and determining whether it reflects a bacterial contamination, colonization, or an actual infection is again decisive for the conduct of adequate anti-infective therapy.

Patients with intra-abdominal sepsis usually present with peritonitis, which implies almost exclusively a polymicrobial infection, often involving a fungal component, usually *Candida spp.* [21,24]. The detection of *Candida* in a primarily sterile part of the organism is defined as a fungal infection, despite lacking proof of invasive fungal growth.

Certain bacteria that are normal constituents of the intestinal microbiota, such as Enterobacteriaceae and *Enterococcus*, are known to cause intra-abdominal infections and are usually identified using culture-based methods. In contrast, anaerobic bacteria, such as *Bacteroides* spp., *Clostridium,* or *Fusobacterium,* die very quickly after collection, and the isolation requires specific environmental growth conditions on standard culture plates. These particular bacterial species are, however, relevant in polymicrobial peritonitis and in the secondary formation of intra-abdominal abscess [21].

Exactly in these areas of constraint to conventional microbiologic diagnostics (identification of polymicrobial infections and difficult to culture pathogens as well as providing rapid diagnostic results), the application of new molecular techniques has proven useful, with an overall higher sensitivity level in microbial identification [11]. Nevertheless, these methods have so far only been evaluated in small studies. Thus, their clinical value is yet to be determined. The current study aimed to compare the results obtained from dHPLC using the WAVE® system (Transgenomic, Omaha, NE) as a culture-independent diagnostic tool with those obtained from standard culture-based microbiologic testing in the clinical setting of severe intra-abdominal sepsis.

Patients and Methods

This observational clinical study was performed in the surgical intensive care unit (ICU) of the University Hospital, Giessen, Germany, after approval by the local ethics committee (Ethics Committee of the Medical Faculty of the Justus-Liebig-University Giessen: Trial code 111/09). Informed consent was obtained from all patients or legal representative. Within two years, 37 patients with severe sepsis or septic shock triggered by an intra-abdominal infection were enrolled in this study. In addition, each patient was treated by abdominal operation or radiologic drainage placement. Severe sepsis or septic shock was defined according to the guidelines of the Surviving Sepsis Campaign [18]. Exclusion criteria were defined as age <18 or >80 years or pregnancy.

Fluid samples of ascites or purulent intra-abdominal material were obtained exclusively during surgical or radiologic therapeutic intervention.

Baseline parameters including patients' gender, age, height, weight, underlying clinical conditions, laboratory values, and intra-abdominal findings (e.g. Mannheim peritonitis score) were recorded. The severity of illness within the first 24 hours after admittance to the intensive care unit was calculated using the Acute Physiology and Chronic Health Evaluation II (APACHE II) score and the rate of organ failure using the Sequential Organ Failure Assessment (SOFA) scoring system. We also included antibiotic therapy, length of hospital stay, length of ICU stay, and hospital deaths.

Patients' treatment was performed according to internal guidelines and the actual guidelines of the Surviving Sepsis Campaign [18]. Adjustments to the antibiotic therapy were based on the results of the antibiotic sensitivity testing of the conventional microbiologic diagnostic.

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Culture-based diagnostic methods

Culture-based diagnostic was performed at the Institute of Medical Microbiology, Justus-Liebig-University, Giessen, Germany. Collected specimens were streaked on MacConkey, blood, chocolate, Schaedler, and Sabouraud agar plates. The agar plates were incubated at 37°C and 5% CO_2 for two days except for Schaedler and Sabouraud agar plates, which were incubated for two weeks. Bacteria grown on agar plates were identified by using Maldi-TOF MS (matrix-assisted laser desorption ionization time of flight mass spectrometry) as described [25]. Results were obtained from the local patient data management system.

Microbiomic analysis

The bacterial deoxyribonucleic acid (DNA) from the collected intra-abdominal specimens was isolated with an alkaline extraction method and with the use of Qiagen-Kits (QIAGEN, Hilden, Germany). A broad-range polymerase chain reaction (PCR) was performed from bacterial 16S rDNA using the forward primer 0933F and the reverse primer 1407R as follows: 5 mcL of template containing 50–100 ng of extracted chromosomal DNA and 95 mcL of a master mix containing 20 pmol of the forward primer 0933F and the reverse primer 1407R, 30 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mcg bovine serum albumin, 1.5 mM MgCl2, 0.4 mM deoxynucleoside triphosphates, and 1.5 units of AmpliTaq DNA polymerase were mixed for the partial amplification of the 16S rRNA and downstream WAVE system analysis.

The PCR products were analyzed using HPLC gradients for the separation of mixed amplicons with the WAVE 3500 DNA fragment analysis system (Transgenomic). DNA sequences of the 16S rRNA were aligned with the Clustal method from MegAlign (DNAStar Inc., Madison, WI). The obtained nucleic acid sequences were analyzed with the algorithm Blastn at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/) and the GOLD genomes online database (wit.integratedgenomics.com / GOLD/) from Integrated Genomics.

Statistical data analysis

The collected data were recorded and processed using SPSS Statistics (Version 16-19, SPSS Inc., IBM), and SAS (Version 9.1, SAS Institute Inc.). Data were analyzed using mean and standard deviation or values and percentages, if appropriate.

Results

Study population

In total, the study included 42 samples of pathologic intra-abdominal fluids, collected from 37 patients. Demographic characteristics of the patients included in the study, as well as markers depicting acute infection are shown in Table 1. The calculated median age for our patient group was 60.4 ± 15.5 years. The study included 23 (62.2%) male subjects. The patients with sepsis with intra-abdominal infections had a calculated mortality rate of 40% equivalent to a median APACHE II score of 21.6 ± 6.2. This was also reflected by significant organ dysfunction, depicted by a median SOFA score of 6.24 ± 1.54. The severity of peritonitis calculated using the Mannheim score was of 24.57 ± 8.87. The mortality rate in the studied cohort was 27%.

Administered antibiotic therapy

At the time the samples were collected, 81% of the patients had already received an anti-infective therapy. When considering the performed adjustments to the antibiotic regimen before sampling, this fraction rises to 95.2%. Almost one third of these patients received a combined antibiotic treatment, whereas 14.3% also had an antifungal agent included in the therapeutic scheme. According to the results of the microbiologic investigations, the empiric antibiotic therapy proved to be inadequate in 12, whereas the adjusted antibiotic

Table 1. Patient Characteristics

Characteristic		*Patients (N = 37)*
Gender: Male		23 (62.2)
Age	Years	60.4 ± 15.5
Height	m	171 ± 9.9
Weight	kg	75.9 ± 20.8
Underlying conditions		
Diabetes mellitus		4 (10.8)
Heart failure		2 (5.4)
Arterial hypertension		21 (56.8)
Coronary artery disease		8 (21.6)
COPD		13 (35.1)
Solid organ transplantation		1 (2.7)
Compensated chronic renal failure		8 (21.6)
Terminal renal failure		4 (10.8)
Acute renal failure		2 (5.4)
Liver cirrhosis		1 (2.7)
Cancer		12 (32.4)
Laboratory values		
White blood cells	1/nL	17.57 ± 10.15
CRP	mg/dL	178.57 ± 106.67
Abdominal surgery before microbiologic sampling		19 (51.4)
Nosocomial peritonitis		28 (75.7)
Mannheim peritonitis score		24.57 ± 8.87
Hospital stay		
Before microbiologic sampling	days	12.5 ± 12.8
ICU	days	14.3 ± 16.1
In total	days	45.2 ± 43.3
Deaths		10 (27.0)

COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; ICU = intensive care unit.
Values shown as numbers (%) or mean ± standard deviation.

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Table 2. Antibiotic Therapy before and after Sampling

	N = *42*
Antibiotic therapy at the time of sampling	34 (81.0)
Antibiotic therapy	
Combined antibiotic therapy	12 (28.6)
Broad-spectrum penicillin	11 (26.2)
Carbapenem	17 (40.5)
Cephalosporin	2 (4.8)
Chinolone	3 (7.1)
Metronidazole	2 (4.8)
Linezolid	3 (7.1)
Vancomycin	6 (14.3)
Tigecycline	2 (4.8)
Clarithromycin	1 (2.4)
Aminoglycoside	1 (2.4)
Antifungal therapy	
Echinocandin	4 (9.5)
Azol	2 (4.8)
Not adequate antibiotic therapy	12 (28.6)
Not adequate antifungal therapy	8 (19.0)
Change in antibiotic therapy at the time of sampling	
None	16 (38.1)
Escalation	22 (52.4)
De-escalation	4 (9.5)
Change in antifungal therapy at the time of sampling	
None	32 (76.2)
Escalation	9 (21.4)
De-escalation	1 (2.4)
Antibiotic therapy after sampling	40 (95.2)
Antibiotic therapy	
Combined antibiotic therapy	13 (30.9)
Broad-spectrum penicillin	9 (21.4)
Carbapenem	29 (69.0)
Cephalosporin	2 (4.8)
Chinolone	2 (4.8)
Metronidazole	1 (2.4)
Linezolid	5 (11.9)
Vancomycin	7 (16.7)
Tigecyclin	3 (7.1)
Clarithromycin	1 (2.4)
Aminoglycoside	0
Antifungal therapy	
Echinocandin	9 (21.4)
Azole	4 (9.5)
Not adequate antibiotic therapy	3 (7.1)
Not adequate antifungal therapy	4 (9.5)

Values shown as numbers (%)

therapy was improper in three of the investigated cases. The empiric and adjusted antifungal therapy failed to cover the necessary spectrum in 8, respectively 4 of the investigated cases. Before time of sampling 8 cases failed to cover the necessary spectrum. After sampling 4 cases failed to cover the necessary spectrum. The anti-infective agents administered before and after microbiologic sampling are depicted in Table 2.

Microbiologic evaluation

In 38.1% of the analyzed samples, the classic, culture-based methods showed no bacterial growth on agar plates, in comparison with the microbiomic analysis in which the proportion of samples with a negative PCR-signal was 31% (Fig. 1). In approximately 40% of the patients, both methods detected one microbiologic agent, whereas in approximately one quarter of the samples, two or more agents were identified. The detection rate of certain bacterial agents such as Enterobacteriacae or *E. faecium* was significantly higher using the microbiomic analysis. Species such as *Haemophilus*, *Lactobacillus*, *Clostridium*, *Methylobacterium*, *Collinsella aerofaciens,* and *Solobacterium moorei* were exclusively detected using microbiomic analysis.

The compared methods provided coinciding results in 26.2%, and similar results in 16.7% of the analyzed samples, whereas in 7.1% of the cases, the methods generated completely different results (Table 3).

Discussion

Sepsis is the most common cause of death among patients in the surgical ICUs. In particular, intra-abdominal infections represent a subgroup of septic syndromes with high number of deaths and the need for prompt and appropriate antimicrobial therapy. Therefore, fast and reliable microbiologic diagnostic evaluation is essential to reduce sepsis-associated deaths [17]. The current study aimed to determine the diagnostic value of dHPLC using the WAVE system as a molecular microbiologic diagnostic method in patients with sepsis with an intra-abdominal infection source. To our knowledge, this study included the largest cohort of patients with intra-abdominal infection that evaluates PCR/electrospray ionization mass spectrometry (ESI-MS) as a molecular microbiologic diagnostic method analyzing pathologic intra-abdominal fluids.

Following this approach, we examined 42 samples of pathologic intra-abdominal fluids, collected from 37 critically ill patients using both conventional culture-dependent

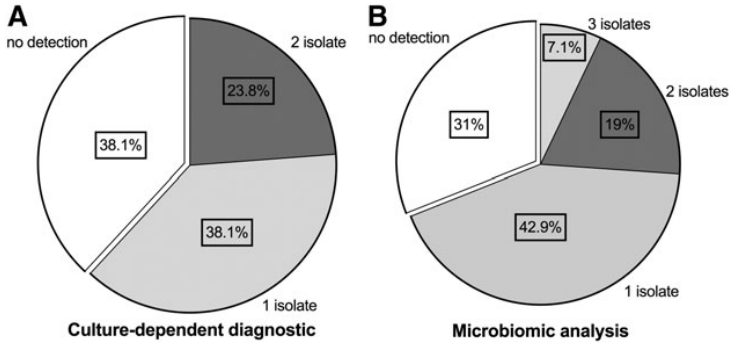

FIG. 1. Comparison of microbiologic isolates in culture-dependent diagnostic (**A**) and microbiomic analysis (**B**).

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Table 3. Frequency of Detection of Micro-Organisms in 42 Intra-Abdominal Fluid Samples

	Culture-dependent diagnostic (n = *42*)	*Microbiomic analysis* (n = *42*)
No microbiologic detection	16 (38.1)	13 (31.0)
1 microbiologic isolate	16 (38.1)	18 (42.9)
2 microbiologic isolates	10 (23.8)	8 (19.0)
3 microbiologic isolates	0	3 (7.1)
No bacterial detection	19 (45.2)	13 (31.0)
1 bacterial isolate	21 (50.0)	18 (42.9)
2 bacterial isolates	2 (4.8)	8 (19.0)
3 bacterial isolates	0	3 (7.1)
Enterobacteriaceae	6 (23.1)[a]	8 (27.6)[c]
Escherichia coli	4 (15.4)[a]	2 (6.9)[c]
Klebsiella pneumoniae	1 (3.8)[a]	0
Proteus vulgaris	2 (7.7)[a]	1 (3.4)[c]
Not specified	0	5 (17.2)[c]
ESBL	1 (3.8)[a]	-
Enterococcus faecium	9 (34.6)[a]	11 (37.9)[c]
Vancomycin-resistant enterococci	5 (19.2)[a]	-
Other gram-positive cocci	4 (15.4)[a]	9 (31.0)[c]
Coagulase-negative staphylococci	2 (7.7)[a]	4 (13.8)[c]
Streptococcus spp.	2 (7.7)[a]	5 (17.2)[c]
Bacteroides spp.	2 (7.7)[a]	1 (3.4)[c]
Non-fermenter	3 (11.5)[a]	3 (10.3)[c]
Other bacterial species	0	12 (41.4)[c]
Haemophilus spp.	0	3 (10.3)[c]
Lactobacillus	0	4 (13.8)[c]
Clostridium spp.	0	2 (6.9)[c]
Methylobacterium spp.	0	1 (3.4)[c]
Collinsella aerofaciens	0	1 (3.4)[c]
Solobacterium moorei	0	1 (3.4)[c]
Multi-resistent bacterial strains	6 (23.1)[a]	-
No fungal detection	31 (73.8)	-
1 fungal isolate	11 (26.2)	-
Candida albicans	6 (54.5)[b]	-
C. glabrata	3 (27.3)[b]	-
C. parapsilosis	1 (9.1)[b]	-
Not specified *Candida spp.*	1 (9.1)[b]	-

ESBL = extended spectrum beta-lactamases.

Percentage of numbers of positive bacterial ([a]): n = 26 or fungal ([b]): n = 11 detections using culture-dependent diagnostic evaluation. Percentage of numbers of positive bacterial ([c]): n = 29 detections using microbiomic analysis. Values shown as numbers (%).

methods and 16S rDNA PCR. The PCR-based method identified one bacterial species in 29 and two species in 11 of the investigated samples, whereas by cultivation on agar plates, the detection rate was slightly lower, with one identified bacterial species in 26 and two bacterial species in 10 of the examined samples. While culture-based methods provided a maximum of two bacterial isolates per sample, three bacterial species per sample were identified in three cases by using the WAVE system. In about one fourth of the samples, both methods showed completely coinciding results and similar results in 16.7% of the cases, while in three of the 42 examined samples, the results provided by the two methods were completely different. The clinical relevance of these little differences in our cohort remains unclear.

Because intra-abdominal infections are most often polymicrobial, it is to be expected for more than one isolate to be identified by microbiologic diagnostic evaluation [15,18]. Consistent with recent literature, fewer than half of the examined samples in the present study were positive for more than one bacterial species. This, however, is consistent with everyday clinical practice in which negative microbiologic results are not unlikely, especially under the circumstance of applied anti-infective therapy before microbiologic sampling [12]. The time to initiation of antibiotic treatment might also explain the higher microbial identification rate of the PCR-based method that, as opposed to conventional culture, can detect even residual DNA from already dead or lysed bacteria. In our study, 21.4% of all detected bacteria were exclusively detected by the WAVE system. In contrast, only 5% of all microbial detections were obtained exclusively by established culture-based diagnostic evaluation.

The lower polymicrobial detection rate of bacterial culture might also partially be because of fastidious, slow-growing bacteria that can elude or delay conventional diagnostic evaluation by requiring special environmental conditions or growing media. This proposition has already been proven valid in studies using metagenomic analysis in examining genitourinary tract infections [5,19]. In one of these studies in which the WAVE system had been applied, more than 10 bacterial species that showed no growth on agar plates were detected via the PCR-based method. Most of these species were anaerobes; others required long incubation times or selective media for cultivation, but nonetheless possessed clinical relevance as known pathogens to cause urinary tract infections [5].

In a similar manner, our study, while examining samples of patients with intra-abdominal infection, turned up six bacterial species as well as certain strains of Enterobacteriaceae solely by using the PCR-based method. These bacterial species (*Haemophilus spp., Lactobacillus, Clostridium spp., Methylobacterium spp, C. aerofaciens, S. moorei*) have only seldom been identified as causative micro-organisms for intra-abdominal infections. In detail, *Haemophilus* has been detected in our study in samples belonging to three different patients, but in the literature has been reported only in very few cases as a microbiologic isolate in patients with peritonitis, mostly related to severe cases of cholangitis or intra-abdominal infections in patients undergoing peritoneal dialysis [20–23]. On the other hand, it is well known that *Haemophilus* colonizes the gastrointestinal tract of children and adults [24,25]. Thus the discrepancy in the turnover rate of this bacterial species between our study and previous literature reports might suggest a lower sensitivity of culture-based methods.

In contrast, in four of our investigated samples, the WAVE system microbiomic analysis even detected *Lactobacillus,* which is known as a normal constituent of the intestinal flora. The relevance of isolating this bacterial strain from the peritoneal cavity thus can be disputed, because it might reflect just colonization after bowel perforation. On the other hand, a growing number of publications have reported infections varying from bacteremia to endocarditis and intra-abdominal abscess, where the main culprit has been identified

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as *Lactobacillus spp.* [26–28]. Therefore, a significant *Lactobacillus* infection also cannot be excluded.

When regarding the major constituents of the bacterial spectrum in our study, differences to key pathogens involved in bacterial peritonitis as reported by literature reviews also occur. The microbial strains that are most often known to be involved in community acquired intra-abdominal infections are coliforms (Enterobacteriaceae, especially *Escherichia coli*) and anaerobes (especially *B. fragilis*). Health-care–associated or nosocomial peritonitis, on the other hand, involves more resistant microbial species such as the nonfermenting gram-negative *Pseudomonas aeruginosa* and *Acinetobacter* species, extended spectrum β-lactamase–producing *Klebsiella* and *E. coli*, *Enterobacter* species, *Proteus spp.,* methicillin-resistant *Staphylococcus aureus*, enterococci, and *Candida spp.* [5,29,30].

In our study, regardless of the methods used, either culture or PCR-based, the most often detected pathogens were enteroccocci (34.6% in culture and 37.9% using microbiomic analysis), Enterobacteriaceae (23.1% in culture and 27.6% using metagenomic analysis), and *Bacteroides* (7.7% in culture and 3.4% using metagenomic analysis). The observed tendency is toward more resistant bacterial specimens, with a relatively low rate of detected Enterobacteriaceae in comparison with a high detection rate for *E. faecium*, which reflects clearly the high percentage of patients with nosocomial peritonitis included in the study (75.7%) as well as the high rate of antibiotic therapy applied before microbiologic sampling (81%). Our findings are in line with the results from several studies that evaluated different PCR/ESI-MS systems during bloodstream infections, pneumonia, and sterile site infections [8,26–28].

Besides the higher bacterial detection rate, albeit a previous antibiotic treatment, another essential benefit that microbiomic analysis provides is a timely result deliverance that might prove crucial in the context of treating a patient with sepsis [10,11]. As mentioned, an inadequate antimicrobial therapy leads to a significant increase in sepsis-associated death. In our study, we found an inadequate anti-infective regimen in 22 (52.4%) patients who had been escalated to a broader-spectrum antibiotic after microbiologic examination of the intra-abdominal samples. Adapting antibiotic treatment is usually performed based on antibiotic resistance testing generated during bacterial culture. Our findings are in line with the results obtained from alternative PCR/ESI-MS systems. Also studies performed with the IRIDICA PCR/ESI-MS system (Abbott Molecular, Des Plaines, IL) demonstrated reliable results in the diagnosis of bloodstream infections, pneumonia, and sterile site infections [26,29–32].

Therefore, the lack of information about antibiotic resistance represents the major disadvantages of microbiomic analysis, because it cannot provide additional information regarding bacterial sensitivity to antibiotic agents. Therefore, the knowledge of regional resistance patterns of specific bacterial strains to antibiotics is needed, and, according to the results of conventional methods, clinical physicians should interpret the results regarding clinical symptoms. Another relevant question that cannot be clarified when using microbiomic analysis is whether the detection of bacterial strains signifies a contamination, colonization, or true infection.

This study has several limitations. First, our study included only a small group of patients, but to our knowledge, it is still the largest cohort of patients with intra-abdominal infection that evaluates dHPLC as a molecular microbiologic diagnostic method analyzing pathologic intra-abdominal fluids. Second, dHPLC can only provide antimicrobial susceptibility information about currently identified resistance genes. Therefore, conventional culture-based methods are needed currently to supply additional information. Third, our recent study does not provide any information about the impact of this approach on clinical and economic outcomes. Further studies are needed to investigate the impact of molecular microbiologic diagnostic evaluation on clinical outcome parameters.

Conclusion

Using the WAVE system to analyze samples originating from patients with sepsis with an intra-abdominal infection source does provide additional information, especially concerning unusual, fastidious bacteria. Further, it has a higher detection rate for polymicrobial infection and delivers results much sooner than conventional methods. Given the broad bacterial spectrum covered by empiric antibiotic regimens, most patients with community-acquired intra-abdominal infections resulting in sepsis do already receive adequate treatment. Therefore, further studies are needed to prove the clinical relevance of higher detection rates for polymicrobial infections and shorter analytic durations of culture independent microbiologic diagnostic evaluation in patients with severe intra-abdominal infections.

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Author Disclosure Statement

No competing financial interests exist.

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8.2 Anlage 2

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ORIGINAL ARTICLE

WILEY mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases

The impact of real life treatment strategies for *Candida* peritonitis—A retrospective analysis

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Summary

Candida species are commonly detected isolates from abdominal foci. The question remains as to who would benefit from early empiric treatment in cases of *Candida* peritonitis. This study collected real-life data on critically ill patients with *Candida* peritonitis to estimate the relevance of the chosen treatment strategy on the outcome of these patients. One hundred and thirty-seven surgical intensive care unit (ICU) patients with intra-abdominal invasive Candidiasis were included in the study. Fifty-six patients did not get any antifungal agent. Twenty-nine patients were empirically treated, and 52 patients were specifically treated. In the group without, with empiric and with specific antifungal treatment, the 30-day mortality rate was 33.9, 48.3 and 44.2 respectively. *Candida albicans* was the most frequently found species. Seven patients in the specific treatment group and one patient in the empiric treatment group emerged with candidaemia. Age, leucocyte count, APACHE II Score and acute liver failure were independent predictors of 30-day mortality in patients with *Candida* peritonitis. Not all patients with *Candida* peritonitis received antifungal treatment in real clinical practice. Patients with higher morbidity more often got antifungals. Early empirical therapy has not been associated with a better 30-day mortality.

KEYWORDS

Candida, candidaemia, intensive care, peritonitis, surgical treatment

1 | INTRODUCTION

Fungal isolates are commonly identified in surgical intensive care unit (ICU) patients. In contrast to patients on medical wards, intra-abdominal pathologies are a major cause of further invasive fungal infections and fungaemia in the critically ill population.[1] Therefore, *Candida* species are one of the most commonly detected isolates from abdominal foci following secondary and tertiary peritonitis.[2-5] Risk factors associated with *Candida* peritonitis include: origin and type of peritonitis, severity of acute illness, gastrointestinal surgery, acute necrotising pancreatitis, postoperative bowel rest, cardiovascular failure, prolonged antibiotic therapy, prolonged ICU stay, catheters and drains and chronic anti-secretory therapy.

Nosocomial *Candida* peritonitis has also been identified as an independent risk factor for mortality.[4] In contrast to primary fungaemia, candidaemia following *Candida* peritonitis is associated with a dramatic increase in mortality.[6] Nevertheless, due to the high rate of clinically unapparent *Candida* positive patients, recent guidelines recommend an antifungal treatment only for high-risk patients with recurrent gastrointestinal perforations or with surgically treated necrotising pancreatitis.[2,7]

Early adequate antifungal treatment is crucial for a beneficial outcome in septic shock due to candidaemia.[8,9] Even though it is clear that a lot of patients with candidaemia receive their antifungal therapy too late, it is still not clear who benefits from an early empiric treatment in cases of *Candida* peritonitis. Azols and echinocandins are recommended antifungals for the management of intra-abdominal *Candida* infections, whereas echinocandins are favoured for critically ill patients, or in cases of suspected azol-resistant species.[7,10]

The aim of this retrospective single-centre cohort study was to collect real life data on critically ill patients with *Candida*-positive

peritonitis, and to estimate the relevance of the chosen treatment strategy (none, empirical or specific treatment) on the outcome of these patients.

2 | MATERIAL AND METHODS

2.1 | Study design

This retrospective observational single-centre cohort study was performed at the surgical ICU of the University Hospital of Giessen after approval by the institutional review board of the medical faculty of the Justus-Liebig-University, Giessen, Germany (AZ 152/11). We analysed data gathered from 2005 through 2011. Included patients were at least 18 years old, had undergone a laparotomy or interventional drainage placement for source control, and had fungi positive cultures recovered from thereby sterile taken samples. Data collection was performed using electronic patient records. Infections were classified as nosocomial when signs and symptoms occurred at least 48 hours after hospital admission. A complicated intra-abdominal infection was defined as an infection that spread through the affected intestinal organ and lead to peritonitis. Sepsis was defined according to the definitions of the "surviving sepsis campaign".[11] The abdominal focus of sepsis was confirmed by surgical interventions such as laparotomy or drainage placement. The associated abdominal focus was specified as epigastric region, small bowel, Colon, or other. Candidaemia was defined as at least one fungi positive blood culture.

2.2 | Data collection

The following data were collected: demographic characteristics (age, gender, height, weight, and body-mass-index), underlying disease (malignancy, diabetes, liver cirrhosis, acute liver failure, acute kidney failure, or/and dialysis), corticosteroid therapy, insertion of a central venous catheter, antibiotic therapy, need for parenteral nutrition, septic shock, SOFA, SAPS and APACHE II Scores, latency between primary surgery and fungal peritonitis, origin of peritonitis, and laboratory data (leucocyte count, thrombocytes, C-reactive protein, procalcitonin, haemoglobin, creatinine, Aspartate-Aminotransferase, Alanine-Aminotransferase and γ-Glutamyltransferase).

2.3 | Outcome

We defined 30-day mortality as the primary outcome criteria. Secondary outcomes were the overall mortality, ICU length of stay, hospital length of stay, incidence of candidaemia and need for another surgical procedure/revision.

2.4 | Anti-infective treatments

Patients in this retrospective analysis were treated according to the local standard of the surgical ICU, University Hospital of Giessen, Germany. Antifungal treatment was considered empiric when the treatment began before a positive fungal sample was reported. Patients in the specific treatment group received a fungal therapy only after the result statement.

2.5 | Statistical analysis

Descriptive statistics were analysed for demographics, clinical characteristics, haemodynamic values and laboratory findings. Continuous variables are presented as means and standard deviations, or medians with interquartile ranges, and categorical variables as numbers and percentages. Comparisons between groups were done by *t* test. Categorical variables were compared by the Pearson χ^2 test. A Cox Regression Analysis was performed to investigate predictors for mortality. The entire statistical analysis was performed with SPSS® version 19 (SPSS Inc, IBM, Chicago, IL, USA).

3 | RESULTS

We identified 137 surgical ICU patients with intra-abdominal invasive Candidiasis due to positive microbiological samples (Table 1). Eighty-four patients were male (61.3%) and 53 were female (38.7%). The mean age was 64.73±14.12 years and almost 90% of the study population had a nosocomial postoperative peritonitis.

About 51.8% suffered from pure fungal infections only (71/137). Concomitant polymicrobial intra-abdominal infections were detected in 66 of 137 patients (48.2%) although no differences existed between the treatment groups (Table 1).

Regarding the antifungal treatment, 29 (21.17%) patients were empirically treated, 52 (37.96%) patients were specifically treated after the microbiological report and 56 (40.88%) patients did not get any antifungal agent. We did, find differences in the antibiotic co-treatment. Patients receiving antifungal medication more often received antibiotics than patients without antifungal therapy. Additionally, there was a greater prevalence of acute liver failure in patients getting antifungals, reflecting their higher transaminases. Overall, patients getting antifungals showed a trend towards higher age, APACHE II scores and a more frequent presence of septic shock.

No significant differences were observed concerning the spectrum of recovered *Candida* species. *Candida albicans* was the most frequently found species in all groups, followed by *Candida glabrata* and *Candida tropicalis*. *Candida krusei* and *Candida parapsilosis* were rare species overall. Only one patient being treated with an empiric therapy developed candidaemia, whereas more patients (13.5%) of the specific antifungal therapy group presented with candidaemia. *C. albicans* was the most frequent species causing candidaemia. Regarding the latency between sampling and clinical report, we found three-quarters of *C. albicans* positive samples were cultured at day 2, whereas this was not true of *C. glabrata* until day three.

Nearly one-half of the empirically treated patients received an echinocandin for primary antifungal therapy (Table 2). However, due to the high rate of *C. albicans* in the group of specifically treated patients, azols were more often administrated to these patients as a primary treatment. Due to the fact that the group with the specific therapy had to wait for

TABLE 1 Baseline characteristics of patients with *Candida* peritonitis

	No antifungals (n=56)	Empiric antifungal therapy (n=29)	Specific antifungal therapy (n=52)	*P*
Age (y)	62.18±15.63	63.66±14.20	68.08±11.93	.08
Gender female	22 (39.3)	12 (41.4)	19 (36.5)	.91
Height (cm)	170.98±9.39	168.52±9.02	169.17±8.66	.61
Weight (kg)	79.24±15.85	77.76±11.67	78.27±23.55	.66
Body mass index (kg/m^2)	27.0±4.55	27.58±5.12	27.18±7.45	.79
Malignoma	31 (55.4)	13 (44.8)	27 (51.9)	.65
Diabetes mellitus	13 (23.2)	3 (20.3)	24 (26.9)	.22
Liver cirrhosis	7 (12.5)	2 (6.9)	5 (9.6)	.71
Acute liver failure	5 (8.9)	8 (27.6)	10 (19.2)	.084
Acute renal failure	21 (37.5)	13 (44.8)	22 (42.3)	.78
Corticosteroid treatment	5 (8.9)	2 (6.9)	5 (9.6)	.91
Hydrocortisone therapy in ICU	15 (26.8)	10 (34.5)	17 (32.7)	.71
Central venous catheter	56 (100)	29 (100)	66 (100)	-
Renal replacement treatment	9 (16.2)	8 (27.6)	12 (23.1)	.430
Antibiotic therapy	46 (82.1)	28 (96.6)	49 (94.2)	.047
Total parenteral nutrition	27 (48.2)	16 (55.2)	33 (63.5)	.23
Partial parenteral nutrition	23 (41.1)	11 (37.9)	29 (55.8)	.16
APACHE II	15.71±5.82	17.62±4.67	18.12±5.84	.06
SOFA	7.44±4.35	8.55±4.36	8.22±3.87	.41
SAPS II	38.29±14.62	43.31±13.78	42.47±11.90	.16
Septic shock	9 (16.1)	8 (27.6)	12 (23.1)	.43
Nosocomial peritonitis	49 (87.5)	26 (89.7)	47 (90.4)	.83
Postoperative peritonitis	47 (83.9)	25 (86.2)	49 (94.2)	.231
Latency between primary surgery and fungal peritonitis (d)	15.20±13.51	11.72±9.45	11.04±8.73	.393
Origin of peritonitis				
Epigastric region	14 (25)	14 (48.3)	21 (40.4)	.278
Small bowel	6 (10.7)	5 (17.2)	6 (11.5)	
Colon	20 (35.7)	7 (24.1)	17 (32.7)	
Other	16 (28.6)	3 (10.3)	8 (15.4)	
Polymicrobial infections	29 (51.8)	13 (44.8)	24 (46.2)	.78
Leucocytes (2/nL)	14.75±9.0	16.7±8.78	15.5±7.59	.47
C-reactive protein (mg/dL)	189.0±321.45	166.85±132.12	198.0±118.68	.1
Procalcitonin (ng/dL)	4.869±15.21	1.872±2.74	7.12±18.84	.24
Haemoglobin (g/L)	10.22±1.59	10.01±1.54	9.88±1.35	.36
Creatinin (mg/dL)	1.35±0.76	1.29±0.71	1.54±0.96	.51
Thrombocytes (2/nL)	249.55±193.55	239.34±190.36	234.48±125.44	.95
Aspartate-Aminotransferase (U/L)	82.39±202.83	450.41±1974.97	50.87±59.72	.025
Alanine-Aminotransferase (U/L)	65.18±113.67	148.83±258.59	45.83±85.15	.041
γ-Glutamyltransferase (U/L)	175.91±251.97	421.55±588.33	167.31±166.30	.024
γ-Glutamyltransferase (U/L)	175.91±251.97	421.55±588.33	167.31±166.30	.024

Values were displayed as count (%) or mean±standard deviation.

the results, it is clear that the latent period between the positive result and the start of therapy was significantly longer in comparison to the empirically treated patients (2.69±2.95 vs −2.55±5.94 days; *P*<.001).

In the empiric treatment group, 17.2% of the patients were deescalated to fluconazole during treatment and only 6.9% were switched from an azol to an echinocandin. Conversely, more patients in the

TABLE 2 Administrated antifungal therapy

	Empiric antifungal therapy (n=29)	Specific antifungal therapy (n=52)	*P*
Primary therapy			
Fluconazole	14 (48.3)	35 (67.3)	<.001
Voriconazole	1 (3.4)	8 (15.4)	.004
Echinocandin	14 (48.3)	9 (17.3)	<.001
Caspofungin	9 (31)	6 (11.5)	<.001
Anidulafungin	5 (17.2)	3 (5.8)	.006
Latency positive result to start of therapy (d)	−2.55±5.94	2.69±2.95	<.001
Secondary therapy	9 (31)	21 (40.4)	<.001
Fluconazole	5 (17.2)	5 (9.6)	.011
Voriconazole	2 (6.9)	7 (13.5)	.019
Echinocandin	2 (6.9)	8 (15.4)	.009
Caspofungin	1 (3.4)	5 (9.6)	.049
Anidulafungin	1 (3.4)	3 (5.8)	.200
Liposomal amphotericin B	0 (0)	1 (1.9)	.440

Values were displayed as count (rate) or mean±standard deviation.

specific treatment group were switched to an echinocandin (15.4%) and only 9.6% to fluconazole. Overall, patients with a specific therapy underwent changes in treatment more often compared to the empiric therapy group (40.4% vs 31%).

Considering 30-day mortality after the verification of the *Candida* species in the abdomen, there was no difference between the three groups. However, the overall mortality rate varied (Table 3). The lowest fatality rates were shown in the group without therapy. Patients with empiric therapy had a mortality rate of 51.7% compared to 61.5% in the patients with a specific treatment. Additionally, length of stay in the ICU, in-hospital length of stay, and the duration of mechanical ventilation were longer in specifically treated patients hinting towards a higher degree of critical illness. Cox Regression Analysis revealed age, leucocyte count, APACHE II Score and acute liver failure were

TABLE 3 Outcome of patients with candida peritonitis (n=137)

		No antifungals (n=56)	Empiric antifungal therapy (n=29)	Specific antifungal therapy (n=52)	*P*
30-d mortality		19 (33.9)	14 (48.3)	23 (44.2)	.37
Overall mortality		21 (37.5)	15 (51.7)	32 (61.5)	.043
Following fungaemia		1 (1.8)	1 (3.4)	7 (13.5)	.037
Latency abd. confirmation until fungaemia	d	7±0	11±0	16.63±18.40	.798
Overall mortality candidaemia		0/2	0/2	4/7 (57.4)	
Length of stay in ICU before result	d	13.39±11.78	14.72±9.02	11.69±8.48	.91
Length of stay in ICU after result	d	14.89±18.37	18.07±18.21	33.69±41.83	.002
Overall length of hospital stay	d	42.07±31.57	45.55±31.484	61.58±50.90	.015
Overall length of hospital stay before result	d	15.66±13.64	14.79±11.60	18.06±18.39	.32
Overall length of hospital stay after result	d	25.45±24.80	29.66±28.93	42.52±45.80	.017
All patients					
Ventilation time in ICU	h	347.57±430.11	427.45±408.53	604.79±814.52	.008
Ventilation time in ICU before result	h	193.92±264.12	248.32±222.73	157.00±153.03	.043
Ventilation time in ICU after result	h	156.44±291.68	192.4±275.37	456.57±787.12	<.001
Only survivors					
Ventilation time in ICU	h	281.27±319.12	346.49±335.96	492.54±362.68	.054
Ventilation time in ICU before result	h	154.90±211.03	165.64±143.76	160.58±177.32	.35
Ventilation time in ICU after result	h	130.09±241.68	210.98±305.77	349.43±350.11	.008

Values were displayed as count (rate) or mean±standard deviation.

TABLE 4 Multivariate Analysis for 30-day Mortality

	P	Hazard-Ratio (95% confidence interval)
Age (y)	.012	1.038 (1.008-1.069)
Leucocyte count (2/nL)	.020	1.043 (1.007-1.081)
APACHE II score	<.001	1.116 (1.055-1.181)
Acute liver failure	<.001	4.443 (2.328-8.480)
Origin of peritonitis		
Epigastric region	.091	
Small bowel	.144	0.535 (0.231-1.239)
Colon	.057	0.505 (0.250-1.021)
Other	.552	1.264 (0.584-2.739)
Antifungal therapy		
No treatment	.075	
Empirical treatment	.512	0.782 (0.374-1.632)
Specific treatment	.026	0.470 (0.242-0.912)

independent predictors of 30-day mortality (Table 4). Despite overall mortality was the highest in the in the specific treatment group, in Cox Regression Analysis the assignment to this group was associated with decreased hazard risk for short-term 30-day mortality.

4 | DISCUSSION

This study collected real-life data in a population of critically ill patients with *Candida* peritonitis, to estimate the effectiveness of each chosen treatment strategy. No difference in 30-day mortality was observed in the treatment arms (none, Empirical, or specific antifungal medication). Overall mortality and morbidity, including following episodes of candidaemia were higher for specifically treated patients, due to a more complicated clinical course after *Candida* detection.

Septic shock and severe sepsis are major causes of ICU admittance, and are associated with high mortality, ranging from 30%-45%.[12,13] A recent prevalence study by Vincent et al.,[13] (EPIC-II Study) showed that 17% of ICU-acquired infections were caused by *Candida* spp, and it was the third most common pathogen to cause infections. The incidence of septic shock caused by *Candida* is around 20%.[9] When septic shock is caused by candidaemia, mortality rates are even higher and can reach up to 60%.[8,14] Kollef et al.[8] showed that adequate source control and prompt antifungal therapy is crucial for patients with septic shock caused by *Candida* infections. Patients who did not meet these criteria (antifungal therapy not within 24 hours, without adequate source control) had a mortality rate of 97.6% compared to 52.8% in those who met these criteria. A recent investigation by Bassetti et al.[9] underlined the importance of source control and adequate antifungal treatment in patients with septic shock caused by candidaemia. Inadequate antifungal therapy, inadequate source control, and higher APACHE II scores were independently associated with higher 30-day mortality. Data from Kollef et al.[8] confirmed these results in a population of patients with septic shock due to candidaemia. In summary, these findings lead to the implementation of protocols for early (empirical or preemptive) antifungal treatment in patients at risk for candidaemia.[7] Actual guidelines suggest the use of echinocandins rather than azols for early antifungal treatment in critically ill high-risk patients.

In our study, overall mortality varied between the groups. The lowest fatality and morbidity rates were seen in the group without antifungal therapy. Patients with complicated clinical courses were more likely to receive antifungal treatment and showed higher mortality rates. Considering the short-term 30-day mortality more likely attributable to the fungal infection, age, leucocyte count, APACHE II Score and acute liver failure were independent predictors for mortality whereas the assignment to the specific treatment group was associated with a reduction in mortality. This on the first sight paradoxically result is probably explained by the fact, that patients with a stable critically illness benefit in the short run from a specific therapy. In the long run, the underlying disease might be more determining for their outcome. Considering the other treatment groups, the absence of an antifungal treatment in stable patients or the empirical treatment in very critically ill patients with organ failure remains without an independent relevance for the short-term outcome. In both patients' groups, other factors might be decisive for a predominantly good or bad outcome, respectively.

Recent studies showed mortality rates for *Candida* peritonitis were between 27% and 38%,[15,16] which is lower than in our studied population (37.5%-61.6%). However, concerning mortality predictors, corresponding studies have shown similar results.[15,17-19]

In patients with recurrent gastrointestinal leakages or infected pancreas necrosis requiring surgical intervention, early antifungal treatment is recommended.[1,20] Nevertheless, current literature does not support a widely established antifungal prophylaxis for patients with primary gastrointestinal (anastomotic) leakage, especially in those without further risk factors. In a prospective multi-centre trial, including 93 patients with *Candida* peritonitis, Montravers et al.[21] could not find a link between mortality and the timing of an adequate antifungal treatment. In the recently published prospective observational multicentre AmarCAND2 Study [22] by Leroy et al. 835 ICU patients with proven or suspected *Candida* infections were evaluated. Almost half of the patients with confirmed fungal infections had intraabdominal candidiasis. The primary reason to initiate empiric antifungal treatment was severe sepsis or septic shock. No significant difference was found between early antifungal therapy and the application of echinocandin for primary treatment. The reported mortality rates were 40.0% in candidaemia, and 25.4% in *Candida* peritonitis. Additionally, in the randomised double-blind placebo-controlled MSG-01 trial by Ostrosky-Zeichner et al.[23] using a targeted caspofungin prophylaxis in unselected ICU patients, there was no benefit found to receiving early treatment. Even though not statistically significant, there was a trend towards reducing the incidence of invasive candidiasis, without any effect on the mortality rate. Even more remarkably, a recently published placebo-controlled study by Knitsch et al.,[24] failed to show any positive effect either on the outcome or the incidence of candidiasis. (This study evaluated early antifungal treatment with micafungin in severely ill patients for the prevention of invasive candidiasis following gastrointestinal surgery for intra-abdominal infections). Moreover, the

absolute rates of non-survivors were higher in both verum study arms (16.7 vs 14.3%; 25.4 vs 22.2%), but this difference was not statistically significant. Regarding this, Bassetti et al.[15] identified the lack of source control as a risk factor for the outcome in patients with intra-abdominal candidiasis.

Our patients in the specific treatment arm had more abdominal complications indicating tertiary peritonitis. Additional co-morbidities/organ failure, and advanced age were also plausible reasons for increased mortality, as could infections that were not responsive to antifungal treatment. Patients with subsequent candidaemias in the specific treated population did not demonstrate an increase in mortality corresponding to other patients in this study arm. Thus, we cannot conclude that early treatment in these patients would have proven to be beneficial. It should also be considered that unnecessary antifungals may be associated with liver toxicity[25] or haemodynamic impairment,[26,27] and thus may contribute to additional morbidity or mortality in severely ill ICU patients. The overall outcome in severely ill patients does not only relate to the antifungal treatment, but also to operative procedure (eg source control). When the patient has signs of infection and intra-abdominal samples are taken, it is crucial to take them from sterile perioperative locations or out of drains which have been in place for less than 24 hours to identify candida peritonitis. In many other cases, yeast detection could be a sign of contamination or colonisation rather than evidence of invasive candidiasis, especially in non-septic patients or patients without a high APACHE II score.

This study has several limitations. First, it is a retrospective single centre study and patient numbers are limited. Due to the retrospective approach, treatment decisions were not controlled, and significant differences between the study arms are present. However, this analysis could provide important health care research data that reflects real-life treatment to analyse clinical decisions.

5 | CONCLUSION

In real clinical practice, not all patients with *Candida* positive abdominal samples taken under sterile conditions received antifungal treatment. Antifungals were more frequently administered to patients with organ failure or severe co-morbidities. The analysed data suggests that empirical antifungal treatment in ICU patients does not contribute to better outcomes. Nevertheless, certain patients might benefit on a short run from a specific treatment. Positive abdominal *Candida* cultures in clinically stable patients might be more likely to represent contamination or transient colonisation and do not necessarily require treatment with antifungals. Further randomised trials evaluating the benefit of specific treatment are mandatory to address this question. Furthermore, age, leucocyte count, APACHE II Score and acute liver failure were independent predictors of 30-day mortality.

CONFLICT OF INTEREST

SD, ML, AH, SW, BHS and RR has no conflicts of interest; CK received congress travel sponsorship from Pfizer and advisory board fees from Astellas Pharma; MAW received speaker fees and advisory fees from Astellas Pharma, MSD Sharp & Dohme, Pfizer Pharma, Novartis, Janssen, Gilead, Bayer, Astra Zeneca, Glaxo Smith Kline, Braun, Biosyn, Eli Lilly, ZLB Behring, Köhler Chemi; CL received speaker fees and advisory fees from Astellas Pharma, MSD Sharp & Dohme and Pfizer Pharma.

ETHICAL STATEMENT

Statement of human rights: All procedures performed in studies involving human participants were done in accordance with the ethical standards of the institutional research committee [approval by the institutional review board of the medical faculty of the Justus-Liebig-University Giessen, Germany (AZ 152/11)] and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

Informed consent: No formal consent was needed due to the retrospective design of the study. No identifying details of patients were published in this study.

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8.3 Anlage 3

Originalien

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Effects of echinocandin preparations on adult rat ventricular cardiomyocytes

Preliminary results of an in vitro study

Background

Candida infections represent a relevant risk for patients in intensive care units (ICU; [1, 2]) resulting in increased mortality [3, 4, 5]. Adequate antifungal treatment is essential in patients with sepsis to prevent irreversible damage due to microbial load, systemic inflammation and organ failure [6].

The echinocandins are large (molecular weight ~1200 kDa) semisynthetic cyclic hexapeptides derived from various natural fungal products [7, 8]. The lipophilic side chain of the echinocandins intercalates with the phospholipid bilayer of the fungal cell membrane where it serves as a non-competitive inhibitor of β-1,3-D-glucan synthase. Deficiency of β-1,3-D-glucan in the fungal cell wall results in osmotic instability and fungal cell lysis. The echinocandins have become the antifungal agents of first choice due to the excellent activity against most *Candida* strains and the favorable safety profile. Guidelines recommend echinocandins as primary therapy for all forms of candidiasis, especially in severely ill patients with organ dysfunction [9, 10]. Development of septic cardiomyopathy reflects a crucial pathogenic part of hemodynamic instability in septic shock which aggravates tissue hypoperfusion and organ failure [11]. Due to cardiac effects following echinocandin administration seen in ICU patients [12] the in vitro effects of echinocandins and fluconazole in clinically relevant concentrations on isolated cardiomyocytes of the rat were examined.

Material and methods

Material

Stock solutions of the three echinocandins anidulafungin (Ecalta®, Pfizer, Illertissen, Germany), micafungin (Mycamine®, Astellas Pharma Europe, Leiden, The Netherlands) and caspofungin (Cancidas®, Merck Sharp & Dohme, Hertfordshire, UK) as well as fluconazole (Diflucan®, Pfizer) were made by reconstitution of licensed preparations according to the product information. These were diluted directly before use with distilled water and 10 µl of this dilution was added to 1 ml culture medium resulting in concentrations of 0.1–100 µg/ml. Control cultures were treated with distilled water only.

Isolation of cardiomyocytes

Animal experiments (sacrificing animals and organ extraction) were performed with approval by the animal welfare officer of the Faculty of Medicine of the Justus-Liebig University Giessen and in accordance with Federation of European Laboratory Animal Science Associations (FELASA) guidelines. Ventricular heart muscle cells were isolated from Lewis rats in a standard procedure as described in greater detail previously [13, 14]. Briefly, hearts from Lewis rats (age 3–4 months) were excised under deep ether anesthesia and mounted on the cannula of a Langendorff perfusion system. Cardiomyocytes were isolated via perfusion with collagenase, followed by mincing, filtering and transfer to culture medium M199 supplemented with carnitine (2 mM), creatine (5 mM) and taurine (5 mM).

Incubation

A time-effect curve was constructed to investigate the representative duration for echinocandin incubation. Cardiomyocytes were incubated with 10 µg/ml caspofungin for 15 min, 90 min and 4 h, respectively. Subsequently, experiments were performed after cardiomyocytes were cultured in the presence of all licensed echinocandin preparations at concentrations of 0 (control), 0.1, 1, 3.3, 10, 33, and 100 µg/ml for 90 min. For fluconazole experiments concentrations of 0 (control), 0.1, 1, 3.3, 10, 20, and 100 µg/ml were used. Due

C. Arens, F. Uhle, M. Wolff, C. Koch, S. Weiterer, K.-D. Schlüter, M.A. Weigand and C. Lichtenstern participated in the study design, laboratory work and data analysis were carried out by C. Arens, F. Uhle, M. Wolff, C. Koch, A. Schulte, S. Weiterer, M. Henrich, M.A. Weigand, K.-D. Schlüter and C. Lichtenstern, statistical analyses and interpretation were performed by C. Arens, F. Uhle, M. Wolff, R. Röhrig, C. Koch, A. Schulte, S. Weiterer, M. Henrich, M.A. Weigand, K.-D. Schlüter, C. Lichtenstern. All authors participated in drafting the manuscript or critical revisionfor important intellectual content. All authors read and approved the final manuscript.

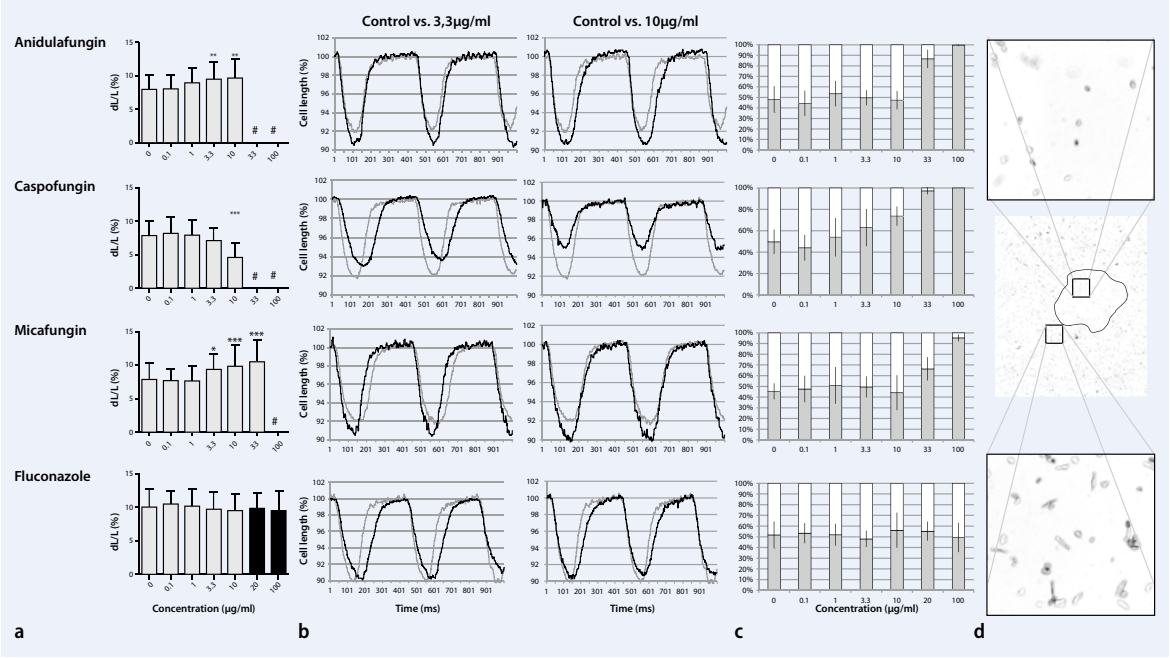

Fig. 1 ▲ Effect of different antifungal preparations and concentrations on cellular parameters of isolated rat cardiomyocytes. **a** Contractility responsiveness measured as shortening ratio (dL/L). Graphs show mean values ± standard deviation. **b** Single cell observation of contractility at 3.3 and 10 µg/ml versus controls. **c** Rounded cells *(grey bars)* and rod-shaped cells *(white bars)* were counted. **d** Representive micrograph of culture dish following high-dose echinocandins given in culture medium containing 10 mg/ml albumin. *Middle picture*: overview, *top and bottom*: magnified areas of overview picture. *Black line* depicts limits of impact area

to the fact that fluconazole is supplied in a ready to use preparation (2 mg/ml), the highest achievable concentration in the experimental setup was 20 µg/ml. Therefore, 100 µg/ml was achieved by adding five times the volume (=50 µl) compared to echinocandin preparations. To mimic plasma conditions two different methods of albumin incubation were used: the first series was performed by adding echinocandin preparations in culture medium containing 10 mg/ml albumin for 15 min followed by replacing the original culture medium by the culture medium-albumin-echinocandin mix and incubating the cells again for 90 min. An albumin concentration of 10 mg/ml was experimentally determined as the highest concentration not affecting cardiomyocyte viability and contractility. The second series was performed by applying echinocandin preparations in culture medium containing 10 mg/ml albumin that has already been in the culture dish. Each experiment was performed in duplicate and in a blinded manner.

Contractility measurements

Cell contraction was investigated using a cell edge detection system as described previously [15]. Briefly, cells were stimulated by biphasic 50 V electrical stimuli of 0.5 ms duration via field stimulation by AgCl electrodes. Each cell was stimulated at 2 Hz for 1 min. Cell contraction was measured at 5 intervals of 15 s and the mean of these 4 measurements was used to define the contractile responsiveness of a given cell. Cell lengths were measured at a rate of 500 Hz via a line camera. Data are expressed as ΔL/L (%) in which the shortening amplitude (ΔL) is expressed as a percentage of the diastolic cell length (L). At least 37 randomly selected cells from 2 independent cardiomyocyte isolations were analyzed for each concentration and substance.

Rod-shaped:round cell ratio

Photographs of cultured cardiomyocytes were taken with a BZ-800K microscope (Keyence, Neu-Isenburg, Germany). From each culture dish five photographs from separate sections were taken and the rod-shaped:round cell ratio was determined. On average approximately 400 cells were analyzed per condition from 6 culture dishes out of 2 preparations. Data were analyzed in a blinded manner.

Statistical analysis

Results are expressed as mean ± standard deviation. Differences between groups were analyzed globally by one-way ANOVA, followed by Dunnet's post-test to compare different treatment groups to controls. A value of $p<0.05$ was regarded as significant. All analyses were done using GraphPad Prism version 5.04 for Mac (GraphPad Software, San Diego CA).

Results

In the experiments anidulafungin concentrations of 3.3, and 10 µg/ml showed a significant increase of contractility re-

Abstract · Zusammenfassung

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Effects of echinocandin preparations on adult rat ventricular cardiomyocytes. Preliminary results of an in vitro study

Abstract

Background. *Candida* infections represent a relevant risk for patients in intensive care units resulting in increased mortality. Echinocandins have become the agents of choice for early and specific antifungal treatment in critically ill patients. Due to cardiac effects following echinocandin administration seen in intensive care unit (ICU) patients the in vitro effects of echinocandins and fluconazole on isolated cardiomyocytes of the rat were examined.

Aim. The study was designed to investigate a possible impact of echinocandins and fluconazole in clinically relevant concentrations on the in vitro contractile responsiveness and shape of isolated rat cardiomyocytes.

Material and methods. Ventricular cardiomyocytes were isolated from Lewis rats. Cardiomyocytes were cultured in the presence of all licensed echinocandin preparations and fluconazol at concentrations of 0 (control), 0.1, 1, 3.3, 10, 33 and 100 µg/ml for 90 min. Cells were stimulated by biphasic electrical stimuli and contractile responsiveness was measured as shortening amplitude. Additionally, the ratio of rod-shaped to round cells was determined.

Results. Anidulafungin concentrations of 3.3 and 10 µg/ml caused a significant increase in contractile responsiveness, caspofungin showed a significant decrease at 10 µg/ml and micafungin concentrations of 3.3–33 µg/ml led to a significant increase in cell shortening. Measurement was not possible at 33 µg/ml for anidulafungin and caspofungin and at 100 µg/ml for all echinocandins due to a majority of round-shaped, non-contracting cardiomyocytes. Fluconazole showed no significant effect on cell shortening at all concentrations tested. For the three echinocandins the ratio of round-shaped, non-contracting versus rod-shaped normal contracting cardiomyocytes increased in a dose-dependent manner.

Conclusions. Echinocandins impact the in vitro contractility of isolated cardiomyocytes of rats. This observation could be of great interest in the context of antifungal treatment.

Keywords

Antifungal agents · Echinocandins · Adverse drug reaction · Cardiomyopathy · Organ failure

Wirkung von Echinocandinpräparaten bei adulten ventrikulären Kardiomyozyten. Erste Ergebnisse einer In-vitro-Studie

Zusammenfassung

Hintergrund. *Candida*-Infektionen stellen ein relevantes Risiko für Patienten einer Intensivstation dar und führen zu einer erhöhten Mortalität. Echinocandine sind mittlerweile Mittel der Wahl für die frühzeitige und spezifische antimykotische Behandlung kritisch kranker Patienten. Aufgrund kardialer Wirkungen nach Echinocandingabe bei den Intensivpatienten der Autoren wurden die In-vitro-Wirkungen von Echinocandinen und Fluconazol auf isolierte Kardiomyozyten der Ratte untersucht.

Ziel der Arbeit. Die Studie wurde durchgeführt, um den möglichen Einfluss von Echinocandinen und Fluconazol in klinisch relevanten Konzentrationen auf die In-vitro-Kontraktilität und die Form isolierter Rattenkardiomyozyten zu bestimmen.

Material und Methoden. Ventrikuläre Kardiomyozyten wurden aus Lewis-Ratten isoliert. Die Kardiomyozyten wurden mit sämtlichen zugelassenen Echinocandinpräparaten und Fluconazol in den Konzentrationen von 0 (Kontrolle); 0,1; 3,3; 10; 33 und 100 µg/ml über 90 min kultiviert. Mit biphasischen elektrischen Reizen erfolgte die Stimulation der Zellen, und die Kontraktilität wurde als Verkürzung der Amplitude gemessen. Außerdem wurde das Verhältnis stäbchenförmiger Zellen zu runden Zellen ermittelt.

Ergebnisse. Eine Anidulafunginkonzentration von 3,3 sowie von 10 µg/ml verursachte einen signifikanten Anstieg der Kontraktilität; bei Caspofungin zeigte sich eine signifikante Verminderung bei 10 µg/ml; und Micafunginkonzentrationen von 3,3–33 µg/ml führten zu einem signifikanten Anstieg der Zellverkürzung. Aufgrund einer Mehrzahl von runden, nichtkontraktilen Kardiomyozyten war die Messung bei einer Konzentration von 33 µg/ml Anidulafungin und Caspofungin sowie für alle Echinocandine bei 100 µg/ml nicht möglich. Bei Fluconazol fand sich in allen analysierten Konzentrationen keine signifikante Wirkung auf die Zellverkürzung. Bei den 3 Echinocandinen stieg das Verhältnis runder, nichtkontraktiler Zellen gegenüber den stäbchenförmigen, normalkontraktilen Kardiomyozyten dosisabhängig an.

Schlussfolgerung. Echinocandine beeinflussen die In-vitro-Kontraktilität isolierter Kardiomyozyten von Ratten. Diese Beobachtung könnte im Rahmen einer antimykotischen Behandlung von hohem Interesse sein.

Schlüsselwörter

Antimykotika · Echinocandine · Nebenwirkung · Kardiomyopathie · Organversagen

sponsiveness in contrast to 0.1 and 1 µg/ml which were not different compared to controls (**Fig. 1a,b**; **Tab. 1**). For caspofungin cell shortening was not different compared to controls for 0.1–3.3 µg/ml; however, incubation with 10 µg/ml showed a significant decrease. Micafungin concentrations of 0.1 and 1 µg/ml showed no effect in contrast to controls while incubation with 3.3–33 µg/m led to a significant increase in cell shortening. Measurement was not possible at 33 µg/ml for anidulafungin and caspofungin and at 100 µg/ml for all echinocandins due to a majority of round-shaped, non-contracting cardiomyocytes. Fluconazole showed no significant effect on cell shortening in all analyzed concentrations.

For all echinocandins the ratio of round-shaped, non-contracting versus rod-shaped normal-contracting cardiomyocytes increased dose-dependently. A stable rate of 44.3–53.5% round-shaped cardiomyocytes was observed for anidulafungin concentrations from 0.1 to 10 µg/ml, whereas 86.6% and 99.8% of cardiomyocytes were round-shaped at 33 µg/

Tab. 1 Values of mean contractility ***(mean dL/L)***, standard deviation ***(SD)***, number of cells ***(N)*** and significance compared to control for all concentrations and preparations

		Concentration (µg/ml])						
		Control	**0.1**	**1**	**3.3**	**10**	**33**	**100**
Anidulafungin	Mean dL/L	7.899	8.096	8.970	9.479	9.627		
	SD	2.101	1.964	2.164	2.646	2.800		
	N	54	54	44	54	37		
	Significance vs. control	ns	ns	ns	**	**		
Caspofungin	Mean dL/L	8.053	8.359	7.951	7.095	4.834		
	SD	1.923	2.372	2.191	1.944	2.079		
	N	54	54	54	54	54		
	Significance vs. control	ns	ns	ns	ns	***		
Micafungin	Mean dL/L	8.033	7.791	7.796	9.436	10.01	10.71	
	SD	2.333	1.775	2.024	2.350	3.156	3.196	
	N	54	54	54	54	53	54	
	Significance vs. control	ns	ns	ns	*	***	***	
Fluconazole	Mean dL/L	10.14	10.55	10.28	9.784	9.518	*9.957*	*9.670*
	SD	2.636	1.990	2.588	2.709	2.585	*2.379*	*2.978*
	N	54	54	54	54	54	*54*	*54*
	Significance vs. control	ns	ns	ns	ns	ns	*ns*	*ns*

Blank cells are depicted where no measurements were possible, **italics** indicate differential treatment regarding fluconazole concentration (33=20 µg/ml and 100 µg/ml concentration was achieved by adding five times the volume of solution than other treatments) ***ns*** not significant*$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$

ml and 100 µg/ml, respectively (**Fig. 1c**; **Tab. 2**). Caspofungin concentrations of 0.1 and 1 µg/ml provided comparable proportions of rounded cardiomyocytes as controls while the proportion increased dose-dependently from 3.3 µg/ml to 100 µg/ml from 63.6% to 73.1%, 97.2% and 100%, respectively. At micafungin concentrations of 0.1–10 µg/ml equivalent fractions of round-shaped cardiomyocytes were seen as in controls; however, at concentrations of 33 and 100 µg/ml cardiomyocytes revealed a rounded shape in 66.4 and 95.3%, respectively. Fluconazole showed no significant effect on shape at all analyzed concentrations.

The rate of round-shaped cardiomyocytes, following high-dose echinocandins given in culture medium containing 10 mg/ml albumin, proceeded according to assumed concentration gradients (**Fig. 1d**). Preincubation of echinocandins with 10 mg/ml albumin led to cancellation of all of the described effects.

Discussion

The results revealed for the first time that echinocandin preparations in contrast to fluconazole have a dose-dependent impact on function and shape of isolated rat cardiomyocytes in vitro. Nevertheless, there has already been evidence of an ex vivo cardiotoxicity in rats performed with a Langendorff heart experiment [16]. Some very recent clinical reports described severe hemodynamic instability during anidulafungin administration in a critically ill ICU patient [17] and a flash pulmonary edema of unknown origin in a 52-year-old male [18]. Cardiac effects following echinocandin administration have also been seen in septic ICU patients [12]. Additionally, Stover et al. [16] summarized four cases from the FDA adverse events reporting system (FAERS) which may reflect the cardiac impact of the echinocandins: two reports of arrhythmia (anidulafungin), one cardiac failure (anidulafungin) and one described as sudden cardiac death (caspofungin; [19]).

Regarding effective tissue concentrations, there are some limits in transferring the effects of isolated cardiomyocytes to an in vivo situation. Cardiomyocytes are protected in vivo by an endothelial barrier; however, disrupted endothelial layers in sepsis might promote increased antifungal tissue concentrations. Consequently trough concentrations of caspofungin in critically ill patients were found to be higher (mean 2.16 µg/ml) than in healthy subjects [20]. Additionally, even in healthy patients caspofungin already reaches peak plasma concentrations up to 20 µg/ml [21] and 9.94 µg/ml in steady state, while heart tissue reaches about 50% of plasma concentrations [22, 23] anidulafungin reaches maximum levels up to 14 µg/ml [24, 25] and the peak micafungin plasma concentration is approximately 8.8 µg/ml [26]. Recommended dose regimens provide loading dosages for caspofungin and anidulafungin which could lead to even higher intramyocardial concentrations. Regarding this, all clinical echinocandin effects which were reported in the case reports [12, 17] were seen according to initial loading dosages (200 mg anidulafungin and 70 mg caspofungin) in critically ill septic patients.

Because echinocandins are highly protein-bound in plasma and thus the total concentration could be many times higher than the free concentration, albumin was added to mimic plasma conditions. Primarily, it has to be considered that serum albumin levels are frequently decreased in patients at risk for invasive fungal infections which would increase the active drug concentration. Additionally, no data concerning the kinetics of protein binding of echinocandins following bolus infusion in humans appear to have been published. Especially in situations when antifungal treatment is needed, it is mostly administered through a central line, which could be followed by high active cardiac concentrations due to less time to bind to proteins. Moreover, standard minimum inhibitory concentrations (MIC) of echinocandins used to predict treatment response are estimated in the absence of albumin. However, albumin addition to this microbiological testing significantly raises the MIC to values above the cut-off that is applied to classify microbiological re-

Tab. 2 Values of cell morphology *(% rounded)*, standard deviation *(SD)* and number of cells *(N)* evaluated for all concentrations and preparations

		Concentration (µg/ml)						
		Control	**0.1**	**1**	**3.3**	**10**	**33**	**100**
Anidulafungin	% Rounded	48.1	44.3	53.5	49.7	47.3	86.6	99.8
	SD	13.0	12.0	12.0	7.0	9.0	9.0	1.0
	N	496	443	498	481	557	591	612
Caspofungin	% Rounded	49.7	44.1	54.0	63.6	73.1	97.2	100
	SD	11.5	12.0	17.9	17.5	9.0	3.3	–
	N	474	440	433	423	431	515	441
Micafungin	% Rounded	45.4	47.5	50.9	49.5	44.1	66.4	95.3
	SD	7.5	12.5	17.2	10.0	16.4	10.9	3.2
	N	495	491	526	492	474	476	476
Fluconazole	% Rounded	51.7	53.3	51.9	48.1	56.1	*55.2*	*49.4*
	SD	12.6	9.5	10.3	7.6	16.5	*8.9*	*13.7*
	N	403	426	481	430	474	*529*	*403*

Figures in *italics* indicate differential treatment regarding fluconazole concentration (33=20 µg/ml and 100 µg/ml concentration was achieved by adding five times the volume of solution than other treatments)

sistance. Data from phase 2 and 3 studies have not described adverse events regarding cardiac failure following echinocandin administration. Product information for caspofungin from the European Medicines Agency (EMA) mentions occasional congestive heart failure. However, most studies on antifungal agents did not include a relevant rate of critically ill patients and therefore, hemodynamic reserves might have been sufficient in the overall study populations. In contrast in septic patients it is possible that a drug-related cardiac failure is wrongly attributed to a progress of illness. According to the drug evaluation documents from EMA and the U.S. Food and Drug Administration (FDA), hypotension and a hemodynamic breakdown are described for an experimental administration of caspofungin in rats. This was associated with vascular histamine release. It has not yet been examined if histamine release is a possible mechanism of the observed effects. Nevertheless, the very recent report from Fink et al. [17] and in vivo observations in three patients [12] lead to the assumption that there must be an additional non-histamine-related pathway which leads to cardiac breakdown. Additionally histamine-releasing cells (e.g. mast cells) as mediators were not observed in the preparations in the current study.

The results of the study do not give the explanation for the mechanism of the observed effects on isolated cardiomyocytes by echinocandins. As Stover et al. [16] based on other publications [27, 28] already mentioned, there are several possible mechanisms of drug-induced cardiomyopathy. The evidence of myocyte and mitochondrial damage was observed using transmission electron microscopy [16]. Furthermore, the observed rounded shape of isolated cardiomyocytes in the current experiments indicates a disturbed intracellular calcium homeostasis. However, the possibility of a receptor mediated mechanism or direct toxic effects, e.g. due to oxidative stress cannot be ruled out. Because of the rapidity in which the effects appeared, alterations in cardiomyocyte gene expression or protein synthesis are not very likely to be the reason for the observed effect. Because of the absence of endothelial cells in the experimental set-up any involvement of endothelial cells in the observed results can be ruled out. Remarkably, lower doses of anidulafungin (3.3 and 10 µg/ml) and micafungin (3.3, 10 and 33 µg/ml) caused an increase in contractility responsiveness whereas caspofungin (10 µg/ml) led to a decrease. However, the same rounded fibrillating shape of the isolated cardiomyocytes without a straightened contraction was observed when exposed to high doses of all echinocandins. Although the molecular structure of the scaffold is very similar, the echinocandins have some differences in the side chains what is assumed to be the reason for such differences [25]. This might also be the reason why the MICs of the echinocandins are not equal [8, 29, 30]. Nevertheless, the rate of cardiomyocytes with a round cell shape consistently increases for all echinocandins dose-dependently.

There are some limitations of the study: First of all, the results from isolated rat cardiomyocytes are not transferable one-to-one to the human clinical setup on the ICU. Isolated cells are not protected by endothelium and higher free echinocandin expositions are even implicated by the absence of albumin in the experiment. Additionally, clinical case reports described effects in septic critically ill patients whereas the experiments were performed in cells harvested from healthy animals. Due to the experimental approach, it is not possible to give decisive recommendations for the clinical choice of any echinocandin preparation or a required modification of the way of administration. Finally, it is not clear whether the observed effects are selective for cardiomyocytes.

Conclusion

Echinocandins impact the in vitro contractility of isolated cardiomyocytes of rats. This observation could be of great interest in the context of antifungal treatment. The setup used is experimental but gives an additional indication that echinocandins could have a potential impact on cardiac function. Further preclinical and clinical studies are necessary to evaluate the impact of echinocandin administration on different disease conditions, such as severe sepsis or septic shock.

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Compliance with ethical guidelines

Conflict of interest. C. Arens received congress travel sponsorship from Gilead and Orion Pharma; F. Uhle, M. Wolff, R. Röhrig, C. Koch and A. Schulte have no conflicting interests; S. Weiterer and M. Henrich received congress travel sponsorship from Astellas Pharma; M.A. Weigand received speaker fees and advisory fees from Astellas Pharma, MSD Sharp & Dohme, Pfizer Pharma, Novartis, Janssen, Gilead, Bayer, Astra Zeneca, Glaxo Smith Kline, Braun, Biosyn, Eli Lilly, ZLB Behring and Köhler Chemie; K.-D. Schlüter has no conflicting interests; C. Lichtenstern received speaker fees and advisory fees from Astellas Pharma, MSD Sharp & Dohme and Pfizer Pharma.

All national guidelines on the care and use of laboratory animals were followed and the necessary approval was obtained from the relevant authorities.

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8.4 Anlage 4

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Cardiac Effects of Echinocandins after Central Venous Administration in Adult Rats

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Echinocandins have become the agents of choice for early and specific antifungal treatment in critically ill patients. *In vitro* studies and clinical case reports revealed a possible impact of echinocandin treatment on cardiac function. The aim of our study was to evaluate echinocandin-induced cardiac failure. Using an *in vivo* rat model, we assessed hemodynamic parameters and time to hemodynamic failure after central venous application (vena jugularis interna) of anidulafungin (low-dose group, 2.5 mg/kg body weight [BW]; high-dose group, 25 mg/kg BW), caspofungin (low-dose group, 0.875 mg/kg BW; high-dose group, 8.75 mg/kg BW), micafungin (low-dose group, 3 mg/kg BW; high-dose group, 30 mg/kg BW), and placebo (0.9% sodium chloride). Left ventricular heart tissue was collected to determine mitochondrial enzyme activity via spectrophotometric measurements. mRNA expression of transcriptional regulators and primary mitochondrial transcripts, mitochondrial DNA (mtDNA) content, and citrate synthase activity were also explored. Animals receiving high-dose anidulafungin or caspofungin showed an immediate decrease in hemodynamic function. All of the subjects in these groups died during the observation period. Every animal in the untreated control group survived ($P < 0.001$). Hemodynamic failure was not noticed in the anidulafungin and caspofungin low-dose groups. Micafungin had no impact on cardiac function. In analyzing mitochondrial enzyme activity and mitochondrial transcripts, we found no association between echinocandin administration and the risk for hemodynamic failure. Further experimental studies are needed to elucidate the underlying mechanisms involved in cardiotoxic echinocandin effects. In addition, randomized controlled clinical trials are needed to explore the clinical impact of echinocandin treatment in critically ill patients.

Fungal infections represent a relevant risk for critically ill patients (1, 2), resulting in prolonged intensive care unit (ICU) stay and increased mortality (3–5). Antifungal therapy is crucial in septic patients to prevent irreversible injuries due to microbial load, systemic inflammation, and organ failure (6). Echinocandins are an established class of antifungal agents with activity against *Candida* and *Aspergillus* species. They are semisynthetic cyclic hexapeptides derived from various natural fungal products presenting a lipophilic side chain that interacts with the phospholipid bilayer of the fungal cell membrane, where they serve as noncompetitive inhibitors of β-1,3-D-glucan synthase (7, 8). The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines and the guidelines of the Infectious Diseases Society of America recommend echinocandins for the primary treatment of candidemia (9, 10). Development of septic cardiomyopathy reflects a crucial pathogenic part of hemodynamic instability in septic shock that aggravates tissue hypoperfusion and organ failure (11). Cardiac effects following echinocandin administration were seen in our ICU patients (12), in isolated rat hearts (Langendorff model) (13), and in isolated cardiomyocytes of rats (14). Following these approaches, we performed hemodynamic measurements after central venous administration of anidulafungin, caspofungin, and micafungin in clinically relevant concentrations in adult male rats to assess echinocandin-induced cardiotoxicity. Previous studies claimed that mitochondrial toxicity is the underlying mechanism behind echinocandin-induced cardiac failure (13, 15). To test this hypothesis in our model, we also determined left ventricular mitochondrial enzyme activity.

MATERIALS AND METHODS

Animal model. A total of 42 male Lewis rats (weighing 275 to 300 g), delivered by Charles River (Sulzfeld, Germany), were used in a randomized controlled model. All procedures involving animals were conducted in compliance with the standards for animal experiments and were approved by the local committee for animal care (GI 20/26 Nr.3/2012; Regierungspraesidium, Giessen, Germany). Studies were performed in rats anesthetized with isoflurane (Baxter, Unterschleissheim, Germany). After endotracheal intubation with a 16-gauge catheter, animals were ventilated with a rodent respirator (Harvard Inspira, MA, USA) using volume-controlled ventilation in a weight-adjusted manner. Heart rate and body temperature were recorded. Ringer solution (10 ml/kg/h; Braun, Melsungen, Germany) and fentanyl (10 μg/kg/h; Ratiopharm, Ulm, Germany) were continuously administered intravenously through the lateral tail vein with a syringe pump (Braun, Melsungen, Germany). Arterial

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TABLE 1 Primer sequences

Primer name	GenBank accession no.	Forward primer	Reverse primer
COX1	FJ355927.1	GATTCTTCGGACACCCAGAA	AGGCTCGCGTGTCTACATCT
Cytochrome *b*	AF295545.1	ATGCTCCATTCCCAACAAAC	AGGAGGTTGGCTACCAGGAT
Tfam	BC062022	CGCCTGTCAGCCTTATCTGT	CCCATCAGCTGACTTGGAGT
NRF-1	NM_001100708	GAACAAAATTGGGCCACATTAC	GTTAAGGGCCATGGTGACAG
PGC-1α	NM_031347	CCGAGAATTCATGGAGCAAT	GTGTGAGGAGGGTCATCGTT
ND-1	EU104724.1	ATTTATTATCGCCCCAACCC	CAGGCGGGGATTAATAGTCA

Primers were designed using the corresponding reference sequences deposited in GenBank.

blood pressure was also measured continuously using a microtip catheter (SPR-1000; Millar Instruments, Houston, TX, USA) inserted in the animal's tail artery. Experimental groups received clinically relevant human doses of echinocandins, and 10-fold higher drug doses were administered over a period of 60 min via a central venous catheter placed in the right jugular vein using a syringe pump (Harvard Apparatus, MA, USA). The animals were randomly divided into 7 groups: anidulafungin low-dose group (2.5 mg/kg body weight [BW] Ecalta; Pfizer, NY); anidulafungin high-dose group (25 mg/kg BW), caspofungin low-dose group (0.875 mg/kg BW Cancidas; Merck and Co., NJ), caspofungin high-dose group (8.75 mg/kg BW), micafungin low-dose group (3 mg/kg BW Mycamine; Astellas Pharma, Inc., IL), micafungin high-dose group (30 mg/kg BW), and untreated controls receiving 0.9% sodium chloride (placebo). Animals were observed for 6 h after echinocandin or placebo administration or until hemodynamic failure. Body temperature was kept at about 37°C throughout the experiment using a feedback-controlled heating pad and an infrared heater. After the rats were euthanized, their hearts were excised, dissected into left and right ventricle, and flash frozen in liquid nitrogen. Storage was carried out at −80°C.

Hemodynamic measurements. Cardiac function of the left ventricle was measured using a pressure-volume conductance catheter (SPR-838, Millar, Houston, TX, USA) (15). After blunt preparation and puncture of the carotid artery, the catheter was inserted, fixed with a suture, and carefully moved into the left ventricle. Pressure-volume signals were recorded using the PowerLab 8/30 signal converter (ADInstruments, Spechbach, Germany) and LabChart7 (ADInstruments). Parallel conductance catheter calibration was performed in each animal, where 30 μl of hypertonic saline (10%) was injected through a polyethylene catheter into the right jugular vein. Once, cuvette calibration to determine blood conductivity was performed with fresh heparinized warm blood. Recorded data were analyzed using PVAN 1.1 (Millar). Hemodynamic parameters, including cardiac output (CO), left ventricular ejection fraction (EF), arterial blood

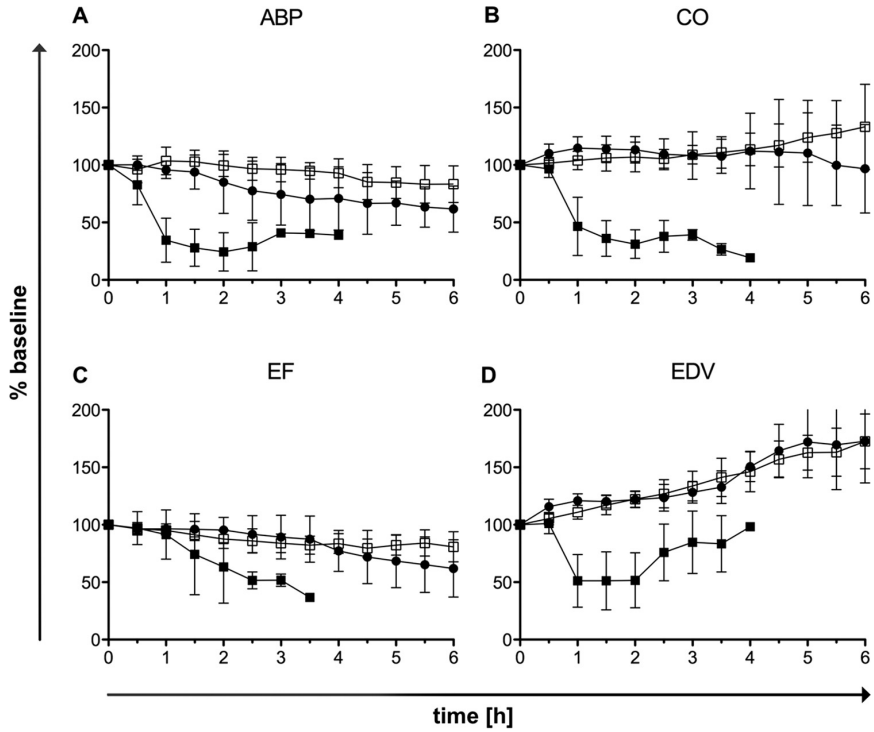

FIG 1 Hemodynamic measurements in control and anidulafungin (ANID) high- and low-dose-treated rats. Animals treated with high-dose anidulafungin showed a large decrease in arterial blood pressure (A), cardiac output (B), and left ventricular ejection fraction (C). (D) Left ventricular end-diastolic volume decreased during the observation. Open squares, sham group; filled circles, low-dose (2.5 mg/kg BW) anidulafungin group; filled squares, high-dose (25 mg/kg BW) anidulafungin group. Data are mean percentages of baseline levels ± SEM; $n = 6$/group.

pressure (ABP), stroke volume (SV), left ventricular end-diastolic volume (EDV), and heart rate (HR) were recorded.

Determination of mitochondrial enzyme activities. Left ventricular (LV) tissues were homogenized in a solution containing 50 mM Tris buffer (pH 7.5), 100 mM potassium chloride, 5 mM $MgCl_2$, and 1 mM EDTA using a glass/glass homogenizer (2 ml, 0.025 mm clearance; Kontes Glass Co., Vineland, NJ). Enzymatic activities were spectrophotometrically measured at 30°C (Cary 50 photometer; Varian, Darmstadt, Germany). Each assay was performed at least in duplicate and normalized to citrate synthase (CS) activity and noncollagen protein (NCP). The analyses of the mitochondrial respiratory chain complexes NAD (NADH):coenzyme Q1 oxidoreductase (complex I), NADH:cytochrome *c* oxidoreductase (complex I + III), succinate:cytochrome *c* oxidoreductase (complex II + III), ubiquinone:cytochrome *c* oxidoreductase (complex III), cytochrome *c* oxidase (complex IV), and citrate synthase as mitochondrial marker enzyme were performed in accordance with a protocol described previously (17).

Real-time PCR analysis. Total RNA was isolated from frozen LV tissue using Tris Fast (Peqlab) according to the manufacturer's instructions. The quality and integrity of the RNA samples were confirmed by agarose gel electrophoresis. RNA concentration was determined by measuring UV absorption (NanoDrop 1000 spectrophotometer; Thermo Fisher Scientific, Wilmington, DE, USA). Reverse transcription of RNA samples (500 ng total RNA) was carried out for 30 min at 42°C using the SuperScript III First-Strand cDNA synthesis kit (Invitrogen). Real-time PCR (primer sequences) (Table 1) and data analysis were performed using the StepOnePlus quantitative PCR system (Applied Biosystems, Life Technologies GmbH, Darmstadt, Germany). Expressed 18S rRNA was used as a housekeeping gene. All PCR results were calculated using the delta threshold cycle (C_T) method and given as relative units to 18S rRNA concentrations. The relative copy number of mitochondrial DNA (mtDNA) per diploid nuclear genome was measured as described previously (18), using a fragment of mtDNA (cytochrome *c* oxidase [COX-1]) and a fragment of hypoxanthine phosphoribosyltransferase 1 (HPRT-1; housekeeping gene).

Statistical analysis. Hemodynamic results are expressed as the mean percentage of baseline levels plus or minus the standard error of the mean (SEM). Enzyme activity data are given as means ± SEMs for animals. All statistical analyses were performed using GraphPad Prism version 5.0 for Mac (GraphPad Software, La Jolla, CA, USA). Kaplan-Meier graphs were drawn for visualization of survival time, and the groups were compared using the log-rank test. For group comparison, we used the global Kruskal-Wallis test followed by Dunn's multiple comparison test. A P value of ≤0.05 was considered significant.

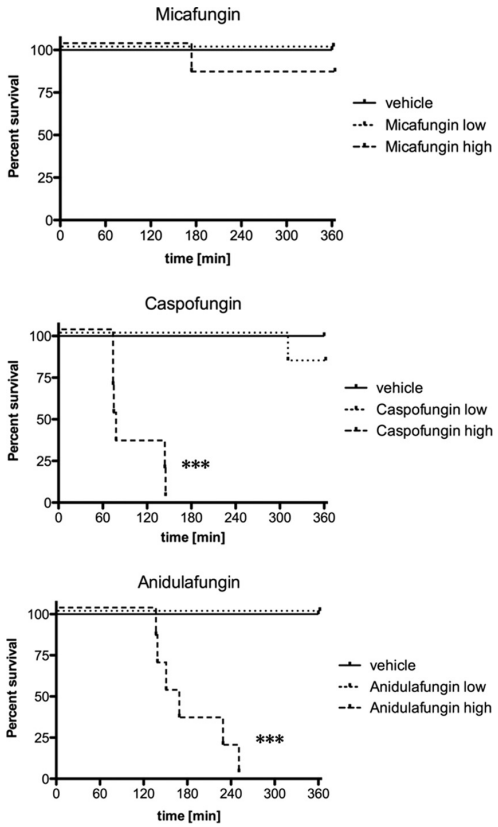

FIG 2 Survival analysis (Kaplan-Meier curves) of controls and rats treated with micafungin, caspofungin, and anidulafungin. (Top) Control and micafungin-treated animals. No animal died in the control group or the group treated with low-dose micafungin. In the high-dose micafungin group, 5 out of 6 animals survived. (Middle) Control and caspofungin-treated animals. Rats treated with high-dose caspofungin died within 150 min after drug administration. Five out of 6 rats in the low-dose group survived during the observation period. (Bottom) Control and anidulafungin-treated animals. Animals treated with low-dose anidulafungin survived during the observation period. Those treated with high-dose anidulafungin showed significantly increased mortality. Data are mean percentages of baseline levels ± SEM. ***, $P < 0.001$.

RESULTS

Hemodynamic measurements in untreated rats over 6 h under anesthesia. All untreated controls ($n = 6$) survived the observation period. We found no change in CO (53 ± 21 ml/min at baseline versus 69 ± 20 ml/min at 6 h) (Fig. 1B), EF (69% ± 9% at baseline versus 55% ± 9% at 6 h) (Fig. 1C), and ABP (63 ± 6 mm Hg at baseline versus 51 ± 11 mm Hg at 6 h) (Fig. 1A). EDV increased slightly over the observation period (200 ± 55 μl at baseline versus 341 ± 47 μl at 6 h) (Fig. 1D).

Time to hemodynamic failure in low-dose- and high-dose-treated rats. All animals in the control group ($n = 6$) survived during the observation period ($t = 360$ min). The times to hemodynamic failure in the high-dose anidulafungin group (175 min versus 360 min; $P < 0.001$) and the high-dose caspofungin group (94 min versus 360 min; $P < 0.001$) were significantly reduced compared to those of the untreated controls (Fig. 2). Among the anidulafungin and caspofungin high-dose groups ($n = 6$ each), hemodynamic failure was observed in all animals. Animals in all the low-dose groups did not show significant differences compared to the placebo group in time to hemodynamic failure. In the high-dose micafungin group, 5 out of 6 rats survived during the observation period, while 1 animal showed hemodynamic failure 150 min after the start of infusion.

Hemodynamic measurements in treated animals. CO, EF, ABP, and EDV did not differ between the low-/high-dose micafungin groups and the nontreated controls during the experiments (Fig. 3A to D). Rats treated with low-dose anidulafungin or caspofungin also showed no significant differences in hemodynamic parameters (Fig. 1 and 4).

Animals treated with high-dose anidulafungin showed significant decreases in ABP (Fig. 1A) and EDV (Fig. 1D) that resulted in a consecutive decrease in CO (Fig. 1B) during our experiments. All animals treated with high-dose anidulafungin died during the observation period, and similar results were obtained after treatment with high-dose caspofungin. In the latter group, rats also showed a massive decrease in ABP (Fig. 4A) and CO (Fig. 4B). Each animal died during the first 120 min after the beginning of

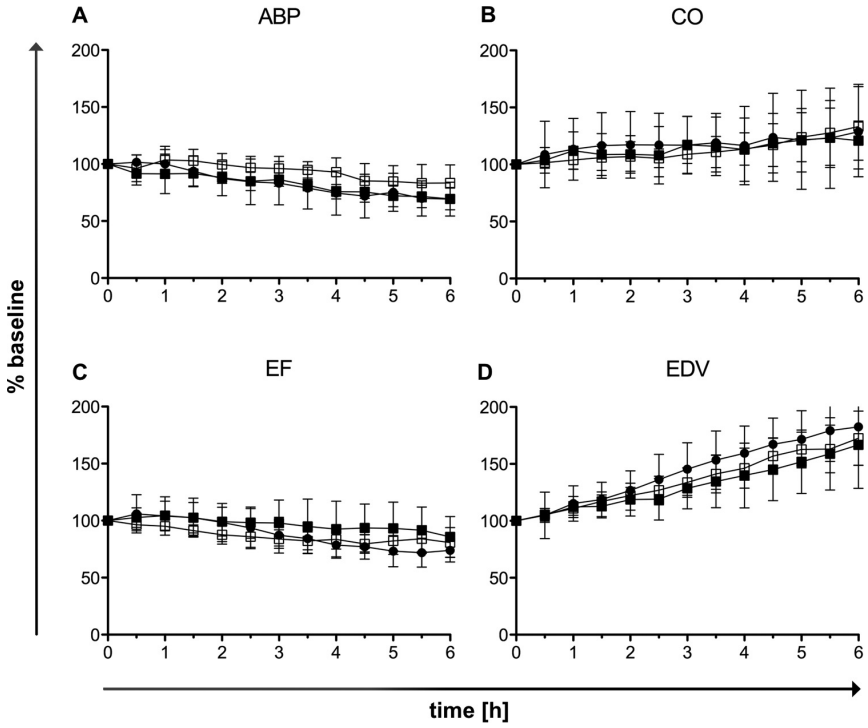

FIG 3 Hemodynamic measurements in controls and rats treated with high- and low-dose micafungin. Low-dose and high-dose groups did not show significant changes compared to controls in arterial blood pressure (A), cardiac output (B), left ventricular ejection fraction (C), or left ventricular end-diastolic volume (D). Open squares, sham group; filled circles, low-dose (3 mg/kg BW) micafungin group; filled squares, high-dose (30 mg/kg BW) micafungin group. Data are mean percentages of baseline levels ± SEM; $n = 6$/group.

drug administration. In contrast to animals receiving high-dose anidulafungin, those receiving high-dose caspofungin presented no significant decrease in EF (Fig. 4C).

Mitochondrial enzyme activities in echinocandin-treated animals. Spectrophotometric measurements of LV mitochondrial enzyme activity (complexes I to III, cytochrome *c* oxidase, succinate dehydrogenase) were performed. Results were normalized to citrate synthase activity. High-/low-dose anidulafungin groups, high-/low-dose caspofungin groups, and the low-dose micafungin group did not differ from the control group (Fig. 5). The high-dose micafungin group showed a significant reduction compared to the control group in complex III activity ($P < 0.05$) (Fig. 5B). No differences in COX-1 were observed between the control and treated groups. Regarding succinate dehydrogenase (SDH), we found a significant decrease in animals treated with low-dose micafungin ($P < 0.05$) (Fig. 5F).

Influence of echinocandin administration on LV mitochondrial gene expression. The mRNA expressions of ND-1 (complex I), cytochrome *b* (CYTB, complex III), COX-1 (complex IV), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), nuclear respiratory factor 1 (NRF-1), and mitochondrial transcription factor A (Tfam) were measured to explore possible influences of echinocandin treatment on mitochondrial gene expression. Treated animals did not show significant changes compared to the control group in mRNA expression. Specifically, there was no induction of ND-1, CYTB, COX-1, PGC-1α, NRF-1, or Tfam (see Table S1 in the supplemental material). Accordingly, no significant differences in mtDNA content were observed between echinocandin-treated animals and the controls (data not shown).

DISCUSSION

Our present study reveals a dose-dependent cardiac depression with anidulafungin and caspofungin treatment in the *in vivo* rat model. The current data also showed significantly higher rates of hemodynamic failure in animals treated with high-dose anidulafungin or caspofungin during the observation period. To our knowledge, this is the first study using a left ventricular catheterization system in rats for longitudinal hemodynamic measurements following echinocandin treatment. Our current results are in line with our previous findings in isolated cardiomyocytes treated with different concentrations of echinocandins (14). In these experiments, we discovered a dose-dependent decrease in contractility after echinocandin administration. In agreement with the data on cardiac side effects seen in our ICU patients after echinocandin administration (12) and case reports of hemodynamic instability (19) and pulmonary edema (20) during anidulafungin administration, we found decreased cardiac function after treatment with anidulafungin and caspofungin. *Ex vivo* cardiotoxicity studies in rats performed with a Langendorff heart model revealed similar findings (13). We searched the FDA Adverse Events Reporting System (FAERS) database and found four

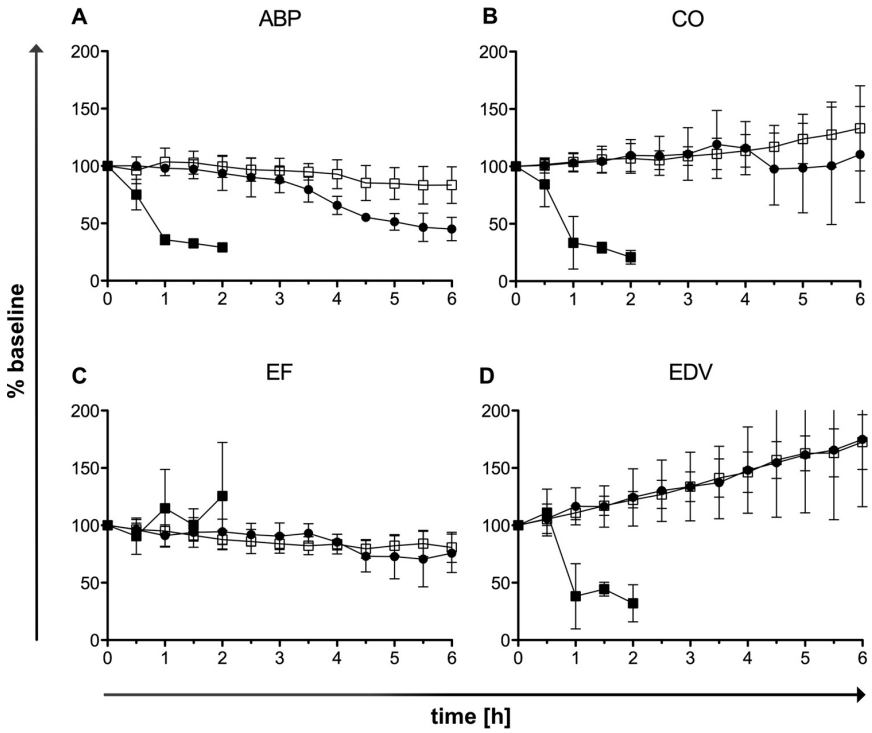

FIG 4 Hemodynamic measurements in controls and rats treated with high- and low-dose caspofungin. Animals treated with high-dose caspofungin showed a strong decrease in arterial blood pressure (A) and cardiac output (B). Left ventricular end-diastolic volume decreased during the observation (D). Rats treated with low-dose caspofungin showed no difference in left ventricular ejection fraction (C) or EDV. Open squares, sham group; filled circles, low-dose (0.875 mg/kg BW) caspofungin group; filled squares, high-dose (8.75 mg/kg BW) caspofungin group. Data are mean percentages of baseline levels ± SEM; $n = 6$/group.

cases of arrhythmias and cardiac failure which may also reflect cardiac events after echinocandin administration (21). However, in a search of phase 2 and 3 drug studies, we did not find reports of adverse cardiac events after echinocandin application. Otherwise, cardiovascular decompensation in critically ill patients is common, so echinocandin-related side effects may have been considered a result of the primary disease. Stover et al. (13, 15) suggest that there are different possible mechanisms of drug-induced cardiotoxicity, including direct toxic effects, alterations in mitochondrial oxidative function, modulation of cardiac gene expression, alterations in myocyte protein synthesis/function, apoptosis, oxidative stress/free-radical generation, neurohormonal activation, and arrhythmia (22, 23).

In our model of continuous *in vivo* hemodynamic measurement in rats, we examined hemodynamic parameters following echinocandin administration. In animals treated with high-dose anidulafungin or caspofungin, we found an immediate decrease in cardiac output starting 30 min after the beginning of drug administration. Correspondingly, SV, LV EDV, and ABP decreased in these two high-dose groups. These findings may reflect an increased ventricular contraction that leads to a reduction in end-diastolic volume and consecutive failure of the Frank-Starling mechanism, which leads to a depression in cardiac output. In contrast to the measured decrease in EF in the high-dose caspofungin group, we found an increase in EF in the high-dose anidulafungin group compared to baseline. This fact might reflect different mechanisms that lead to impaired left ventricular function.

We performed spectrophotometric measurements of LV mitochondrial enzyme activity in cardiac tissue samples in order to investigate the underlying mechanisms of echinocandin-induced cardiac failure. We did not see an association between LV mitochondrial enzyme activity and hemodynamic dysfunction. Further analyses of mRNA did not demonstrate echinocandin-induced changes in mitochondrial gene expression in the study animals.

Animals treated with low-dose echinocandins, at a dosage commonly used in patients, showed no significant decrease in cardiac function. Because of these findings, we suggest a dose-dependent component in echinocandin-induced cardiac toxicity, but the underlying mechanisms of the described effects need further evaluation. Nevertheless, according to the findings of Stover et al. (13), micafungin did not cause relevant changes in cardiac function. Stover and colleagues hypothesized that micafungin, which is water soluble compared with the other two lipophilic agents, would be unable to penetrate the tissue and cause serious cell damage.

In our experiments, we found no evidence for the hypothesis that arrhythmia, as a side effect of echinocandin treatment, was the reason for the demonstrated changes in cardiac function. Arrhythmias were not described after antifungal drug administration in the Langendorff studies or in our ICU patients (12, 13). The effects of echinocandin treatment observed in our *in vivo* and *in vitro* studies, in the Langendorff heart studies, and in the published case reports were rapid. Therefore, modulations of cellular

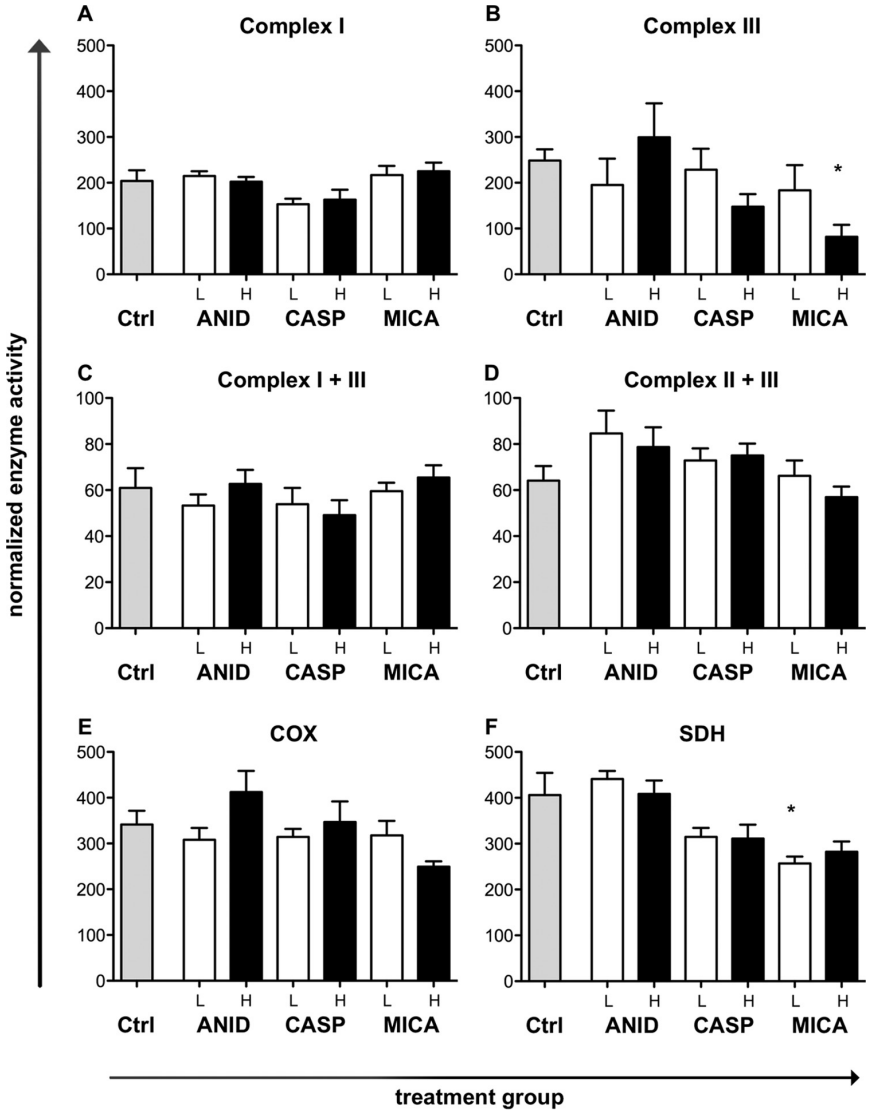

FIG 5 Spectrophotometric measurement of LV mitochondrial enzyme activity (complexes I to III, cytochrome *c* oxidase, succinate dehydrogenase) in left ventricular tissue samples of echinocandin-treated rats. Ctrl, sham animals; L, low dose; H, high dose. Data are means ± SEM; $n = 6$/group. *, $P < 0.05$ compared to control.

gene expression, alterations in cardiac protein synthesis, and neurohormonal activation are unlikely causes of the observed cardiac effects. Other possible mechanisms of toxicity are oxidative stress and the generation of free radicals, which were not analyzed in the present study. In summary, our findings revealed a dose-dependent cardiac depression in an *in vivo* rat model. The drug concentrations used in our studies were equal to or 10-fold higher than clinical dosages used in humans. Previous studies explored concentrations of caspofungin in ICU patients that were even higher than those used in healthy volunteers (24). Also, caspofungin peak plasma concentrations in healthy patients can reach 20 μg/ml (25), and preclinical distribution studies in animals revealed that heart tissue reaches about 30% of plasma concentrations (26–28). Anidulafungin plasma peak concentrations were measured up to 14 μg/ml. Micafungin peak plasma concentrations have reached 8.8 μg/ml (29).We conclude that rapid infusion of loading or maintenance doses via central venous catheters in critically ill patients with impaired cardiac function might lead to high echinocandin peak concentrations and cardiac depression in humans. Patients with sepsis-induced cardiomyopathy especially may be at risk for further aggravation of cardiac dysfunction following echinocandin administration due to the existing inflammation-induced endothelial damage. Nevertheless, our study only provides data for doses clinically equivalent to those used in humans and

for doses that are 10-fold higher. Further studies are necessary to delineate the potential dose dependence of the effect. Moreover, we did not reveal the mechanism that is responsible for our findings. Taking into consideration the results in isolated cardiomyocytes treated with echinocandins from our earlier *in vitro* study (14), we hypothesize that the observed effect might be driven by echinocandin-induced alterations in calcium homeostasis or direct toxic effects, but these mechanisms need further exploration. Randomized controlled trials are needed to explore drug-induced cardiac toxicity in critically ill patients and advise safe and effective antifungal treatment. Mechanisms leading to echinocandin-induced heart failure also need further evaluation.

In conclusion, echinocandin administration in an *in vivo* rat model of hemodynamic measurement reduces cardiac function and time to hemodynamic failure. Changes in mitochondrial function or mitochondrial biogenesis are unlikely causes of this cardiac dysfunction. This study may influence antifungal treatment of critically ill patients with impaired cardiac function. Further randomized controlled trials in ICU patients are needed to determine the cardiotoxicity of echinocandins.

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8.5 Anlage 5

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Cardiac Effects of Echinocandins in Endotoxemic Rats

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Echinocandins are known as effective and safe agents for the prophylaxis and treatment of different cohorts of patients with fungal infections. Recent studies revealed that certain pharmacokinetics of echinocandin antifungals might impact clinical efficacy and safety in special patient populations. The aim of our study was to evaluate echinocandin-induced aggravation of cardiac impairment in septic shock. Using an *in vivo* endotoxemic shock model in rats, we assessed hemodynamic parameters and time to hemodynamic failure (THF) after additional central-venous application of anidulafungin (2.5 mg/kg of body weight [BW]), caspofungin (0.875 mg/kg BW), micafungin (3 mg/kg BW), and control (0.9% sodium chloride). In addition, echinocandin-induced cytotoxicity was evaluated in isolated rat cardiac myocytes. THF of the animals in the caspofungin group ($n = 7$) was significantly reduced compared to that in the control ($n = 6$) (136 min versus 180 min; $P = 0.0209$). The anidulafungin group ($n = 7$) also showed a trend of reduced THF (136 min versus 180 min; log-rank test $P = 0.0578$). Animals in the micafungin group ($n = 7$) did not show significant differences in THF compared to those in the control. Control group animals and also micafungin group animals did not show altered cardiac output (CO) during our experiments. In contrast, administration of anidulafungin or caspofungin induced a decrease in CO. We also revealed a dose-dependent increase of cytotoxicity in anidulafungin- and caspofungin-treated cardiac myocytes. Treatment with micafungin did not cause significantly increased cytotoxicity. Further studies are needed to explore the underlying mechanism.

Sepsis is one of the most serious and urgent infectious conditions in clinical practice. Although bacterial infections are the main cause of sepsis, fungal infections also represent a relevant risk factor for critically ill intensive care unit (ICU) patients (1, 2). Echinocandins (anidulafungin [ANID], caspofungin [CASP], and micafungin [MICA]) are an established class of antifungal agents recommended for the empirical and specific treatment of invasive *Candida* infections and aspergillosis (3, 4).

Randomized controlled trials and meta-analysis described echinocandins as effective and safe agents for the prophylaxis and treatment of different cohorts of patients with (suspected) fungal infections (5). Nevertheless, evidence is mounting that the pharmacokinetics of echinocandins in special patient populations prone to systemic inflammatory responses (e.g., critical ill patients, burn patients, patients with severe organ dysfunction) may impact clinical efficacy and safety (6).

Recent case reports describe adverse cardiac effects following echinocandin administration, especially in certain cohorts of critical ill patients (7–9). Also, in experimental studies using isolated rat hearts (Langendorff model) (10) or isolated cardiomyocytes of the rat (11), echinocandins were found to impair function properties.

Hemodynamic measurements after central venous administration of high doses of anidulafungin or caspofungin in adult rats provided evidence of significantly reduced cardiac output, which was associated with a significantly reduced survival rate compared to that in control animals (12). These results, which suggest a dose-depending mechanism of echinocandin-induced cardiac depression, were also confirmed by a study of rats treated with different doses of echinocandins (13). Previous investigations claimed mitochondrial toxicity to represent the underlying mechanism behind echinocandin-induced cardiac failure (10, 13, 14, 28). However, spectrophotometric measurements in echinocandin-treated rats did not reveal any altered mitochondrial enzyme activity (12).

Septic shock is characterized by impaired hemodynamic function, microcirculatory alterations, and mitochondrial damage, which all reduce cellular energy production. The mechanisms of sepsis-induced cardiac dysfunction include the attenuation of the adrenergic response on the cardiomyocyte level, alterations of intracellular calcium trafficking, and blunted calcium sensitivity of contractile proteins (15). Hypothesizing an echinocandin-induced aggravation of cardiac impairment in patients already suffering from septic shock, we performed *in vivo* hemodynamic measurements in endotoxemic rats.

MATERIALS AND METHODS

Animals. Male Lewis rats (275 to 300 g) were obtained from Charles River (Sulzfeld, Germany). All procedures involving animals were conducted in compliance with standards for animal experiments and were approved by the local committee for animal care (GI 20/26 Nr.3/2012, JLU-Nr. 540_M; Regierungspraesidium Giessen, Germany). Studies were performed in anesthetized rats using isoflurane (Baxter, Unterschleissheim, Germany).

Experimental groups. Animals were randomly assigned into 4 study groups: the anidulafungin (ANID) group, which received 2.5 mg/kg of body weight (BW) (Ecalta; Pfizer, NY, USA) plus lipopolysaccharides (LPS) (1 mg/kg BW) ($n = 7$) (16); the caspofungin (CASP) group, which received 0.875 mg/kg BW (Cancidas; Merck, NJ, USA) plus LPS (1 mg/kg

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BW) ($n = 7$) (17); the micafungin (MICA) group, which received 3 mg/kg BW (Mycamine; Astellas Parma, Inc., IL, USA) plus LPS (1 mg/kg BW) ($n = 7$) (18); and the control group, which received 0.9% sodium chloride (NaCl) plus LPS (1 mg/kg BW) ($n = 6$). The echinocandins and LPS were dissolved in 0.9% sodium chloride, and drug dosage was adjusted to follow the specifications given by the manufacturers. As these clinically used dosages are not adjusted to the patient's body weight, we supposed a "standard patient" of 75 kg for our calculations, resulting in the concentrations given above. Hemodynamic data were measured over a period of 1 h or until hemodynamic failure after echinocandin or placebo administration. After surgical treatment and a stabilization period of 30 min (but before drug administration), the baseline of each parameter was measured and was used for measurement normalization in each animal individually. Survival time was recorded over a period of 3 h after echinocandin or placebo administration.

***In vivo* hemodynamic rat model.** Animals were handled and hemodynamic data were obtained as described previously (12, 27). Briefly, after endotracheal intubation with a 16G catheter, animals were ventilated with a rodent respirator (Inspira; Harvard Apparatus, MA, USA) using volume-controlled ventilation in a weight-adjusted manner. Ringer solution (10 ml/kg/h; Braun, Melsungen, Germany) and fentanyl (10 μg/kg/h; Ratiopharm, Ulm, Germany) were continuously administered intravenously over the lateral tail vein with a syringe pump (Braun, Melsungen, Germany). Experimental agents (anidulafungin, caspofungin, or micafungin) were administered via a central venous catheter placed in the right jugular vein using a syringe pump (Harvard Apparatus, MA, USA) over a period of 1 h. Simultaneously, animals received a lipopolysaccharide (LPS) bolus (LPS-EB Ultrapure from *Escherichia coli* O111:B4 strain TLR4 ligand; InvivoGen, San Diego, CA, USA) (1 mg/kg in 1 ml saline intravenously over tail vein) for >20 min via a syringe pump (Harvard Apparatus). LPS are the major constituents of the outer membrane of Gram-negative bacteria and are recognized by the Toll-like receptor 4 (TLR4), which is expressed from many cell types (e.g., monocytes), leading to a strong activation of the immune cells and to subsequent cytokine secretion. As a result of their amphiphile structure, LPS are capable of forming micelles in aqueous solution. Therefore, it was sonicated for 30 min before injection. Body temperature was kept at about 37°C during the experiment using a feedback-controlled heat pad and an infrared heater. Arterial blood pressure was also measured continuously using a Mikro-Tip catheter (SPR-1000; Millar Instruments, Houston, TX, USA) inserted in the animal's tail artery. Cardiac function of the left ventricle was measured using a pressure-volume conductance catheter (SPR-838; Millar, Houston, TX, USA) (20). Recorded data were analyzed using PVAN 1.1 (Millar Instruments, Houston, TX, USA). Hemodynamic parameters, including stroke volume (SV; volume of blood pumped from the left ventricle per contraction), cardiac output (CO; cumulative volume of blood pumped from the left ventricle per time unit, given in this study in milliliters per minute), left ventricular ejection fraction (EF; fraction [%] of outbound blood pumped from the left ventricle with each contraction), arterial blood pressure (ABP), left ventricular end-diastolic volume (EDV; volume of blood within the left ventricle at the end of the filling phase [diastole]), and heart rate (HR), were recorded. After euthanasia, blood samples were collected and centrifuged. The plasma was stored at −80°C.

Isolation of cardiomyocytes. Ventricular cardiac myocytes were isolated from Lewis rats as described previously (21). Briefly, hearts from Lewis rats (age 3 to 4 months) were excised under deep isoflurane anesthesia and were mounted on the cannula of a Langendorff perfusion system. Cardiomyocytes were isolated via perfusion with collagenase, which was followed by mincing and filtering and by transfer to culture medium M199 supplemented with carnitine (2 mM), creatine (5 mM), and taurine (5 mM).

Cell viability assay and LDH plasma level. The effect of different dosages of echinocandins on rat cardiac myocyte cytotoxicity was determined using the lactate dehydrogenase (LDH) assay method. Freshly isolated rat cardiac myocytes were seeded at a density of 3,200 cells per well in a 96-well plate and were incubated for 2 h. They were then treated in triplicate with ANID, CASP, or MICA concentrations of 1, 2.5, 5, 10, 15, 20, 50, or 100 μg/ml and were incubated for another 20 h. The LDH released from cells was measured by a cytotoxicity detection kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions. The background control values were subtracted, and the mean percentage of treatment-induced cytotoxicity for each sample was calculated using the following equation: 100 × [(experimental value − spontaneous release)/(maximum release − spontaneous release)]. Spontaneous release was defined as the LDH activity in the supernatant of untreated samples, while maximum release was measured from untreated cell samples lysed by Triton X-100, reflecting the total capacity of intracellular LDH within the sample. Plasma LDH levels were also analyzed using the described cytotoxicity assay. At the end of the observation period or at the appearance of hemodynamic breakdown, animal blood samples were taken and plasma was separated. Samples were diluted 1:10 and optical density (OD) was measured. The absorbance at 490 nm was read using an automated plate reader (Epoch; BioTek Instruments GmbH, Heilbronn, Germany).

Statistical analysis. Hemodynamic results are expressed as the mean percentage of the baseline level plus or minus the standard error of the mean (SEM). Cytotoxicity data are given as a normalized percentage of cytotoxicity of each LDH test sample plus or minus the SEM. Plasma LDH levels were expressed as mean OD plus or minus the SEM. All statistical analyses were performed using GraphPad Prism version 5.0 for Mac (GraphPad Software, La Jolla, CA, USA). Kaplan-Meier graphs were drawn for the visualization of survival time, and the groups were compared using the log-rank test. For group comparison, we used a global Kruskal-Wallis test followed by a Dunn's multiple comparison test. A P value of 0.05 or smaller was regarded as significant.

RESULTS

Hemodynamic measurements in control animals. Among the control animals receiving 0.9% sodium chloride (NaCl) plus LPS (1 mg/kg BW) ($n = 6$), all animals survived the whole observation period. We observed only slight changes in CO (t_0 versus t_1, 6.34% ± 11.07%) (Fig. 1A), EF (t_0 versus t_1, −8% ± 4.62%) (Fig. 1B), and ABP (t_0 versus t_1, −10.94% ± 4.34%) (Fig. 1C). EDV (t_0, 19.16% ± 6.14%) (Fig. 1D) increased over the observation period.

Time to hemodynamic failure. All animals ($n = 6$) of the control group (LPS plus 0.9% saline) survived the complete observation period ($t = 180$ min). The time to hemodynamic failure of the endotoxemic animals in the caspofungin group ($n = 7$) was significantly reduced compared to that in the control animals (Fig. 2B; log-rank test, $P = 0.0209$). The anidulafungin group ($n = 7$) also showed a trend to reduced time to hemodynamic failure (Fig. 2A; $P = 0.0578$). Animals of the micafungin group ($n = 7$) did not show any significant difference in time to hemodynamic failure compared to that in the animals of the control group (Fig. 2C).

Hemodynamic measurements. Hemodynamic data were recorded during the first hour of each experiment. All animals survived during that period. In control group animals and in micafungin group animals, we found an increased CO during the experiments, while the CO decreased in anidulafungin and caspofungin group animals compared to baseline CO (Fig. 1A). Similar results were found regarding the animals' SV (data not shown). Further, our experiments revealed no alterations to the EF of the control group or of the micafungin group animals, while anidulafungin and caspofungin group animals showed a reduced EF (Fig. 1B). Common over all experimental groups, we observed a time-dependent decrease of ABP during the observation period (Fig. 1C). Also, all groups showed an at least slight tendency toward an increased EDV (Fig. 1D). Finally, we did not find any changes in

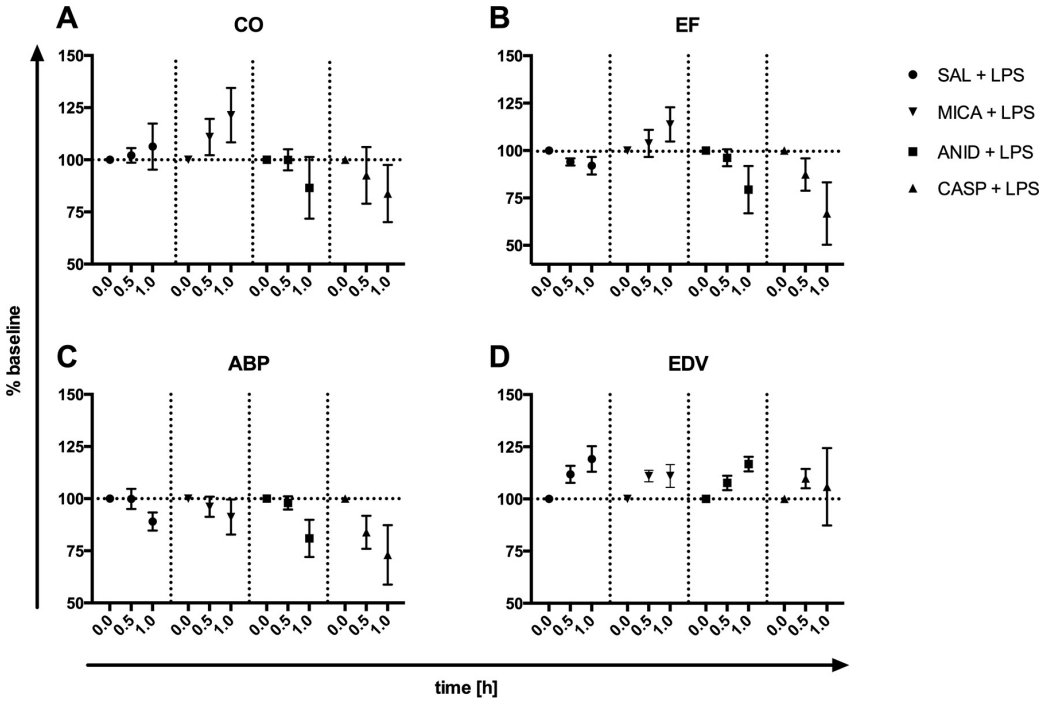

FIG 1 Hemodynamic measurements in endotoxemic rats. Hemodynamic measurements in the control (SAL+LPS; 0.9% NaCl + 1 mg/kg BW lipopolysaccharide [LPS]; $n = 6$), the anidulafungin group (ANID+LPS; 2.5 mg/kg BW + 1 mg/kg BW, respectively; $n = 7$), the caspofungin group (CASP+LPS; 0.875 mg/kg BW + 1 mg/kg BW, respectively; $n = 7$), and the micafungin group (MICA+LPS; 3 mg/kg BW + 1 mg/kg BW, respectively; $n = 7$). (A) Cardiac output (CO). (B) Left ventricular ejection fraction (EF). (C) Arterial blood pressure (ABP). (D) Left ventricular end-diastolic volume (EDV). SAL, saline (0.9% sodium chloride). Data are the mean percentage of the baseline level ± SEM.

HR among the different groups during the observation period (data not shown).

Plasma lactate dehydrogenase. At the end of the observation period or at the appearance of hemodynamic breakdown, blood samples of each study animal were taken and serum LDH levels were measured and expressed as mean OD. The highest values were found among the anidulafungin group animals. The mean OD values of the caspofungin group and of the micafungin group did not differ from that of the control group (Fig. 3A).

Cell viability assay. In order to link the observed effects to an actual influence of echinocandins on the cardiomyocytes, we assessed the cytotoxicity of the compounds by the release of LDH on freshly isolated rat cardiac myocytes. This revealed a dose-dependent increase of cytotoxicity in anidulafungin- ($n = 4$) and caspofungin-treated ($n = 6$) cardiac myocytes. Treatment with micafungin ($n = 4$) did not cause a significant increase in cytotoxicity (Fig. 3B).

DISCUSSION

Echinocandins are known as effective and safe agents for the prophylaxis and treatment of different cohorts of patients with fungal infections (5). Recent studies revealed that the pharmacokinetics of echinocandins in special patient populations might impact clinical efficacy and safety (6). Septic cardiomyopathy is a well-known organ dysfunction in sepsis, which is associated with reduced left ventricular contractility (19). Our study demonstrates cardiac depression and a reduced time to hemodynamic failure after administration of clinically used doses of anidulafungin (2.5 mg/kg BW) and caspofungin (0.875 mg/kg BW) in endotoxemic rats. In contrast, administration of micafungin (3 mg/kg BW) did not alter cardiac function or survival time.

Recent clinical case reports demonstrated unsuspected side effects after echinocandin treatment (7–9). In our previous studies, we discovered a dose-dependent decrease of contractility after echinocandin administration in isolated cardiomyocytes (11). Furthermore, in our model of continuous *in vivo* hemodynamic measurement in rats, we examined hemodynamic parameters following echinocandin administration. In animals treated with high doses of anidulafungin (25 mg/kg BW) or caspofungin (8.75 mg/kg BW), we found an immediate decrease of CO and a reduced survival time compared to those of the control and those of animals treated with low doses (anidulafungin group, 2.5 mg/kg BW; caspofungin group, 0.875 mg/kg BW). Micafungin (low-dose group, 3 mg/kg BW; high-dose group, 30 mg/kg BW) administration had no effect on cardiac function or survival time (12), a finding which holds true in this study using endotoxemic rats. Furthermore, *ex vivo* cardiotoxicity studies in rats performed with a Langendorff heart model and hemodynamic measurements in rats using echocardiography revealed similar findings (10, 13). The echinocandin doses used in our experiments reflect manufacturers' recommendations for human use (16–18). Other authors used even higher echinocandin concentrations in murine models to take into account the higher metabolism rate, the higher heart

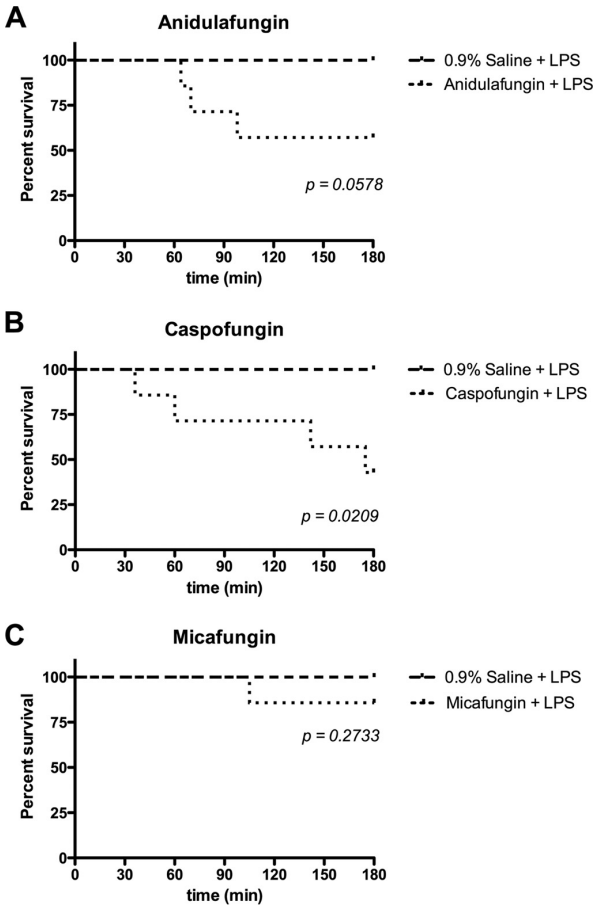

FIG 2 Time to hemodynamic failure in endotoxemic rats. Kaplan-Meier curves of control (0.9% NaCl + 1 mg/kg BW LPS; $n = 6$) and echinocandin-treated animals. (A) In the anidulafungin group (2.5 mg/kg BW + 1 mg/kg BW LPS; $n = 7$) versus the control group, 3 out of 7 animals died during the observation period (log-rank test, $P = 0.0578$). (B) In the caspofungin group (0.875 mg/kg BW + 1 mg/kg BW LPS; $n = 7$) versus the control group, 4 out of 7 animals died during the observation period (log-rank test, $P = 0.0209$). (C) In the micafungin group (3 mg/kg BW + 1 mg/kg BW LPS; $n = 7$) versus the control group, 1 out of 7 animals died during the observation period (log-rank test $P = 0.2733$). Data are mean percentages of survival.

frequency, and different plasma protein binding (13). In the absence of reliable metabolism data in rats, especially in systemic inflammatory conditions, we decided to test concentrations comparable to clinical use.

Especially in surgical ICU patients, bacterial infections are the main cause of sepsis. Nevertheless, mixed infections of bacterial and fungal pathogens are also very common in these patients. In clinical routine, the discrimination between bacteria- and fungal-induced systemic immune response is not feasible. Due to that fact, empirical echinocandin therapy in patients that may suffer from bacterial or mixed infections is common practice in ICUs. Considering this fact, and in order to use a standardized and well-known model, we chose LPS as the immunological agent to induce systemic inflammation and shock for our experiments. LPS are the major component of the outer membrane of Gram-negative bacteria and act as potent antigens for the immune system causing inflammatory reactions in mammalians after recognition by the Toll-like receptor 4 (TLR4), which is expressed from many cell types but especially from monocytes, dendritic cells, macrophages, and B cells. Binding promotes the secretion of proinflammatory cytokines, nitric oxide, and eicosanoids.

Performing hemodynamic measurement in endotoxemic rats, we aimed to evaluate hemodynamic alterations following echinocandin administration in an endotoxin shock model. Central venous administration of anidulafungin plus LPS or caspofungin plus LPS caused immediate decreases in CO, SV, ABP, and EDV. These results may reflect impaired cardiac contractility together with LPS-induced peripheral vasodilatation. This fatal combination can synergistically result in a reduction of end-diastolic volume and in consecutive failure of the Frank-Starling mechanism, which leads to decreased CO. Investigating our hypothesis of a dose-dependent mechanism, we performed cytotoxicity studies using an LDH assay in freshly isolated rat cardiac myocytes. Corresponding to the results of our hemodynamic measurements, we again found a dose-dependent increase in cytotoxicity in cells treated with anidulafungin or caspofungin. Interestingly, according to our *in vivo* data, micafungin-treated cells did not show an increased rate of cytotoxic death. Regarding the LDH serum levels of our *in vivo* rat studies, we only found a trend toward elevated LDH values in anidulafungin-treated animals compared to that in control group animals. These results are in line with data form previous studies that did not observe micafungin-induced cardiac impairment (10–13). This fact may be explained by the hypothesis that micafungin, which is water-soluble compared with the other two lipophilic agents, would be unable to penetrate the tissue and cause serious cell damage.

Investigating the underlying mechanism leading to echinocandin-induced cardiac failure, we previously searched for alterations in cardiac mitochondrial function. Performing spectrophotometric measurements of rat left ventricular cardiac tissue after echinocandin treatment, we were not able to detect any altered mitochondrial enzyme activity (12). Previous studies on septic cardiomyopathy demonstrated the importance of mitochondrial dysfunction and reduced ATP generation (22, 23). Moreover, studies using isolated cardiomyocytes found that endotoxins alter or suppress the L channel-dependent calcium flow, possibly through changes in autonomic regulation of this channel (24, 25). These mechanisms caused a reduced concentration of intracellular calcium and a decrease in fiber contractility. With respect to our earlier results regarding isolated cardiomyocytes treated with echinocandins (14) and the results of our hemodynamic measurements, we would hypothesize that alterations in calcium homeostasis induced by echinocandins might be one potential harmful mechanism of action.

Beside these effects, vascular pathologies may contribute to the described results. Endotoxemia also has been described to induce capillary leakage by loosening epithelial tight junctions (26). Impaired vascular barrier function during endotoxemia may lead to elevated drug levels in the myocardium. Thus, vascular dysfunction may represent another important factor that aggravates echinocandin-induced cardiac impairment. Manufacturers recommend slow intravenous infusion of all echinocandins (anidulafungin, caspofungin, micafungin) over about 1 h (16–18). Rapid infusion of loading or maintenance doses over central venous

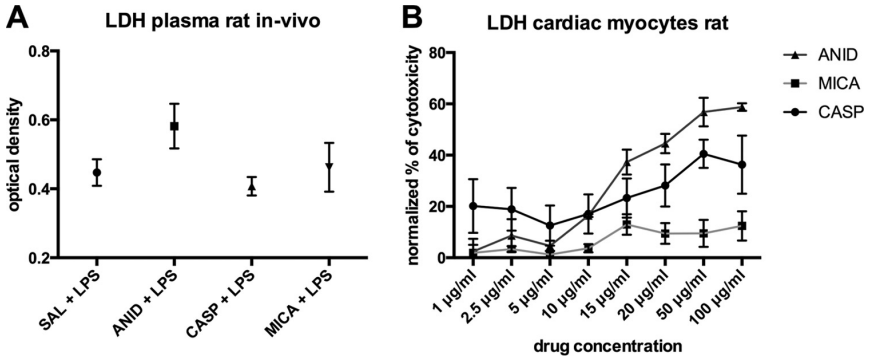

FIG 3 Plasma lactate dehydrogenase and cytotoxicity analyses. (A) Lactate dehydrogenase (LDH) plasma measurements in the control group (SAL+LPS; 0.9% NaCl + 1 mg/kg BW, respectively; $n = 6$), the anidulafungin group (ANID+LPS; 2.5 mg/kg BW + 1 mg/kg BW, respectively; $n = 7$), the caspofungin group (CASP+LPS; 0.875 mg/kg BW + 1 mg/kg BW LPS, respectively; $n = 7$), and the micafungin group (MICA+LPS; 3 mg/kg BW + 1 mg/kg BW LPS, respectively; $n = 7$) animals after appearance of hemodynamic failure or at the end of the observation period. Data are mean optical density (OD) ± SEM. (B) Cytotoxicity analyses in isolated rat cardiac myocytes after incubation with different doses of anidulafungin (ANID), caspofungin (CASP), or micafungin (MICA). Data are normalized percentages of cytotoxicity ± SEM.

catheters that lead to a high echinocandin peak concentration may increase the risk for cardiac depression.

In summary, our results revealed that anidulafungin and caspofungin dosages that were similar to clinically used dosages in humans caused acute cardiac dysfunction in endotoxemic rats. In addition, we showed that anidulafungin and caspofungin induced dose-dependent cytotoxic effects in isolated rat cardiac myocytes. We conclude that patients suffering from severe infections may be at risk for a further aggravation of cardiac dysfunction following echinocandin administration.

Nevertheless, our study has some limitations. Pharmacokinetic and pharmacodynamic parameters vary between rats and humans, resulting in changed peak concentrations. Despite a higher basal rate of metabolization in rats, we found cardiac depression even after administration of comparable low dosages, which may be important information for health care professionals treating critically ill patients. At this time, we are not able to track down the harmful molecular mechanism that is responsible for the observed effects. Regarding the primary results of contractility studies in isolated cardiomyocytes, hemodynamic measurements, mitochondrial enzyme analyses, and cytotoxicity measurements, we would suggest that the described results may be triggered by alterations in calcium homeostasis or direct toxic effects. The mechanisms leading to echinocandin-induced heart failure will need further careful evaluation.

In conclusion, in our model of continuous hemodynamic measurements in endotoxemic rats, intravenous administration of anidulafungin or caspofungin was associated with acute cardiac dysfunction. Second, administration of anidulafungin or caspofungin in our model reduced survival. In addition, our studies revealed dose-dependent cytotoxicity in isolated cardiac myocytes exposed to anidulafungin or caspofungin. Further studies are needed to explore the underlying mechanism.

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8.6 Anlage 6

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MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS

Caspofungin Modulates Ryanodine Receptor-Mediated Calcium Release in Human Cardiac Myocytes

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ABSTRACT Recent studies showed that critically ill patients might be at risk for hemodynamic impairment during caspofungin (CAS) therapy. The aim of our present study was to examine the mechanisms behind CAS-induced cardiac alterations. We revealed a dose-dependent increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) after CAS treatment. Ca^{2+} ions were found to be released from intracellular caffeine-sensitive stores, most probably via the activation of ryanodine receptors.

KEYWORDS antifungal agents, adverse drug reaction, cardiac output, cardiac toxicity

Caspofungin (CAS) is known as the first echinocandin antifungal agent recommended for the empirical and specific treatment of invasive *Candida* infections and aspergillosis (1, 2). According to several clinical case reports describing adverse cardiac events during echinocandin therapy in critically ill patients, animal studies revealed an altered contractility of isolated rat cardiomyocytes after CAS administration (3–6). According to these results, studies in isolated heart models treated with CAS also found impaired cardiac function (7). In line with this, after central venous administration of CAS, rats were found to exhibit impaired left ventricular function associated with reduced survival rates (8, 9). Furthermore, hemodynamic measurement in endotoxemic rats, mimicking the clinical setting, revealed that CAS application induced cardiac impairment even at subclinical concentrations (10). Therefore, we suggested that mechanisms similar to septic cardiomyopathy might be causative (4–6, 10–13). The aim of our study was to investigate the impact of CAS on intracellular calcium homeostasis in rat and human cardiac myocytes.

First, we identified CAS-induced human cardiac myocyte (HCM) cytotoxicity using a cell viability assay of cultured HCMs (C-12810; PromoCell GmbH, Heidelberg, Germany). Cytotoxicity increased dose-dependently after the administration of dosages of 50 μg/ml CAS and higher (13.50% $\pm$ 3.42%; $n = 4$). Dosages of >100 μg/ml were associated with severe cytotoxicity (100 μg/ml, 76.15% $\pm$ 7.03%; 140 μg/ml, 80.93% $\pm$ 10.79%; 150 μg/ml, 85.63% $\pm$ 8.89%; 200 μg/ml, 82.52% $\pm$ 11.84%; all $n = 4$).

Second, we showed a dose-dependent increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) oscillation frequency in freshly isolated rat cardiomyocytes, with significant effects at CAS concentrations of $\geq$50 μg/ml, by performing Ca^{2+} imaging with 2.5 μM Fura-2-AM-microscopy (methodology as described earlier [10]) (Fig. 1; $n_{CTRL} = 24$; $n_{CAS} = 10$ to 22 per group; 12.5 μg/ml, fold change [FC] = 1.13, $P = 0.2$; 25 μg/ml, FC = 1.11, $P = 0.5$; 50 μg/ml, FC = 1.60, $P < 0.001$; 100 μg/ml, FC = 2.28, $P < 0.01$; 200 μg/ml, FC = 2.74, $P < 0.001$). All experiments were approved by the local committee for animal care (JLU-no. 540_M; Regierungspraesidium, Giessen, Germany). CAS treatment (75 to 200 μg/ml) in HCMs caused a dose-dependent increase in $[Ca^{2+}]_i$ in physiologic calcium-containing buffer medium. Dosages of >130 μg/ml

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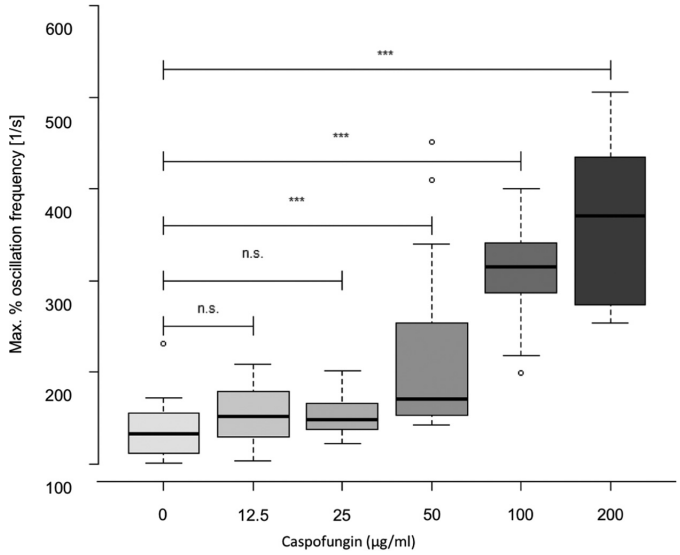

FIG 1 Boxplot diagram showing maximum (Max.) of % oscillation frequency in $1/s^{-1}$ under the influence of different dosages of CAS in rat cardiomyocytes. Boxplot whiskers represent confidence intervals of median. ***, $P < 0.001$; n.s., not significant.

were associated with a significant elevation in the Fura-2-AM ratio attributed to $[Ca^{2+}]_i$ ($n_{CTRL} = 20$; $n_{CAS} = 29$ to 40 per group; $P < 0.01$) of $[Ca^{2+}]_i$ with CAS >130 μg/ml and increased $[Ca^{2+}]_i$ between 1.6- and 3.3-fold (control <1-fold; Fig. 2). Furthermore, the CAS-dependent increase in $[Ca^{2+}]_i$ was also found in experiments involving Ca^{2+}-free buffer medium ($n_{CTRL} = 20$; $n_{CAS} = 30$ to 50 per group; $P < 0.01$) of $[Ca^{2+}]_i$ with CAS at >100 μg/ml and increased $[Ca^{2+}]_i$ between 2.2- and 2.4-fold (control <1-fold)) (Fig. 3).

Third, we were able to identify potential mechanisms of $[Ca^{2+}]_i$ release by measuring the ratio of Fura-2-AM fluorescence in HCM. While CAS-induced elevation in $[Ca^{2+}]_i$ was found in physiological and Ca^{2+}-free buffer media (Fig. 2A), CAS-induced (140 μg/ml) elevation of $[Ca^{2+}]_i$ was significantly reduced in the presence of caffeine (CAF; 30 mM) ($n_{CTRL} = 20$, $n_{Effect} = 40$; CAF+ versus CAF−, FC = 1.04; $P < 0.001$; Fig. 2B). Application of ryanodine (RYN; 40 μM) to inhibit ryanodine receptors prior to 140

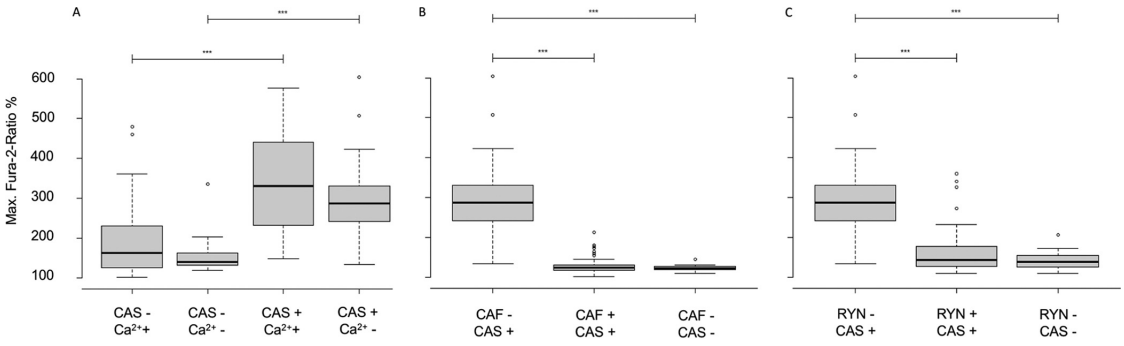

FIG 2 Boxplot diagram demonstrating the influence of physiological and Ca^{2+}-free buffer media, CAF, RYN, and CAS on changes in intracellular Ca^{2+} levels in individual rat cardiomyocytes determined by fluorescence microscopy of Fura-2-AM signal. Administration of substances is characterized with + (CAS +, 140 μg/ml CAS; Ca^{2+} +, 2.5 mM Ca^{2+}; RYN +, 40 μM RYN) and −. ***, $P < 0.001$.

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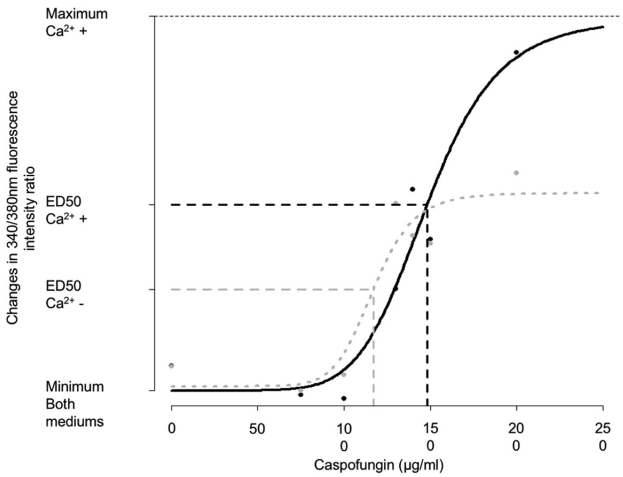

FIG 3 Dose-response curve of intracellular calcium levels measured with fluorescence microscopy in relation to different dosages of CAS. Ca^{2+} + and Ca^{2+} − symbolize Ca^{2+}-containing and Ca^{2+}-free buffer media, respectively. ED_{50}, 50% effective dose.

μg/ml CAS administration resulted in a significant suppression of $[Ca^{2+}]_i$ release (RYN $n_{CTRL} = 20$, $n_{Effect} = 40$; RYN+ versus RYN−, FC = 1.15; $P < 0.01$; Fig. 2C).

Echinocandins represent a well-established group of antifungal agents which are widely used for intensive care treatment in critically ill patients (1, 2). However, recent data raise doubts about their safe use in critically ill patients with regard to hemodynamic stability (14–16). Several cases of severe hemodynamic instability following echinocandin infusion were reported, which led to the definition of an antifungal-associated drug-induced cardiac disease by Cleary et al. (4–6, 11). Cardiac impairment following echinocandin administration was reported in isolated rat cardiomyocytes (3). Especially, high-dose central-venous application of anidulafungin or caspofungin in adult rats resulted in a significant reduction in cardiac output and was also associated with reduced survival rate compared to control animals. These effects might be explained by a rapid onset of high peak levels of echinocandins (8). Since adverse effects of echinocandins have been observed especially in septic patients, endotoxemic rats were examined subsequently. While control and micafungin-treated animals did not show hemodynamic alterations, administration of anidulafungin or CAS in clinically used dosages led to a significant and dose-dependent decrease in cardiac output (10). Mitochondrial damage was suspected as a result of analysis of isolated rat hearts in transmission electron microscopy following echinocandin infusion (7). In contrast to these findings, we were not able to identify mitochondrial damage in spectrophotometric measurements of rat left ventricular cardiac tissue or altered mitochondrial enzyme activity after echinocandin treatment (8, 10). Therefore, we hypothesized that the mechanism behind CAS-induced cardiac alterations was not yet fully identified. With respect to the hemodynamic impairment of endotoxemic rat hearts following echinocandin infusion and studies supporting an endotoxin-induced suppression of the L-channel-dependent calcium flow, we hypothesized that alterations in Ca^{2+} homeostasis induced by echinocandins might be one potential harmful mechanism (10, 17–19).

CAS is the oldest and a well-established echinocandin and was therefore chosen as the substrate in this study. Beyond, CAS offers lipophilic features, in contrast to micafungin, and is therefore able to penetrate cell membranes. Our results might explain some of the observed detrimental effects of echinocandins in former studies.

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Long-term incubation of CAS in freshly isolated rat cardiomyocytes leads to severe cell toxicity. Therefore, all further results were performed by short-time application of CAS. In HCMs, the dose-dependent increase in $[Ca^{2+}]_i$ and maximum oscillation frequency prove a CAS-induced intracellular Ca^{2+} increase. Ca^{2+}-free buffer medium led to a reduced maximum but still dose-dependent effect of CAS-induced increase of $[Ca^{2+}]_i$, supporting the hypothesis of Ca^{2+} release from intracellular Ca^{2+} stores. The computed 50% effective dose (ED_{50}) of CAS in Ca^{2+}-containing buffer medium amounted to 148.1 μg/ml, which is approximately 7.5-fold higher than plasma levels of healthy probands (20). These findings are in line with cardiac impairment in high-dose-caspofungin-treated rats (8). However, critically ill patients suffering from multiple organ failure might be at high risk for excessive plasma levels, especially if CAS is infused rapidly via a central venous catheter. This might explain previous observations of sustained cardiac impairment following standard-dose administration of echinocandins in endotoxemic rats (10).

Subsequently, we addressed the mechanism of Ca^{2+} release. Caffeine (CAF) is well known to release Ca^{2+} most probably from sarcoplasmic reticulum (SR) by activation of RYN receptors. Therefore, depleting caffeine-sensitive SR Ca^{2+} stores was found to inhibit CAS (140 μg/ml)-induced Ca^{2+} release (21, 22). Also, RYN used in doses that inhibit ryanodine receptors was able to inhibit CAS-induced increase in $[Ca^{2+}]_i$. These results lead to the conclusion that CAS interferes with intracellular caffeine-sensitive stores, most probably via the activation of ryanodine receptors. Interestingly, these effects were found in physiological and Ca^{2+}-free buffer media and therefore are independent of the influence of extracellular Ca^{2+}, further supporting the hypothesis of Ca^{2+} release from the SR.

However, our study exhibits some limitations. Variations in pharmacokinetic and pharmacodynamic parameters might vary between HCMs and rat cardiac myocytes (RCMs). HCM-derived cell lines might feature different reactions to substrates than native cells. Furthermore, isolated cells are not able to represent physiologic *in vivo* reactions because of their lack of cell-to-cell or other physiologic interactions.

In conclusion, first, we were able to prove a severe grade of toxicity of long-term application of CAS in HCMs, which is in line with the findings of earlier studies (7, 8). Second, measurements in the oscillation frequency of cytosolic Ca^{2+} showed a dose-dependent impact of CAS with an increase in oscillation frequency. Third, we addressed these results and asked for the reasons of elevation in oscillation frequency and found a dose-dependent increase in $[Ca^{2+}]_i$ following CAS treatment in a 340/380 nm fluorescence intensity ratio. Fourth, Ca^{2+} ions were found to be released from intracellular caffeine-sensitive stores, most probably via the activation of ryanodine receptors.

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RESEARCH ARTICLE

Hemodynamic changes in surgical intensive care unit patients undergoing echinocandin treatment

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Abstract

Background Echinocandins are well-established agents for the treatment of patients with fungal infections, but growing evidence questions their safety in special patient populations prone to systemic inflammatory responses. *Objective* The study aimed to analyse early hemodynamic changes during echinocandin therapy in critically ill surgical patients. *Setting* The study was conducted at the surgical intensive care unit at the University Hospital of Giessen, Germany. *Methods* This single-centre retrospective study includes data from critically ill patients who underwent primary antifungal treatment during 2009–2013. *Main outcome measures* Hemodynamic parameters, need for vasopressor/inotropic therapy, and dose of vasopressor/inotropic therapy were recorded 2 h before and 2 h after the onset of antifungal treatment. Comparisons of echinocandins to azoles and analysis of a combined endpoint (decrease of mean arterial pressure ≥ 10 mmHg and/or new or increased dosages of norepinephrine, epinephrine, or dobutamine) were performed. *Results* We found 342 episodes of intravenous antifungal treatment (33 [9.6%] anidulafungin, 116 [33.9%] caspofungin, 132 [38.6%] fluconazole, 17 [5%] micafungin, 44 [12.9%] voriconazole). Group comparisons revealed no significant differences of hemodynamic parameters, need for vasopressor/inotropic therapy, and dose of vasopressor/inotropic therapy, expect for a decreased dose of norepinephrine in the fluconazole group ($p < 0.001$). The combined endpoint occurred in 58 (50%) caspofungin-, 16 (48.5%) anidulafungin-, 4 (23.5%) micafungin-, 23 (17.4%) fluconazole-, and 15 (34.1%) voriconazole treatment episodes. Secondary analysis of the combined anidulafungin/caspofungin group to the azoles group (fluconazole, voriconazole) showed a significant decrease of mean arterial pressure ≥ 10 mmHg (n = 37 [25%] vs. n = 27 [15%], OR = 1.8, $p = 0.04$), increased use of norepinephrine (n = 38 [26%] vs. n = 12 [7%], OR = 4.7, $p \leq 0.001$), increased use of dobutamine (n = 12 [8%] vs. n = 4 [2%], OR = 3.8, $p = 0.02$), and the combined endpoint (n = 74 [50%] vs. n = 38 [21%], OR = 3.6, $p \leq 0.001$). *Conclusion* Our retrospective data might demonstrate clinically relevant hemodynamic-depressing effects of anidulafungin and caspofungin. Further prospective acquisition of clinical data will be necessary to evaluate their impact on hemodynamic function.

Keywords Adverse drug reaction · Cardiac output · Cardiac toxicity · Echinocandins

Impacts on practice

- Critically ill postsurgical patients might be at risk for hemodynamic depression as a result of anidulafungin and caspofungin treatment.
- Micafungin treatment was not associated with elevated percentages of hemodynamic side effects.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11096-019-00939-8) contains supplementary material, which is available to authorized users.

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Introduction

Although bacteria remain the main cause of severe infections, fungal infections are an emerging problem in the treatment of critically ill patients, resulting in significant risk for patients in the surgical intensive care unit (ICU) [1–3]. Several treatment guidelines address antifungal therapy for severely ill patients, including echinocandins (anidulafungin, caspofungin, and micafungin) for the empiric and specific treatment of invasive *Candida* infections [4, 5]. Furthermore, echinocandins are also recommended as salvage therapy for patients suffering from invasive aspergillosis [6]. Echinocandins are well-established agents for the prophylaxis and treatment of patients with proven or suspected fungal infections, but growing evidence questions their safety in special patient populations prone to systemic inflammatory responses (e.g., critically ill patients, burn patients, patients with severe organ dysfunction). These concerns are hypothesized to be due to the high pharmacokinetic variability of echinocandins related to age, body surface area, disease status, and body integrity [4, 7, 8]. Adverse cardiac effects as a result of echinocandin administration were first described in case reports of critically ill and neutropenic patients [9–11]. In contrast, a recent prospective study of fifteen medical patients suffering from septic shock showed a significant increase in mean arterial and diastolic blood pressures immediately after application of echinocandins, while 4 h after administration differences were no longer present. Further analysis of the amount of catecholamine therapy as well as all transpulmonary thermodilution monitoring parameters did not indicate any significant changes immediately and 4 h after echinocandin administration [12]. Previous experimental studies analysing isolated cardiomyocytes (using the Langendorff model) and isolated rat hearts confirmed an echinocandin-induced cardiac impairment [13, 14]. Central venous high dose administration of anidulafungin or caspofungin in adult rats led to a significant reduction of cardiac output and was associated with a reduced survival rate compared to control animals, which further studies showed to be a dose-dependent mechanism [14, 15]. In contrast, micafungin did not cause cardiac impairment in rats or isolated cardiac myocytes as shown by Stover et al., who demonstrated that even high-dose micafungin treatment was free from cardiac toxicities [14–17]. One possible explanation for these findings might be the different pharmacological properties of micafungin compared to the other echinocandins. Since micafungin is water-soluble, in contrast to the lipophilic properties of anidulafungin and caspofungin, it does not penetrate the cell membrane and might therefore cause less cell damage [13].

Aim of the study

The aim of this study was to analyse early hemodynamic changes during echinocandin therapy in critically ill surgical patients.

Ethics approval

The study was approved by the institutional review board of the medical faculty of the Justus-Liebig-University, Giessen, Germany (AZ 117/13).

Methods

Study cohort

This retrospective observational single-centre cohort study was performed at the surgical ICU of the University Hospital of Giessen, Germany. We included the data of each adult patient (≥ 18 years) who received intravenous antifungal treatment during the period from 2009 to 2013. Patients with suspected or proven aspergillosis were excluded. All patients were treated according to the local standard of the surgical ICU, University Hospital of Giessen, Germany, and according to the guidelines of the European Society of Clinical Microbiology and Infectious Disease for the diagnosis and management of *Candida* diseases in non-neutropenic adult patients [4]. Empiric antifungal therapy was defined as the initiation of antifungal therapy prior to documented microbiological detection of *Candida* species, while specific treatment was defined as the initiation of antifungal therapy after documented microbiological detection of *Candida* species. Prophylactic treatment was referred to a pre-emptive therapy in patients with high risk for fungal infections (e.g., after lung transplantation). Drug dosage and speed of intravenous administration of antifungal agents were carried out according to manufacturers' recommendations.

Data collection

Study patients were reviewed and validated for inclusion and exclusion criteria as well as for plausibility by analysing the ICU Patient Data Management System (PDMS) ICUData (IMESO® GmbH, Giessen, Germany). Data were recorded in an external database using Statistical Package for the Social Sciences (SPSS) Statistics, version 24 (IBM, Armonk, NY, USA). Baseline parameters included age, body mass index (BMI), gender, and history of surgical treatment. We also recorded the severity of illness using the sequential organ failure assessment (SOFA) score, simplified

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acute physiology score (SAPS II) score, and acute physiology and chronic health evaluation score (APACHE II) score. Hemodynamic parameters including systolic, diastolic, and mean arterial blood pressure (MAP), heart rate (HR), cardiac index (CI), central venous pressure (CVP), need for vasopressor/inotropic therapy, and dosage of vasopressor/inotropic therapy were recorded 2 h before and 2 h after the onset of antifungal treatment. We also recorded length of ICU stay, length of hospital stay, and 30-days mortality.

Combined endpoint definition

A combined endpoint was defined to analyse hemodynamic depression and/or the need for medical hemodynamic support after antifungal administration. The combined endpoint included:

- decrease of MAP ≥ 10 mmHg

and/or

- new or increased dosages of norepinephrine, epinephrine, or dobutamine.

Furthermore, the lipophilic echinocandins anidulafungin and caspofungin were merged for further analysis and compared to a combined azole group (voriconazole- and fluconazole-treated patients). The analysis included single endpoints (decrease of MAP ≥ 10 mmHg, new or increased dosages of norepinephrine, epinephrine, or dobutamine) and the combined endpoint.

Statistical analysis

Descriptive statistics were performed for demographics, clinical characteristics, and hemodynamic data. Continuous variables are presented as mean and standard deviations or medians with interquartile ranges, and categorical variables as numbers and percentages. First, normal distribution of the data was tested using the Shapiro test, then comparisons of hemodynamic parameters and vasopressor-/inotrope therapy between the study groups were analysed using paired *t* tests or Wilcoxon signed-rank tests for matched samples. *P* values of statistical differences for single- and combined endpoints were calculated by using the Fisher's exact test. A two-tailed value of $p < 0.05$ was considered to be statistically significant. Furthermore, odds ratios were calculated for all group comparisons. All statistical analyses were performed using *R* statistical software version 3.4.2 (www.r-project.org) and Graphpad Prism version 5.0 for Mac (GraphPad Software, La Jolla, CA, USA).

Results

Baseline characteristics

We identified 270 surgical ICU patients who received antifungal treatment (Table 1). Of all the patients, 172 were male (63.7%). The mean age was 63.6 years. Within our cohort, we found 342 episodes of intravenous antifungal treatment (33 [9.6%] anidulafungin, 116 [33.9%] caspofungin, 132 [38.6%] fluconazole, 17 [5%] micafungin, 44 [12.9%] voriconazole). Patient demographics and clinical characteristics are presented in Table 1.

Microbiological findings

We observed positive fungal cultures in 89.8% of all patients included in the study. *C. albicans* was most frequently found within the microbiological results (60.1%) (Supplement 1). We found that 58.8% of patients suffered from invasive *Candida* infections including severe infections such as candidemia (7.3%) and intra-abdominal infections (29.5%), while 29.5% were treated with antifungals without proven *Candida* infection. Additionally, 11.7% received prophylactic antifungal treatment after lung transplantation. Invasive *Candida* infections were associated with a significantly elevated mortality

Table 1 Study characteristics

		n = 342
Gender male		172 (63.2)
Age	years	63.6 ± 13.3
Height	cm	171 ± 10
Weight	kg	81 ± 22
BMI	kg/m^2	28 ± 8
Length of hospital stay	days	54.66 ± 43.42
Length of ICU stay	days	26.38 ± 24.98
APACHE II		21 [18–25]
SOFA Score		6 [5–8]
SAPS II		44 [34–52]
30 days mortality		120 (35.1)
Antifungal treatment	*Anidulafungin*	33 (9.6)
	Caspofungin	116 (33.9)
	Fluconazole	132 (38.6)
	Micafungin	17 (5)
	Voriconazol	44 (12.9)

Data are shown as number of treatment episodes n (%), mean (± SD) or median [IQR]

BMI Body Mass Index, *ICU* Intensive Care Unit, *APACHE II*: Acute Physiology and Chronic Health Evaluation Score, *SOFA Score* Sequential Organ Failure Assessment Score, *SAPS II* Simplified Acute Physiology Score

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compared to patients without proven *Candida* infection (14.6% vs. 27.5%; $p = 0.048$).

Hemodynamic measurements

We analysed 342 episodes of intravenous antifungal treatment at baseline 2 h before administration of antifungal therapy and 2 h after the administration of antifungal medication. Patients receiving anidulafungin, caspofungin, fluconazole, micafungin, or voriconazole showed no changes in hemodynamic function (CI, HR, blood pressure, CVP) (Table 2). Except for a decreased dose of norepinephrine in the fluconazol group ($p < 0.001$), analysis of single echinocandins found that the median infusion rate of continuously administrated norepinephrine, epinephrine, and dobutamine also did not differ between baseline values and 2 h after antifungal administration within each group (Table 3).

Combined endpoint analysis

Analysing these factors, the combined endpoint occurred in 58 caspofungin treatment episodes (50%). Additionally, following anidulafungin administration the combined endpoint occurred in 16 episodes (48.5%) (Fig. 1a). In contrast, the combined endpoint was achieved only in 4 micafungin treatment episodes (23.5%), 23 fluconazole treatment episodes (17.4%) and 15 voriconazole treatment episodes (34.1%) (Fig. 1a). Combining the echinocandins anidulafungin and caspofungin resulted in a significant decrease of MAP ≥ 10 mmHg (n = 37 [25%] vs. n = 27 [15%], OR = 1.8, $p = 0.04$), increased use of norepinephrine (n = 38 [26%] vs. n = 12 [7%], OR = 4.7, $p \leq 0.001$), and increased use of dobutamine (n = 12 [8%] vs. n = 4 [2%], OR = 3.8, $p = 0.02$) compared to the azole group (Fig. 1b). Furthermore, the combined endpoint occurred in 74 cases (50%) of the combined anidulafungin/caspofungin group compared to 38 cases (21%) of the azoles group (OR = 3.6, $p < 0.001$) (Fig. 1b).

Comparison of the micafungin group (n = 17) to the azole group (n = 176) did not reveal significant differences in the decrease of MAP ≥ 10 mmHg (n = 2 [11%] vs. n = 27 [15%], OR = 0.74, $p = 1$), the use of norepinephrine (n = 3 [18%] vs. n = 12 [7%], OR = 2.91, $p = 0.13$), the use of dobutamine (n = 0 vs. n = 4 [2%], OR = not applicable, $p = 1$) or the combined endpoint (n = 4 [24%] vs. n = 38 [22%], OR = 1.11, $p = 0.77$) (data not shown).

Table 2 Hemodynamic findings

	2 h before therapy		2 h after therapy		*p*
	Median	IQR	Median	IQR	
Cardiac index (l/min/m²)					
Anidulafungin	3.35	2.39–4.25	3.34	2.51–3.93	1.00
Caspofungin	3.44	2.83–4.84	3.46	2.96–5.38	0.92
Fluconazole	3.35	3.19–4.91	4.33	3.61–5.59	0.50
Micafungin	3.99	2.61–4.17	3.63	3.17–4.92	0.24
Voriconazole	2.60	1.90–3.31	2.62	2.31–3.21	0.95
Heart rate (bpm)					
Anidulafungin	101	85–118	97	86–122	0.84
Caspofungin	100	83–112	99	82–115	0.52
Fluconazole	104	94–118	102	92–122	0.88
Micafungin	89	74–107	88	75–101	0.12
Voriconazole	95	80–107	96	82–108	0.83
Diastolic blood pressure (mmHg)					
Anidulafungin	61	49–68	62	54–68	0.71
Caspofungin	60	53–71	59	52–70	0.35
Fluconazole	60	54–64	58	53–62	0.42
Micafungin	62	52–70	64	56–72	0.3
Voriconazole	68	62–77	66	59–72	0.6
Mean arterial pressure (mmHg)					
Anidulafungin	82	67–93	84	74–87	0.53
Caspofungin	79	71–91	79	72–92	0.5
Fluconazole	75	71–84	77	69–82	0.54
Micafungin	85	73–94	85	76–96	0.14
Voriconazole	85	77–97	85	73–94	0.69
Systolic blood pressure (mmHg)					
Anidulafungin	113	102–141	121	108–138	0.38
Caspofungin	117	102–131	120	105–132	0.66
Fluconazole	113	100–131	113	101–126	0.75
Micafungin	127	111–141	132	117–149	0.08
Voriconazole	119	106–132	119	104–140	0.93
Central venous pressure (mmHg)					
Anidulafungin	16	9–28	17	11–77	0.65
Caspofungin	16	9–23	16	12–20	0.67
Fluconazole	20	15–22	17	16–55	0.39
Micafungin	15	11–17	17	12–21	0.22
Voriconazole	14	10–17	15	10–17	0.42

Hemodynamic findings 2 h before– and 2 h after antifungal therapy. Values are shown as median with interquartile range (IQR). Number of antifungal treatment episodes n (%) per group: anidulafungin 33 (9.6), caspofungin: 116 (33.9), fluconazole: 132 (38.6), micafungin: 17 (5), voriconazole: 44 (12.9)

Discussion

In the current study our aim was to analyse early hemodynamic changes during echinocandin therapy in 342 antifungal treatment episodes from 270 surgical ICU patients. Considering the severity of illness reflected by the SOFA, SAPS, and APACHE II scores, the patients suffered from severe impairment of their general clinical status. This could be explained by the high rate of invasive *Candida* infections, patients' comorbidities, and the high-risk constellation in a major surgery setting. Accordingly, invasive *Candida* infections were associated with significantly elevated mortality. However, it has been demonstrated that perioperative hypotension is also

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Table 3 Comparison of inotropes and vasopressor therapy before and after antifungal therapy

	n (all)	2 h before therapy			2 h after therapy			*p*
		n (%) (dose > 0)	Median [IQR] (all)	Median [IQR] (dose > 0)	n (%) (dose > 0)	Median [IQR] (all)	Median [IQR] (dose > 0)	
*Norepinephrine (µg/kg*min)*								
Anidulafungin	33	19 (58)	0.12 [0–0.31]	0.30 [0.15–0.41]	21 (64)	0.12[0–0.28]	0.21 [0.15–0.41]	0.86
Caspofungin	116	71 (61)	0.06 [0–0.32]	0.19 [0.09–0.49]	73 (63)	0.06 [0–0.31	0.14 [0.07–0.48]	0.24
Fluconazole	132	53 (40)	0 [0–0.06]	0.07 [0.04–0.13]	47 (36)	0 [0–0.05]	0.07 [0.04–0.1]	< 0.001
Micafungin	17	13 (76)	0.19 [0.02–0.64]	0.56 [0.14–0.78]	13 (76)	0.22 [0.02–0.72]	0.51 [0.11–0.73]	0.64
Voriconazole	44	29 (66)	0.05 [0–0.11]	0.10 [0.05–0.18]	31 (70)	0.05 [0–0.15]	0.11 [0.05–0.2]	0.37
*Epinephrine (µg/kg*min)*								
Anidulafungin	33	1 (3)	0	0.02 [0.02–0.02]	1 (3)	0	0.02 [0.02–0.02]	NA
Caspofungin	116	3 (3)	0	0.22 [0.15–0.3]	6 (5)	0	0.23 [0.13–0.34]	0.18
Fluconazole	132	2 (2)	0	0.05 [0.04–0.06]	2 (2)	0	0.07 [0.05–0.08]	1
Micafungin	17	1 (6)	0	0.02 [0.02–0.02]	2 (12)	0	0.17 [0.1–0.24]	1
Voriconazole	44	6 (14)	0	0.03 [0.02–0.05]	5 (11)	0	0.01 [0.01–0.04]	0.18
*Dobutamin (µg/kg*min)*								
Anidulafungin	33	5 (15)	0	0.005 [0.002–2.31]	6 (18)	0	1.16	0.10
Caspofungin	116	14 (12)	0	2.23 [1.01–2.63]	19 (16)	0	2.08	0.18
Fluconazole	132	7 (5)	0	1.11 [0.52–3.58]	7 (5)	0	2.22	1
Micafungin	17	4 (24)	0	1.68 [1.06–2.12]	4 (24)	0	1.68	1
Voriconazole	44	7 (16)	0	2.62 [1.45–2.8]	8 (18)	0	2.39	0.67

Values are shown as median with interquartile range [IQR] or numbers with percentage n (%), n: number of patients per group, n (dose > 0): Number of patients receiving medication of interest per group, % (dose > 0): Percentage of patients receiving medication of interest per group, (all): entire group including patients without receiving medication of interest NA: not applicable, not enough data for statistical test. Data were analyzed using the Wilcoxon signed-rank test

associated with an increase in morbidity and mortality. Since the ideal individual blood pressure of critically ill patients has not yet been well-defined, various definitions of intra- and postoperative hypotension have been published. Futier et al. found that a reduction of MAP of 6 mmHg in a cohort of patients undergoing major surgery lasting 2 h or longer was significantly associated with elevated rates of postoperative organ dysfunction [18]. Furthermore, Salmasi et al. assessed that in a cohort of patients after non-cardiac surgery MAP values below an absolute threshold of 65 mmHg or a relative threshold of 20% reduction of MAP from preoperative baseline values were progressively related to both myocardial- and kidney injury [19]. The *European Best Practice Guidelines on Hemodynamic Instability* by Kooman et al. defined an absolute decrease of MAP ≥ 10 mmHg as a clinically relevant hemodynamic instability in patients during hemodialysis [20]. Similarly, Khanna et al. determined that in a cohort of postsurgical ICU patients, an absolute decrease of MAP ≥ 10 mmHg was significantly associated with the occurrence of acute kidney injury [21]. Furthermore, a pre-existing history of hypertension as well as the duration of the hypotensive episode might affect patients' outcomes. Therefore, we chose the combination of endpoints MAP ≥ 10 mmHg and changes of hemodynamic treatment in order to reflect the clinicians' daily routine evaluation and management of hemodynamic alterations. Due to the retrospective study design, we also defined the start of a new hemodynamic medication or the increase (independent from the relative percentage change) in continuously administered catecholamines as clinically relevant outcome parameters that might reflect clinicians' treatment of possible echinocandin-induced hemodynamic suppression.

Expect for a minor but significantly decreased dose of norepinephrine in the fluconazol group, the individual analyses of hemodynamic parameters (blood pressure, CI, HR, CVP) as well as the use of vasopressors or inotropes revealed no significant changes of hemodynamic stability within 2 h after echinocandin administration. It is possible that short-term clinical interventions might have ameliorated the effect of echinocandins on blood pressure, CI, HR, and CVP. Therefore, fluid administration, vasopressor therapy, or other therapeutic interventions might have attenuated echinocandin-induced hemodynamic depression. Additionally, fluconazole- and voriconazole therapy caused no changes in hemodynamic parameters. However, examining the combined endpoint consisting of the reduction in MAP (≥ 10 mmHg) and/or the need for new or increased vasopressor/inotrope therapy could reveal important differences between the

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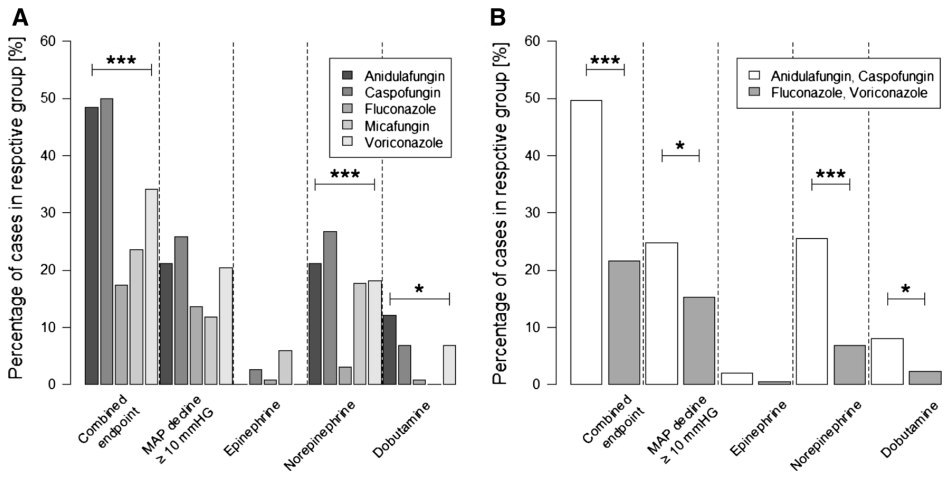

Fig. 1 Combined endpoint analysis **a** comparison of individual antifungals. Number of antifungal treatment episodes n (%) per group: anidulafungin 33 (9.6), caspofungin: 116 (33.9), fluconazole 132 (38.6), micafungin 17 (5), voriconazole 44 (12.9). MAP: mean arterial pressure. *$=p<0.05$; **$=p<0.01$; ***$=p<0.001$. The p values have been obtained from applying fisher's exact test for count data. **b** Comparison of anidulafungin/caspofungin vs. fluconazole/voriconazole group. Number of antifungal treatment episodes n (%) per group: anidulafungin/caspofungin (total n = 149; combined endpoint: n = 74; MAP ≥ 10 mmHg: n = 37; epinephrine: n = 3; norepinephrine: n = 38; dobutamine: n = 12), fluconazole/voriconazole (total n = 176, combined endpoint: n = 38; MAP ≥ 10 mmHg: n = 27; epinephrine: n = 1; norepinephrine: n = 12; dobutamine: n = 4). Combined endpoint included decline of MAP ≥ 10 mmHg and/or new- or increased dosages of norepinephrine, epinephrine or dobutamine. Norepinephrine group: new- or increased dosages, epinephrine group: new- or increased dosages, dobutamine group: new- or increased dosages. *$=p<0.05$; **$=p<0.01$; ***$=p<0.001$. The p values have been obtained from applying fisher's exact test for count data

different antifungal agents. The combined endpoint was found in 48.5% of the anidulafungin treatment episodes and in 50% of the caspofungin treatment episodes. In contrast, the combined endpoint was only found in 24% after micafungin treatment. These results are in line with animal studies [13, 14, 17, 22, 23]. One possible explanation might be the different pharmacological features of micafungin compared to the other echinocandins. Since micafungin is water-soluble in contrast to the lipophilic properties of anidulafungin and caspofungin, it does not penetrate the cell membrane and might therefore cause less cell damage [14–16]. Furthermore, Stover et al. suggest from their studies that micafungin alone represents an effective, high-dose regimen that is compared to anidulafungin and caspofungin free from cardiac toxicities [17]. Therefore, we combined anidulafungin and caspofungin for further analysis and identified a significant decrease of MAP (≥ 10 mmHg) and a significant increase of the use of norepinephrine, epinephrine, and dobutamine in the echinocandin group compared to the azole group (voriconazole- and fluconazole-treated patients).

Previously, we were able to demonstrate a dose-dependent decrease of contractility after echinocandin administration in isolated cardiomyocytes using the Langendorff model, which has been confirmed in another ex vivo study [13, 15, 16]. Subsequently, we infused echinocandins in dosages equivalent to standard clinical human therapy as well as in high dosages via central lines into adult rats while monitoring the hemodynamic changes with an in vivo assessment device. The results indicated that high dose administration of anidulafungin [25 mg/kg bodyweight (BW)] and caspofungin (8.75 mg/kg BW) effectively reduced cardiac activity, and survival time in contrast to the lower dose study group (anidulafungin group: 2.5 mg/kg BW; caspofungin group: 0.875 mg/kg BW) [14]. Similar results have been described following administration of clinically relevant doses of anidulafungin and caspofungin in an in vivo endotoxemic shock rat model [22]. However, the detailed pathomechanism of echinocandin-induced hemodynamic and cardiac impairment remains unclear. Cleary et al. identified toxic effects of echinocandins on cardiac mitochondrial function and adenosine triphosphate (ATP) synthesis [13, 23, 24]. In contrast to these results, our study group was not able to detect any altered mitochondrial enzyme activity in spectrophotometric analyses of rat left ventricular cardiac tissue [22]. We assumed an echinocandin-induced dysregulation of intracardial calcium homeostasis based on previous study results and similar findings concerning septic cardiac failure [22, 25, 26]. Recently, our group was able to demonstrate that caspofungin therapy induces a dose-dependent increase of intracellular calcium in human cardiac myocytes. However,

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calcium ions were found to be released from intracellular caffeine-sensitive stores likely via activation of ryanodine receptors [27]. Therefore, dysregulation of calcium homeostasis might be one possible explanation for our current results. In order to shed light into the pathomechanism of echinocandin-toxicity further experimental and clinical studies are necessary, especially to differentiate echinocandin effects from the influence of other drugs and the underlying severe disease in the clinical context.

In contrast to our findings, Lahmer et al. were not able to detect a significant impairment of hemodynamic status after echinocandin administration but observed a transient increase of the diastolic and mean arterial pressure [12]. Other hemodynamic parameters deriving from transpulmonary thermodilution and the need for vasopressors did not change significantly, even though the dosage of echinocandins were identical to our recent study. However, it is possible that differences observed between our study and Lahmer et al.'s study may be due to differences in the sizes of the populations studied [12].

Our study has several limitations. First, due to the retrospective design, no causal effect of echinocandins on negative hemodynamic function can be verified. Second, data concerning the type of intravenous application (central vs. peripheral line) is lacking, particularly since our data supports a possible aggravating effect of centrally administered echinocandins as well as fast injection of echinocandins. Third, due to the dose-dependent prolonged infusion scheme (90–180 min) that is suggested for anidulafungin treatment, some of the study patients might not have received the complete medication at the observation point (2 h after onset of antifungal treatment). Fourth, we analysed an unsorted cohort of postsurgical patients with diverse comorbidities and severity of illness. Due to the retrospective character of our study, we were not able to detect special cohorts of patients at risk. Fifth, sedation, fluid management, and other drugs might also have had an impact on our hemodynamic findings that we were not able to exclude. Last, due to the exploratory character of the study, we did not predefine the comparison between echinocandins and azoles, even though the severity of illness might have affected the choice of antifungal therapy and therefore influenced the study´s results. However, we believe that our findings might affect daily clinical awareness during antifungal therapy in critically ill postsurgical patients and justify further prospective studies.

Conclusion

In summary, this retrospective study yields data on critically ill postsurgical patients that might demonstrate an important hemodynamic-depressing effect of anidulafungin and caspofungin. These findings are in line with published case reports and experimental data. Further prospective acquisition of clinical data will be necessary to evaluate their impact on hemodynamic function.

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