

NON-INTERVENTIONAL (NI) STUDY REPORT

PASS information

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Joint PASS	No
Research question and objectives	To quantify the risk for severe hepatotoxicity in users of anidulafungin relative to other marketed echinocandins.

Country(-ies) of study	United States
Author	Pfizer Ltd (anonymous), Ramsgate Road, Sandwich, CT130NJ, UK

Marketing Authorisation Holder(s)

Marketing Authorisation Holder(s)	Pfizer Limited Ramsgate Road, Sandwich, Kent CT130NJ United Kingdom
MAH contact person	Lisa Weiss, PhD, MPH

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TABLE OF CONTENTS

1. ABSTRACT (STAND-ALONE DOCUMENT)	6
2. LIST OF ABBREVIATIONS.....	11
3. INVESTIGATORS	13
4. OTHER RESPONSIBLE PARTIES.....	13
5. MILESTONES.....	14
6. RATIONALE AND BACKGROUND.....	15
7. RESEARCH QUESTION AND OBJECTIVES	16
8. AMENDMENTS AND UPDATES.....	17
9. RESEARCH METHODS	18
9.1. Study Design	19
9.2. Setting.....	19
9.3. Subjects	19
9.4. Variables.....	20
9.4.1. Outcome 20	
9.4.2. Exposure 21	
9.4.3. Confounders and Effect Modifiers.....	22
9.5. Data Sources and Measurement	26
9.6. Bias.....	27
9.7. Study Size.....	28
9.8. Data Transformation	28
9.9. Statistical Methods	29
9.9.1. Main Summary Measures	29
9.9.2. Main Statistical Methods	30
9.9.3. Missing Values.....	34
9.9.4. Sensitivity Analyses.....	34
9.9.5. Amendments to the Statistical Analysis Plan	36
9.10. Quality Control.....	37
9.11. Protection of Human Subjects.....	37
10. RESULTS	38
10.1. Participants	38

10.2. Descriptive Data	40
10.3. Outcome Data	42
10.4. Main Results	42
10.5. Other Analyses	46
10.5.1. Sensitivity Analyses	46
10.6. Adverse Events / Adverse Reactions	48
11. DISCUSSION	49
11.1. Key Results	49
11.2. Limitations	52
11.3. Interpretation	55
11.4. Generalizability	57
12. OTHER INFORMATION	57
13. CONCLUSIONS	57
15. LIST OF SOURCE TABLES AND FIGURES	61

LIST OF IN-TEXT TABLES AND FIGURES

No table of figures entries found.

Annex 1. List of stand-alone documents

Appendix 1.	SIGNATURES
Appendix 2.	PROTOCOL
Appendix 3.	REFER TO SECTION 3 INVESTIGATORS AND SECTION 5 MILESTONES
Appendix 4.	ICD-9-CM, HCPCS, GPI, AND CPT-4 CODES FOR IDENTIFYING COMORBIDITIES AND MEDICATIONS

1. ABSTRACT (STAND-ALONE DOCUMENT)

Title

A Retrospective Cohort Study of the Risk of Severe Hepatotoxicity in Hospitalized Patients Treated with Echinocandins

Mei Sheng Duh, MPH, ScD, Analysis Group, Inc, and Harvard T.H. Chan School of Public Health, Boston, Massachusetts

20 April 2015

Keywords

Echinocandin; Anidulafungin; Caspofungin; Micafungin; Severe hepatotoxicity.

Rationale and Background

Anidulafungin, caspofungin, and micafungin are echinocandins used for treating invasive candidiasis. Among these, anidulafungin is the only echinocandin that is not metabolized by the liver and does not require dose adjustment in patients with severe hepatic impairment.

Research Question and Objectives

1. To estimate the unadjusted and adjusted risk of severe hepatotoxicity in patients treated with anidulafungin, caspofungin, and micafungin
2. To evaluate clinical and demographic features associated with the type of echinocandin received
3. To estimate the unadjusted and adjusted risk ratios of severe hepatotoxicity in patients treated with anidulafungin versus those in patients treated with caspofungin or micafungin

Study Design

A retrospective observational cohort study.

Setting

Hospitalized patients treated with anidulafungin, caspofungin or micafungin identified in hospital-based electronic medical records (EMR) data in the United States (US).

Subject and Study Size, Including Dropouts

Patients ≥ 18 years of age receiving ≥ 1 intravenous infusion of echinocandins during the hospitalization were included in the study (N = 12,678). The date of the treatment initiation was defined as the index date. The baseline period included the time between the hospital admission date and the index date, inclusive, and the observation period included the time from the index date until the earliest event of severe hepatotoxicity, hospital discharge or death. Patients were required to have liver function test (LFT, ie, aspartate transaminase [AST], alanine aminotransferase [ALT], total bilirubin) values both in the baseline and observation periods. LFTs were graded per modified Clinical Islet Transplantation study - Terminology Criteria for Adverse Events in trials of adult pancreatic islet transplantation (CIT-TCAE). Severe hepatotoxicity was defined as the first occurrence of a Grade ≥ 3 LFT in the observation period.

Variables and Data Sources

Data were obtained from two US-based hospital EMR databases, Humedica and Cerner Health Facts, pooled into a single dataset. Exposure to echinocandins among hospitalized patients was identified using Healthcare Common Procedure Coding System and National Drug Codes in the EMR data.

For Objective 1, the unadjusted absolute risk (ie, cumulative incidence) of severe hepatotoxicity was calculated as the number of patients with severe hepatotoxicity divided by the total number of patients exposed to each type of echinocandin. The unadjusted incidence rate for each echinocandin group was calculated as the number of patients with severe hepatotoxicity divided by the total person-days of observation in that group, and reported per 30 person-days. Adjusted absolute risk and incidence rate of severe hepatotoxicity in each echinocandin group were computed using regression-based indirect standardization methodology. For Objective 2, factors associated with the type of echinocandin that patients received were identified from multivariate logistic regression models. For Objective 3, relative risks (RRs) and incidence rate ratios (IRRs), used to measure the association between the echinocandin and severe hepatotoxicity, were estimated for anidulafungin versus caspofungin and anidulafungin versus micafungin using log binomial (for RRs) and negative binomial (for IRRs) regressions, adjusting for demographic, baseline LFT (except for the subgroup analysis on patients with normal LFT at baseline), other labs, and clinical covariates.

Several sensitivity and subgroup analyses were conducted for Objective 3: (1) inclusion of patients without baseline LFT values, (2) patients with normal, or mildly or moderately elevated LFT at baseline (ie, Grade 0-2, and Grade 0), (3) Cerner sub-sample, and by baseline LFT grades and treatment duration, (4) Humedica sub-sample, and by baseline LFT grades and integrated delivery network, (5) severe hepatotoxicity outcome defined based on the first occurrence of an event of Grades ≥ 4 , and (6) severe hepatotoxicity outcome defined based on the occurrence of an event of Grade 5.

Results

A total of 12,678 eligible patients were identified (anidulafungin: 1700; caspofungin: 4431; micafungin: 6547), among whom 9161 patients had normal to moderately elevated LFT at baseline (anidulafungin: 1012; caspofungin: 3281; micafungin: 4868). At baseline, compared to patients receiving caspofungin and micafungin, anidulafungin patients had statistically significantly more elevated LFT (proportion LFT Grade ≥ 3 , 40.4% vs 25.9% and 25.6%), critical care admissions (75.3% vs 52.6% and 48.6%), surgeries (41.1% vs 33.7% and 27.1%), use of central venous catheters (43.8% vs 13.3% and 19.3%) and immunosuppressive drugs (14.6% vs 4.4% and 5.9%), and higher rates of comorbidities (eg, organ failures: 69.4% vs 46.7% and 51.5%; sepsis or septic shock: 68.5% vs 46.9% and 47.9%; cardiovascular disease (CVD): 71.1% vs 42.1% and 49.8%; kidney disease: 40.2% vs 17.5% and 21.2%). All comparisons yielded p-values less than 0.05.

In Objective 1 analyses, the unadjusted absolute risk of severe hepatotoxicity was 37.2% (95% CI: 34.3-40.1), 22.4% (95% CI: 21.0-23.8), and 23.3% (95% CI: 22.1-24.4) in the anidulafungin, caspofungin and micafungin groups, respectively. After adjustment, the absolute risk of severe hepatotoxicity decreased to 25.7% (95% CI: 24.7-26.7) in the anidulafungin group, and increased to 24.3% (95% CI: 23.4-25.2) and 24.8% (95% CI: 23.9-25.6) in the caspofungin and micafungin groups, respectively. A similar trend was observed in incidence rates after adjustment. The adjusted incidence rate of severe hepatotoxicity was 0.47 (95% CI: 0.44-0.51) in the anidulafungin group, 0.41 (95% CI: 0.38-0.44) in the caspofungin group, and 0.45 [95% CI: 0.43-0.48] in the micafungin group.

In Objective 2 analyses, baseline clinical features found to be significantly associated with an increased probability of receiving anidulafungin vs caspofungin or micafungin, included higher grade of baseline bilirubin, use of extended-spectrum azoles, having ≥ 2 fungal infection sites, having critical care admission, using immunosuppressive therapy, using antiretroviral drugs known to have hepatotoxic effects, using central venous catheter, and the presence of comorbid CVD, hypertension, kidney disease, endocarditis, sepsis or septic shock. Clinical features associated with decreased probability of receiving anidulafungin vs caspofungin or micafungin included emergency admission to the index hospitalization, use of antibiotics known to have hepatotoxic events and the presence of comorbid gastro-oesophageal reflux disease.

Table 1 below summarizes the main results from Objective 3 analyses. The adjusted RRs and IRRs are presented for both the main study sample and the subgroup of patients with baseline LFT Grade ≤ 2 . The LFT Grade ≤ 2 subgroup was chosen for the summary of results because this group excluded patients who had severe hepatotoxicity pre-treatment, allowing the assessment of newly developed severe hepatotoxicity during treatment. The results showed no statistically significant differences in severe hepatotoxicity between anidulafungin and caspofungin/micafungin in the majority of the analyses. The only statistically significant effect was observed in the IRR model for the anidulafungin versus caspofungin comparison in the main study sample (IRR 1.43, 95% CI 1.14-1.79). All subgroup analyses on patients with baseline LFT Grade ≤ 2 were not statistically

significant. In particular, the IRR for the anidulafungin versus caspofungin was no longer statistically significant (IRR 1.46, 95% CI 0.91-2.37).

Table 1. Adjusted Relative Risks and Incidence Rate Ratios of Severe Hepatotoxicity Between Anidulafungin and Caspofungin or Micafungin

	Anidulafungin vs Caspofungin	Anidulafungin vs Micafungin
Main Study Sample		
Adjusted RR (95% CI)	1.07 (0.95-1.20)	1.03 (0.93-1.15)
Adjusted IRR (95% CI)	1.43 (1.14-1.79)*	1.19 (0.92-1.54)
Baseline LFT Grades 0-2 Subgroup		
Adjusted RR (95% CI)	1.11 (0.88-1.41)	1.08 (0.87-1.34)
Adjusted IRR (95% CI)	1.46 (0.91-2.37)	1.62 (0.95-2.77)

* p <0.05.

CI = confidence interval; IRR = incidence rate ratio; LFT = liver function test; RR = relative risk.

Similarly, the majority of sensitivity analyses for Objective 3 yielded adjusted RRs and IRRs estimates that were not statistically different from 1 (ie, no difference in risk between anidulafungin and the comparison echinocandin). The exception included the adjusted RRs and IRRs for the anidulafungin versus caspofungin comparison in the Humedica subsample, and the adjusted RR for the anidulafungin versus micafungin comparison in the sensitivity analysis for Grade 5 hepatotoxicity events, which suggested higher risk of severe hepatotoxicity in anidulafungin patients. Among patients with Grade 5 events, those in the anidulafungin group had significantly worse prognosis for death at baseline than those in the caspofungin and micafungin groups: 86.0% were admitted to critical care (vs 59.6% [p <0.001], 58.0% [p <0.001]), 61.3% had surgeries (vs 34.0% [p <0.001], 35.4% [p <0.001]), 90.7% had organ failures (vs 66.7% [p <0.001], 71.7% [p <0.001]) and 92.0% had sepsis or septic shock (vs 64.5% [p <0.001], 70.4% [p <0.001]).

Discussion

Based on real-world hospital practice data, the majority of the current study analyses showed that adjusted RRs and IRRs estimates were not statistically different from 1, suggesting that anidulafungin was not associated with a statistically significantly higher absolute risk or incidence rate for severe hepatotoxicity, as compared to caspofungin and micafungin. In the IRR comparison to caspofungin, however, there was a statistically significantly higher incidence rate in anidulafungin in the main study sample, although the statistical significance was not present in the subgroup of baseline LFT Grades 0-2.

It is important to note that the baseline data demonstrated the channelling of anidulafungin treatment towards patients with impaired liver function and higher mortality prognosis based on comorbidity profiles; this is especially notable among patients with Grade 5 hepatotoxicity events. This confounding by indication bias is well-known in epidemiology literature and adjustment is methodologically challenging. Attempts to control for differences in the severity profile of patients in the current study were limited to the information available in the databases. Thus, residual confounding due to unobserved factors is possible. In subgroup analyses on patients with normal or mildly/moderately elevated LFT at baseline (Grades 0-2), which used restriction as a method to homogenize the baseline LFT risk across the treatment groups, no evidence was found to indicate significant differences in the risk of severe hepatotoxicity between patients treated with anidulafungin and patients treated with caspofungin or micafungin.

Marketing Authorization Holder (s)

Pfizer Ltd

Names and Affiliations of Principal Investigators

Name, degree(s)	Title	Affiliation
Mei Sheng Duh, MPH, ScD	Managing Principal and Chief Epidemiologist	Analysis Group, Inc
	Visiting Scholar	Harvard T.H. Chan School of Public Health
Lisa Weiss, PhD, MPH	Director, Epidemiology	Pfizer Inc
Francis Vekeman, MA	Vice President	Groupe d'analyse, Ltée
Wendy Cheng, MPH, MPhil	Manager	Analysis Group, Inc
Raluca Ionescu-Ittu, PhD	Economist	Groupe d'analyse, Ltée
Yongling Xiao, PhD	Economist	Groupe d'analyse, Ltée
Rachel Bhak, MS	Analyst	Analysis Group, Inc

2. LIST OF ABBREVIATIONS

Abbreviation	Definition
ALT	Alanine aminotransferase
AST	Aspartate transaminase
CCI	Charlson comorbidity index
CCU	Critical/Coronary care unit
CI	Confidence interval
CIT-TCAE	Clinical Islet Transplantation-Terminology Criteria for Adverse Events
CKD	Chronic kidney disease
CPT	Current Procedural Terminology
CTCAE	Common Terminology Criteria for Adverse Events
CVD	Cardiovascular disease
ED	Emergency department
EMA	European Medicines Agency
EMR	Electronic medical records
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
FDA	Food and Drug Administration
GERD	Gastro-oesophageal reflux disease
GFR	Glomerular filtration rate
GPP	Good Pharmacoepidemiology Practices
HCPCS	Healthcare Common Procedure Coding System
HIPAA	Health Insurance Portability and Accountability Act
ICD-9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification

ICU	Intensive care unit
IDN	Integrated Delivery Network
IEC	Independent Ethics Committee
INR	International normalized ratio
IQR	Inter-quartile range
IRB	Institutional Review Board
IRR	Incidence rate ratio
ISPE	International Society for Pharmacoepidemiology
IV	Intravenous
LFT	Liver function test
MAH	Marketing authorisation holder
NCI	National Cancer Institute
NDC	National Drug Code
NSAID	Non-steroidal anti-inflammatory drug
PASS	Post-Authorisation Safety Study
PS	Propensity score
RR	Relative risk
SD	Standard deviation
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase
SmPC	Summary of product characteristics
ULN	Upper limit of normal
US	United States

3. INVESTIGATORS

The names, affiliations, and contact information of the investigators at each study site are listed in Appendix 3.1.

Principal Investigator(s) of the Protocol

Name, degree(s)	Title	Affiliation
Mei Sheng Duh, MPH, ScD	Managing Principal and Chief Epidemiologist Visiting Scholar	Analysis Group, Inc Harvard T.H. Chan School of Public Health
Lisa Weiss, PhD, MPH	Director, Epidemiology	Pfizer Inc
Francis Vekeman, MA	Vice President	Groupe d'analyse, Ltée
Wendy Cheng, MPH, MPhil	Manager	Analysis Group, Inc
Raluca Ionescu-Ittu, PhD	Economist	Groupe d'analyse, Ltée
Yongling Xiao, PhD	Economist	Groupe d'analyse, Ltée
Rachel Bhak, MS	Analyst	Analysis Group, Inc

4. OTHER RESPONSIBLE PARTIES

Not applicable—not a Joint PASS and no other parties were involved.

5. MILESTONES

Milestone	Planned date	Actual date	Comments
Date of Institutional Review Board (IRB) approval of protocol		19 November 2013	
Data obtained for analysis	December 2013	Cerner: 24 April 2014 Humedica: 25 April 2014	Retrospective data were received from two sources: Humedica and Cerner. No data were actively collected.
Final report of study results	6 April 2015	20 April 2015	

6. RATIONALE AND BACKGROUND

Echinocandins are a class of antifungal medications that inhibit 1,3- β -D-glucan synthesis, an essential component of fungal cell walls.¹ The deprivation of this major structural component results in the disruption of the cell wall and fungal cell lysis. Given that glucan synthase is absent in mammalian cells, it is an attractive target for antifungal activity. Currently available echinocandins in the United States and the European Union include anidulafungin (Eraxis®/Ecalta®, Pfizer), caspofungin (Cancidas, Merck) and micafungin (Mycamine, Astellas Pharma).

Anidulafungin is unique among the echinocandins in that it undergoes elimination by chemical degradation and nonspecific peptidases in the plasma,² while caspofungin and micafungin are metabolized to some degree in the liver. As a result, the use of caspofungin and micafungin may require dose adjustment, restrictions on food and beverage, and monitoring of drug-drug interactions, while anidulafungin is considered to be safe to use for patients with liver impairment without dosing requirement. Specifically, anidulafungin is used at a uniform dosage across all patients regardless of body weight, organ functions (including hepatic impairment), or concurrent use of other medications, whereas caspofungin and micafungin both require dosage adjustment based on body weight, liver function, and concomitant medications.

While the safety and tolerability of the echinocandins are generally favourable, liver enzyme abnormalities have been reported as an adverse event according to clinical studies and as labelled in summaries of product characteristics (SmPC).³⁻⁵ However, rates of elevated liver enzymes in echinocandins were generally lower than those for other antifungals. For instance, in a pivotal trial of anidulafungin against fluconazole, 1.5% of patients on anidulafungin vs 7.2% of patients on fluconazole experienced hepatic enzyme abnormalities ($p = 0.03$).⁶ In a Phase III trial of caspofungin versus amphotericin B, 2.8% of patients on caspofungin experienced elevated bilirubin, compared to 8.9% of patients on amphotericin B. Nonetheless, in clinical trials of micafungin, increases in AST, ALT, and bilirubin have been reported in both healthy volunteers and patients.³ In some patients, more severe hepatic dysfunction and hepatic failure, including fatal cases, have been reported. As a result, the SmPC for micafungin includes a special warning and precaution for use in patients with elevated liver enzymes. The European Medicines Agency (EMA) restricted the use of micafungin only after other antifungals are deemed inappropriate.³

While few head-to-head comparisons of the safety profiles of echinocandins have been conducted to date, a recent meta-analysis and systematic review published in 2010 of 39 randomized controlled trials evaluating the tolerability and hepatotoxicity of antifungals found that the use of echinocandins was associated with a lower risk of liver injury compared to other antifungal treatments.⁷ Specifically, 2.0% of echinocandin users, as compared to 19.7% of voriconazole users, 17.4% of itraconazole users, and 9.3% of fluconazole users had elevated serum liver enzyme levels. Additionally, among the echinocandins, 0.8% of anidulafungin users experienced abnormal liver function tests (LFTs), as compared to 0.2% of caspofungin users and 2.7% of micafungin users.

An auxiliary analysis was performed in the same study adding 37 reports of nonrandomized controlled trials, case series, and cohort studies to the meta-analysis to increase the generalizability.⁷ This analysis found similar results in that the anidulafungin, caspofungin, and micafungin had lower risks of elevated liver enzymes (1.7%, 6.9%, 7.8%, respectively) than amphotericin B, itraconazole, fluconazole, and voriconazole (16.3%, 20.2%, 8.3%, 29.7%, respectively). Anidulafungin showed the lowest risk of hepatotoxicity among the other echinocandins and other antifungal treatments.

In post-marketing spontaneous reports, isolated cases of significant hepatic dysfunction or acute liver failure have been noted, but the causal relationship between anidulafungin and hepatic dysfunction has not been established. The present epidemiology study aimed to quantify and compare the risk for severe hepatotoxicity among hospitalized patients treated with echinocandins in real-world populations. This non-interventional study was designated as a Post-Authorization Safety Study (PASS) and was a commitment to the EMA.

7. RESEARCH QUESTION AND OBJECTIVES

The primary objective of the study was to estimate the risk of severe hepatotoxicity associated with exposure to echinocandins, and to compare the risk of severe hepatotoxicity in hospitalized patients treated with anidulafungin to that of hospitalized patients treated with other echinocandins (caspofungin and micafungin) in a real-world setting. Specific aims were as follows:

- Objective 1 – To estimate the unadjusted and adjusted risk of severe hepatotoxicity in hospitalized patients treated with echinocandins (ie, anidulafungin, caspofungin, and micafungin);
- Objective 2 – To evaluate clinical and demographic features associated with the type of echinocandin received (ie, anidulafungin, caspofungin, and micafungin) during the hospitalization;
- Objective 3 – To estimate the unadjusted and adjusted risk ratios of severe hepatotoxicity in hospitalized patients treated with anidulafungin to those in hospitalized patients treated with caspofungin or micafungin.

The risks were evaluated in the forms of absolute risk (ie, cumulative incidence) and incidence rate. The risk ratios were evaluated in the forms of relative risk and incidence rate ratio.

The null hypotheses tested were that the risk of severe hepatotoxicity in hospitalized patients treated with anidulafungin was not statistically different from that in hospitalized patients treated with caspofungin or micafungin. Specifically:

Hypothesis 1:

$H_0: P_{\text{anidulafungin}} = P_{\text{caspofungin}}$

$H_a: P_{\text{anidulafungin}} \neq P_{\text{caspofungin}}$

Hypothesis 2:

$$H_0: P_{\text{anidulafungin}} = P_{\text{micafungin}}$$

$$H_a: P_{\text{anidulafungin}} \neq P_{\text{micafungin}}$$

Where P is the absolute risk or incidence rate of severe hepatotoxicity.

8. AMENDMENTS AND UPDATES

Amendment number	Date	Substantial or administrative amendment	Protocol section(s) changed	Summary of amendment	Reason
1	21 October 2014	Administrative	9.2, 9.7, 9.9	The proposal to remove duplicate records between Cerner and Humedica databases was removed. The core analysis would be based on the combined datasets. Sensitivity analyses would be conducted on the Cerner and Humedica databases separately.	Hospital ID and other hospital-level information necessary to identify duplicates were not available in the Humedica database.
2	21 October 2014	Administrative	9.3	The proposal to account for the dosage of echinocandin treatment was removed.	The variables used to calculate the total daily dose were either unreliable or mostly missing (71%-93%) in both the Cerner and Humedica databases.
3	21 October 2014	Administrative	9.3, 9.7	The proposal to account for the duration of echinocandin treatment in the main analyses using the combined datasets was removed. The analyses would only account for the duration of echinocandin treatment in the stratified analyses based on the Cerner database.	Duration of echinocandin treatment exposure was not available in the Humedica database, as there was only a drug order date variable without an end date indicator.
4	21 October 2014	Administrative	9.3, 9.7	The proposal to adjust for patient comorbidities at baseline in the multivariate analyses	The Cerner database only contained rolled-up diagnosis records without dates, which could not be used to

				was expanded to adjust for comorbidities recorded during the full hospitalization.	identify comorbidities that had occurred prior to the initiation of treatment.
5	21 October 2014	Administrative	4, 9.9	The proposal to adjust for severity and progression of fungal infection during hospitalization was removed.	Candidiasis diagnosis records in both Cerner and Humedica databases were severely under-coded; only 16-20% of echinocandin users had a record of a candidiasis diagnosis code.
6	21 October 2014	Administrative	9.7	The proposal to account for intensive care unit (ICU) length of stay in the calculation of incidence rate of severe hepatotoxicity was removed.	The Cerner database did not have the variables to allow for the calculation of ICU length of stay.
7	19 December 2014	Administrative	9.2, 9.3, 9.9	The definition of Grade 5 severe hepatotoxicity (death due to hepatic causes) was changed from a death with a primary or secondary discharge diagnosis containing at least one diagnosis code for liver diseases to a death with at least one Grade 4 LFT lab result between the index date and death.	In both the Cerner and Humedica databases, no discharge diagnoses were available. The Cerner database contained rolled-up diagnosis codes during a whole hospitalization episode without date indicators, and while the Humedica databases had diagnosis codes by date, no principal or secondary discharge diagnoses could be identified. The new definition would allow for the identification of severe hepatotoxicity temporally associated with an echinocandin treatment, because LFT results contained associated test dates in both databases.

9. RESEARCH METHODS

Detailed methodology for research methods are documented in the final protocol, which is dated, filed and maintained by the sponsor (Appendix 2).

9.1. Study Design

A retrospective cohort design was used. The study was implemented by pooling two US hospital-based electronic medical records (EMR) databases, Humedica and Cerner Health Facts (“Cerner”).

A patient’s most recent hospitalization that included echinocandin treatment and satisfied all study inclusion criteria (see Section 9.3) was included in the analysis and defined as the patient’s index hospitalization. Any intensive care unit (ICU) or critical care unit (CCU) encounters that occurred between the admission and discharge of the index hospitalization were included in the analysis. The date of the first recorded echinocandin administration during the index hospitalization was designated as the index date. The baseline period was defined as between the date of admission to the index hospitalization and the index date, inclusive. The observation period was defined as the period from the index date until the earlier event of severe hepatotoxicity, date of discharge from the index hospitalization or death. Given the pharmacokinetics of intravenous (IV) injections ($C_{\max}$ is reached within 3-10 hours, half-life ranges from 9 to 50 hours), no induction period was imposed and patients’ follow-up was not censored at the end of echinocandin treatment. The study design, depicted in Figure 1, ensured that only at-risk time periods were included in the denominator of the risk (ie, absolute risk or incidence rate).

9.2. Setting

The study sample was derived from two large US-based hospital EMR databases, Humedica and Cerner, which had data sourced from multiple care delivery sites, including hospitals, large multi-specialty practices, group practices and physician offices. In 2012, the Humedica database covered >13 million unique patients treated in >135 hospitals and other medical centres across all US census regions. In 2014, the Cerner database covered >58 million unique patients treated in >480 facilities across all US census regions. More detailed information on the databases can be found in Section 9.5.

9.3. Subjects

The source population for the study sample included patients from (1) Humedica database: those who had at least one record for one of the echinocandins between 1 January 2007 and 30 September 2013, and (2) Cerner database: those who had at least one record for one of the echinocandins between 1 January 2006 and 30 June 2013. The end dates for these two databases were longer than what were presented in the study protocol approved by EMA (ie, 30 September 2014 for Humedica and 30 June 2013 for Cerner, respectively), because more data became available following the EMA approval. The study sample was selected from the source population by applying the inclusion and exclusion criteria described below.

Inclusion criteria

1. ≥ 1 hospital admission or acute care admission;

2. ≥ 1 dose of echinocandin as defined by the following Healthcare Common Procedure Coding System (HCPCS) and National Drug Code (NDC) codes during hospitalization:
 - a. Anidulafungin

HCPCS code: J0348 and NDC codes: 00049011428, 00049011528, 00049011628, 00049101028;
 - b. Caspofungin

HCPCS code: J0637 and NDC codes: 00006382210, 00006382310
 - c. Micafungin

HCPCS code: J2248 and NDC codes: 00469321110, 00469325010;
3. ≥ 18 years of age at the time of the hospital admission;
4. ≥ 1 LFT (ie, alanine aminotransferase (ALT), aspartate transaminase (AST) or total bilirubin) result during the baseline period. This criterion was imposed here to facilitate causality assessment of the role of echinocandin on severe hepatotoxicity by evaluating LFT results before and after the drug administration. In sensitivity analyses, this criterion was removed to be inclusive of all echinocandin users;
5. ≥ 1 LFT result following the index date during the observation period. Because the outcome definition was based on LFT results (see Section 9.4.1), this criterion was imposed to allow for the identification of severe hepatotoxicity events.

Exclusion criteria

1. Exposure to more than one type of echinocandin during hospitalization.

For patients having multiple hospitalizations meeting eligibility criteria, the most recent hospitalization was selected for analysis and defined as the index hospitalization. Figure 2 illustrates the process for sample selection.

9.4. Variables

9.4.1. Outcome

The study outcome was defined as the first severe hepatotoxicity event in the observation period, regardless of whether there were any known aetiologies involved, consistent with EMA guidelines. Known aetiologies were considered as confounders, as described in Section 9.4.3. Severe hepatotoxicity was ascertained based on the first LFT of Grades 3, 4, or 5 in the observation period. For this study, the definition of the LFT grades was adapted from the Clinical Islet Transplantation study - Terminology Criteria for Adverse Events in trials of adult pancreatic islet transplantation (CIT-TCAE) Version 5.0,⁸ which

are modified standards of those set forth in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE).⁹ Specifically, the following operational definitions were applied to define severe hepatotoxicity events:

	Grade		
	3	4	5
AST or serum glutamic-oxaloacetic transaminase (SGOT) Or ALT or serum glutamic-pyruvic transaminase (SGPT)	>5.0 to 20.0 times the upper limit of normal (ULN)	Evidence of fulminant hepatic failure (ICD-9-CM diagnosis code 572.2), with international normalized ratio (INR) ≥ 2.5 and AST/ALT $\geq 20.0 \times$ ULN	Death due to hepatic causes, defined as death preceded by a Grade 4 LFT in the observation period
Total bilirubin	>3.0 – 10.0 x ULN	>10.0 x ULN	

Death due to hepatic causes was identified based on the presence of a Grade 4 LFT rather than cause of death or the primary or secondary discharge diagnoses, because neither cause of death nor the primary or secondary discharge diagnoses could be identified in the Cerner and Humedica data (see Section 8).

9.4.2. Exposure

Exposure to echinocandins was determined based on following HCPCS procedure and NDC drug codes during the index hospitalization:

- Anidulafungin codes:
 - HCPCS: J0348,
 - NDC: 00049011428, 00049011528, 00049011628, 00049101028;
- Caspofungin codes:
 - HCPCS: J0637,
 - NDC: 00006382210, 00006382310;
- Micafungin codes:
 - HCPCS: J2248,

- NDC: 00469321110, 00469325010.

The index date for exposure to echinocandins was based on the first recorded administration of an echinocandin in the EMR data during the index hospitalization.

Because the risk of severe hepatotoxicity may increase with extended exposure to echinocandins, the duration of the echinocandin exposure (ie, 1-3, 4-7, and 8+ days) was used as a stratification variable for Objective 3 analyses among the subgroup of patients from the Cerner database (Note: duration of the echinocandin exposure was not available in the Humedica database).

9.4.3. Confounders and Effect Modifiers

All available potential confounders and effect modifiers were measured in the baseline period, with the exception of the diagnosis-based comorbidities that were measured over the full hospitalization period (because diagnoses made during a hospitalization could not be linked to specific dates in the Cerner data). Diagnostic (ICD-9-CM), procedure (ICD-9-CM, CPT, HCPCS) and drug (NDC, HCPCS) codes used for the measurement of confounders and effect modifiers are presented in Appendix 4.

The following confounders and effect modifiers were selected a priori based on published literature (ie, at the time of the protocol development, with the exception of mild liver disease, pancreatitis, alcohol abuse and endocarditis, which were added at the time of the statistical analysis plan development upon review of the actual data availability from Humedica and Cerner and additional literature on risk factors for severe hepatotoxicity) and were all considered in the analyses. During the analysis phase, the associations of these confounders with the exposure (ie, echinocandin) and the outcome (ie, severe hepatotoxicity) were also assessed to check if these variables fulfilled the statistical criteria for confounding. The final list of confounders included in the analyses was based on both statistical significance and clinical relevance according to extant literature as described in Main Statistical Methods (Section 9.9.2). The list of variables selected a priori is as follows:

- Age at admission to index hospitalization, extracted from the demographic records in each data source
- Sex, extracted from demographic records
- Race and/or ethnicity, extracted from demographic records
- Data source (Cerner vs Humedica)
- A proxy measure of hospital formulary for echinocandin, which was based on all types of echinocandins observed in the data for a given hospital or hospital grouping in the year when the patient had the index admission:
 - All three echinocandins (anidulafungin, caspofungin and micafungin)
 - Anidulafungin and caspofungin only

- Anidulafungin and micafungin only
- Caspofungin and micafungin only
- Anidulafungin only
- Caspofungin only
- Micafungin only
- Admission to acute care settings (eg, ICU, CCU), extracted from EMR codes indicating the setting of the service
- Admission through emergency department (ED) for the index hospitalization, extracted from records indicating admission type
- Use of other antifungal agents, identified in the data using NDC codes: amphotericin B, fluconazole, extended-spectrum azoles (itraconazole, voriconazole, posaconazole)
- Fungal infection severity, including:
 - Type of fungal infection
 - Invasive fungal infection, identified in the data using ICD-9-CM diagnosis codes for candidiasis of lung, systemic candidiasis, candida endocarditis, candida meningitis, and candidal enteritis;
 - Non-invasive fungal infection, identified in the data using ICD-9-CM diagnosis codes for candidiasis of mouth, candidiasis of vulva and vagina, candidiasis of other urogenital sites, candidiasis of skin and nails, candida otitis externa, and candida esophagitis;
 - Fungal infection of unknown site, identified in the data using ICD-9-CM diagnosis codes for candidiasis of unspecified site and candidiasis of other sites;
 - Number of fungal infection sites, measured as the number of distinct candidiasis ICD-9-CM diagnosis codes involving different organs
- Risk factors for fungal infections
 - Central venous catheter and catheter removed within 24 hours of hospitalization, identified in the data using CPT and HCPCS procedure codes and ICD-9-CM diagnosis codes;
 - Broad-spectrum antibiotics (any type, regardless of whether they were known or not to have a hepatotoxic effect), identified in the data using NDC codes;
 - Surgery, identified in the data using HCPCS and CPT procedure codes for both major and minor surgery;

- Hyperalimentation, identified in the data using ICD-9-CM diagnosis codes;
- Immunosuppressive therapy, identified in the data using NDC codes.
- LFT results at baseline - for each type of LFT, a grade was determined based on the highest value that was observed across all days in the baseline period using ULN cut-offs based on NCI-CTCAE criteria (within each day, if multiple values were observed, the value that was the most common in the day was selected):
 - ALT: Grade 0 if test result $\leq$ ULN; Grade 1 if test result $>$ ULN - 2.5 x ULN; Grade 2 if test result $>$ 2.5 - 5.0 x ULN; Grade 3 if test result $>$ 5.0 - 20.0 x ULN; and Grade 4 if test result $>$ 20.0 x ULN
 - AST (same cut-offs as ALT)
 - Total bilirubin: Grade 0 if test result $\leq$ ULN; Grade 1 if test result $>$ ULN - 1.5 x ULN; Grade 2 if test result $>$ 1.5 - 3.0 x ULN; Grade 3 if test result $>$ 3.0 - 10.0 x ULN; and Grade 4 if test result $>$ 10.0 x ULN

In addition, an overall measure of LFT (ie, combining ALT, AST and bilirubin into a single LFT measure) during the baseline period was measured for descriptive purposes using the same criteria as for the outcome definition (see Section 9.4.1)

- Overall mortality prognosis - based on comorbidity profiles measured by the Charlson comorbidity index (CCI),^{10, 11} which is a score between 0 and 33 calculated based on the presence of ICD-9-CM diagnosis codes for 17 comorbidities associated with high risk of death, such as cancer, myocardial infarction, congestive heart failure, diabetes and others; higher scores indicate higher mortality prognosis.
- Binary indicators of relevant comorbid conditions, identified in the data based on the ICD-9-CM diagnosis codes:
 - Alcohol abuse
 - Cardiovascular diseases (CVD)
 - Diabetes mellitus
 - Endocarditis
 - Oesophageal varices
 - Gastro-oesophageal reflux disease (GERD)
 - Hypertension
 - Kidney diseases

- Mild liver disease (ie, chronic liver disease, cirrhosis, chronic hepatitis)
- Obesity
- Organ failures (ie, heart failure, kidney failure)
- Pancreatitis
- Sepsis or septic shock
- Prior use of echinocandin based on information from prior hospitalizations (where available)
- Neutropenia, defined based on the results of absolute neutrophil count laboratory test (ie, ≤ 500 per cubic millimetre)
- Chronic kidney disease (CKD) stage, calculated from the results of the serum creatinine lab and estimated glomerular filtration rate (GFR) values , categorized as follows,¹²:
 - Stage 1 - normal or slightly diminished kidney function, defined as GFR (mL/min/1.73 m²) ≥ 90
 - Stage 2 - mildly reduced kidney function, defined as GFR 60-89
 - Stage 3 - moderately reduced kidney function, defined as GFR 30-59
 - Stage 4 - severely reduced kidney function, defined as GFR 15-29
 - Stage 5 - very severe, or end stage kidney failure, defined as GFR <15
- Aetiologies of hepatotoxicity, identified in the data based on the ICD-9-CM diagnosis codes:
 - Acute hepatic diagnoses:
 - Viral hepatitis;
 - Liver disease secondary to biliary pathologies;
 - Liver malignancy;
 - Acute and subacute necrosis of liver associated with cardiovascular causes (paired with codes for right heart failure or hypotension);
 - Hepatitis associated with viral infections (paired with codes for mononucleosis or other viral infections);

- Hepatitis in other infectious diseases classified elsewhere (paired with codes for underlying malaria).
- Drugs with known hepatotoxicity,^{13, 14} identified through NDC and HCPCS codes:
 - Grouped by classes:
 - Chemotherapies (eg, methotrexate, azathioprine);
 - Non-steroidal anti-inflammatory drug (NSAID) (eg, diclofenac);
 - Antiretrovirals (eg, zidovudine, didanosine, stavudine);
 - Psychotropics (eg, paroxetine, nefazodone, valproic acid);
 - Antibiotics (eg, amoxicillin, telithromycin);
 - Antimycobacterials (eg, isoniazid, rifampin);
 - Antidiabetics (eg, rosiglitazone, pioglitazone);
 - Acetaminophen.
 - Number of distinct drugs with known hepatotoxicity effect used in the baseline period.

9.5. Data Sources and Measurement

As there were no known population-based databases in Europe with the necessary variables to address the research objectives when this study was initiated, this study used two major US-based hospital databases: Humedica and Cerner databases. The two databases were combined in order to obtain sufficiently large cohorts and increase the statistical power of the study.

Humedica

The Humedica data were available from 1 January 2007 to 30 September 2013 and contained information on demographic characteristics, type of healthcare provider (specialty), medical history and diagnoses (ICD-9-CM codes) for all types of encounters within the network, detailed area of care during hospitalization (ICU, ED, ward, etc.), in-hospital procedures (ICD-9-CM procedure, HCPCS and CPT codes), inpatient medications including injectable and oral medications (NDC codes), physician prescriptions, and laboratory data (including date and time of test, result value).

Humedica patients belonging to Integrated Delivery Networks (IDNs) received all care through the IDN, resulting in more complete records of services received outside of the index hospitalization. In sensitivity analyses, the baseline period was extended for these patients to include the 6-month period prior to the index admission. Both comorbidities and health

resource utilization (binary indicators for inpatient visit, outpatient visit, ED visit and other health resource utilization) were measured in the extended baseline period.

Cerner

The Cerner data were available from 1 January 2006 to 30 June 2013 and contained information on demographic characteristics, detailed area of care during hospitalization (ICU, ED, ward, etc.), medical history, comorbidities, in-hospital procedures (mainly ICD-9-CM codes), laboratory data (including date and time of test, result value), inpatient medications including injectable and oral medications, physician prescriptions, in-hospital mortality, and hospital characteristics.

Data collection in Cerner and Humedica databases

Both Cerner and Humedica databases sourced data from multiple care delivery sites, including hospitals, large multi-specialty practices, group practices and physician offices. The EMR data from each care delivery site were transferred to Cerner and Humedica data centres, respectively, where they were cleaned (ie, duplicates removed) and standardized across care delivery sources by mapping to a common nomenclature. To comply with Health Insurance Portability and Accountability Act (HIPAA) guidelines, the data were de-identified prior to the submission to third parties for analysis.

Overlap between the Humedica and Cerner databases

Given that some of the hospitals in the Humedica database were Cerner hospitals, duplicate records were expected. However, the extent of the overlap between Cerner and Humedica databases was unknown. While duplicate records could not be removed due to the lack of a unique hospital identifier in Humedica, sensitivity analyses were conducted, stratified by database, in order to assess the association of echinocandin and severe hepatotoxicity within each of the two databases separately. However, by not accounting for the correlation of the duplicate observations in the main analyses in which the Cerner and Humedica data was pooled, the variance of point estimates may have been underestimated, resulting in inflated Type I errors and yielding results that were more likely to be found statistically significant.

9.6. Bias

Confounding

To evaluate and statistically reduce the effect of confounding, all potential confounders available in the pooled dataset were selected to be used as adjustment variables in analyses that estimated the effect of echinocandins on the absolute risk and incidence rate of severe hepatotoxicity. Particular attention was given to the patients' baseline LFT results, which were expected to be a strong confounder (anidulafungin is the only echinocandin that is not metabolized by the liver and, as such, a strong confounding by indication bias was expected as physicians channel patients with or at high risk for impaired liver function to anidulafungin¹⁵⁻¹⁷). Despite measuring and adjusting in the analysis for many potential confounders, some potential confounders could not be adequately measured with EMR data

(eg, LFTs may not perfectly characterize clinical liver dysfunction, hospital formulary for echinocandin could only be measured by a proxy, alcohol use data may be underreported). However, given that anidulafungin appeared to be channelled towards sicker patients with hepatic impairment, it is likely that residual confounding would result in conservative estimates for the effect of anidulafungin versus caspofungin and micafungin on severe hepatotoxicity (ie, artificially higher risk for the anidulafungin group).

Selection and Informational Bias

To avoid the possibility of immortal person-time bias, only at-risk time periods were included in the denominator of the absolute risk or incidence rate calculation. The at-risk time period was defined as from the initiation of the treatment (index date), instead of from the hospital admission date, until the earliest observation of a severe hepatotoxicity event, hospital discharge, or recorded death. This design ensured that only at-risk time periods were included in the calculation of the observation period.

To increase generalizability, a sensitivity analysis included all echinocandin-treated patients, regardless of the availability of LFT before starting therapy. However, this sensitivity analysis may have introduced bias in the etiological assessment of the role of echinocandin on severe hepatotoxicity given that missing baseline LFT results were not random across the three echinocandin groups (5.2% in anidulafungin, 9.1% in caspofungin, and 8.9% in micafungin).

9.7. Study Size

Sample size calculations were conducted at the time of the protocol development to assess the sample size needed in Objective 3 to detect a range of relative risks (RR) with at least 80% power and a 5% two-sided alpha assuming various absolute risks of severe hepatotoxicity in the micafungin and caspofungin cohorts. According to pooled estimates from a meta-analysis of clinical trials by Wang et al.,⁷ 0.2% and 2.7% patients receiving caspofungin and micafungin had elevated liver enzymes that were on average $\geq 5 \times$ ULN and required treatment termination. However, the severe hepatotoxicity definition used in the current study was broader than that of Wang et al. Therefore, a higher absolute risk of severe hepatotoxicity was expected at the time of the protocol development.

Assuming an absolute risk of severe hepatotoxicity of 2% in the micafungin or caspofungin cohorts and a RR of 2.0, it was estimated using the PASS software¹⁸ that about 1239 subjects per treatment group were required to achieve 80% power. Given the preliminary count of 1917 hospitalized patients receiving echinocandins in the smallest cohort (anidulafungin), the sample size was expected to be powered to detect a RR of 1.5-2.0 (please see Appendix 2 [protocol, Table 4] for all scenarios tested in the sample size calculation). Of note, the actual statistical power of the study was higher, as the absolute risk of severe hepatotoxicity in the micafungin and caspofungin cohorts observed in the data was $\sim 20\%$.

9.8. Data Transformation

All variables used in the analyses were similarly measured in the Cerner and Humedica databases, except that duration of exposure to echinocandins was not available in the

Humedica database. Continuous variables were categorized if a non-linear effect was expected for either the effect of the covariate on the echinocandin treatment selection or for the effect of the covariate on the absolute risk /incidence rate of severe hepatotoxicity. Categorization cut-offs for continuous variables were selected based on clinical significance and data distribution. For example, age was categorized using the following three categories: 18-49 years (23% of sample), 50-64 years (34% of the sample) and 65+ years (43% of the sample). Other variables that were categorized included: CCI and number of distinct drugs with known hepatotoxicity effect used in the baseline period.

9.9. Statistical Methods

Detailed information on the statistical methods was documented in the statistical analysis plan.

9.9.1. Main Summary Measures

Descriptive Measures

- Frequencies and proportions for categorical variables
- Means, standard deviations (SD), medians, and inter-quartile ranges (IQR) for continuous variables

Main Analysis Measures

- Adjusted odds of anidulafungin treatment (vs caspofungin or micafungin treatment) associated with different demographic, baseline LFT (except for the subgroup analysis on patients with normal LFT at baseline), other labs, and clinical characteristics and corresponding 95% confidence intervals (CI)
- Unadjusted and adjusted absolute risk of severe hepatotoxicity of each echinocandin and its 95% CI
- Unadjusted and adjusted RR of severe hepatotoxicity: comparing severe hepatotoxicity absolute risks between patients treated with anidulafungin versus caspofungin and those treated with anidulafungin versus micafungin
- Unadjusted and adjusted incidence rate of patients with severe hepatotoxicity over the total person-time of observation in each group and its 95% CI
- Unadjusted and adjusted incidence rate ratio (IRR) comparing severe hepatotoxicity incidence rates between patients treated with anidulafungin versus caspofungin and those treated with anidulafungin versus micafungin
- Unadjusted and adjusted Kaplan-Meier curves for the time to first severe hepatotoxicity event among patients treated with anidulafungin versus caspofungin and anidulafungin versus micafungin

9.9.2. Main Statistical Methods

Analytic Samples

Analyses related to Objective 1 were performed in each echinocandin group separately. Analyses related to Objectives 2 and 3 were performed in two analytic samples: one analytic sample that included the patients in the anidulafungin and caspofungin groups and one analytic sample that included the patients in the anidulafungin and micafungin groups.

Descriptive Analyses

The Chi-squared test or Fisher exact test was conducted to compare categorical variables between the echinocandin groups (Fisher exact test was used when the lowest cell count was <5 patients) and the non-parametric Wilcoxon rank-sum test was conducted to compare continuous variables between echinocandin groups.

Unadjusted and Adjusted Estimates Of Absolute Risk and Incidence Rate of Severe Hepatotoxicity in Hospitalized Patients Treated With Echinocandins (Objective 1)

Absolute Risk Of Severe Hepatotoxicity

The unadjusted absolute risk or proportion of severe hepatotoxicity diagnosed after treatment among patients treated with each type of echinocandin was calculated as the number of patients with severe hepatotoxicity divided by the total number of patients exposed to each type of echinocandin. The 95% CI for the unadjusted absolute risk of severe hepatotoxicity was calculated based on a binomial distribution.

Adjusted absolute risk of severe hepatotoxicity among patients treated with each type of echinocandin was computed using regression-based indirect standardization methodology. For each specific echinocandin treatment group, the expected number of severe hepatotoxicity was obtained by summing the predicted probabilities of severe hepatotoxicity (derived from a multivariable logistic model) for all patients. The adjusted absolute risk of severe hepatotoxicity for each specific echinocandin treatment group was calculated as the ratio of the observed number of severe hepatotoxicity to the expected number of severe hepatotoxicity cases in that group and then rescaled by multiplying by the absolute risk observed in the entire echinocandin population. The resulting adjusted absolute risk can be interpreted as the absolute risk that would have been observed in each specific echinocandin group if the patients in that group had the same patient characteristics as in the entire echinocandin population. The 95% CI for the adjusted absolute risk of severe hepatotoxicity was calculated using non-parametric bootstrap methodology with 500 replicates.

Incidence Rate Of Severe Hepatotoxicity

To account for patients' different observation durations in the observation period, defined as the period from the index date until the earliest event of severe hepatotoxicity, hospital discharge or death, the incidence rate for each echinocandin group was calculated as the number of patients with severe hepatotoxicity divided by the total person-days of observation in that group. Incidence rates were then reported as severe hepatotoxicity events per 30

person-days. The 95% CI for the incidence rate was calculated based on a Poisson distribution.

Adjusted incidence rate of severe hepatotoxicity among patients treated with each type of echinocandin was computed using regression-based indirect standardization methodology. For each specific echinocandin treatment group, the expected number of severe hepatotoxicity was obtained by summing the predicted probabilities of severe hepatotoxicity (derived from a multivariable Poisson model) for all patients. The adjusted incidence rate of severe hepatotoxicity for each specific echinocandin treatment group was calculated as the ratio of the observed number of severe hepatotoxicity to the expected number of severe hepatotoxicity in that group and then rescaled by multiplying by the incidence rate observed in the entire echinocandin population. The resulting adjusted incidence rate can be interpreted as the incidence rate that would have been observed in each specific echinocandin group if the patients in that group had the same patient characteristics as in the entire echinocandin population. The 95% CI for the adjusted incidence rate of severe hepatotoxicity was calculated using non-parametric bootstrap methodology with 500 replicates.

Time to First Severe Hepatotoxicity Event

Kaplan-Meier analysis was conducted to compare the distribution of the time to event between echinocandin groups. The time to event was calculated as the time from the index date to the first occurrence of a severe hepatotoxicity event (for those who had an event) or to hospital discharge or non-hepatic death (censored for those who did not have an event). The median time to severe hepatotoxicity was not reached for any of the echinocandin groups. The log-rank test was used to compare the time to severe hepatotoxicity between anidulafungin versus caspofungin and anidulafungin versus micafungin.

Since the conventional Kaplan-Meier analysis would only provide an unadjusted comparison of time to first severe hepatotoxicity event, the current Kaplan-Meier curves were adjusted for potential confounders by using inverse probability weights methodology. The resulting adjusted Kaplan-Meier curves illustrated how the Kaplan-Meier curves would look if patients in the anidulafungin, caspofungin, and micafungin groups were to have the same distribution of covariates as observed in the combined echinocandin groups (ie, curves were standardized to the characteristics of the full main study sample).

Clinical and Demographic Features Associated With the Type Of Echinocandin Received (Objective 2)

Factors associated with physician's choice of treatment with anidulafungin versus caspofungin and anidulafungin versus micafungin were identified from multivariate logistic regression models. Final predictors in the model were selected using stepwise selection methodology, with significance level of 0.25 as covariate entry cut-off, and 0.10 as covariate retaining cut-off. Variables from the list presented in Section 9.4.3 were selected by the stepwise statistical criteria (with the exception of hospital formulary for echinocandin that

could not be included in the list of potential predictors because it had a deterministic impact on the treatment choice). In addition, seven covariates were included in the model based on clinical relevance: highest baseline AST grade (categorical: 0 to 4 and unknown), highest baseline ALT grade (categorical: 0 to 4 and unknown), highest baseline total bilirubin grade (categorical: 0 to 4 and unknown), age at the hospital admission (categorical: 18-49, 60-64 and 65+ years), gender, Cerner vs Humedica dataset and mortality prognosis measured by CCI (categorical: 0 to 3 and 4+).

Relative Risks and Incidence Rate Ratios Of Severe Hepatotoxicity in Hospitalized Patients Treated With Anidulafungin Versus Caspofungin and Micafungin (Objective 3)

Relative Risk

The RR of severe hepatotoxicity between two groups of echinocandin was defined as the ratio of the absolute risk of severe hepatotoxicity occurring in the anidulafungin group to the absolute risk of severe hepatotoxicity occurring in the caspofungin and micafungin groups (separately).

Unadjusted RRs and corresponding 95% CIs were estimated using univariate log-binomial regression models with a binary indicator of severe hepatotoxicity as the dependent variable and a binary indicator for echinocandin treatment (anidulafungin vs caspofungin in one model and anidulafungin vs micafungin in the second model) as the independent variable.

Adjusted RRs and corresponding 95% CIs were estimated for each treatment comparison using multivariate log-binomial regression models. The multivariate log-binomial regression models adjusted for all covariates described in Section 9.4.3 did not converge, because log-binomial models often have convergence problems when multiple covariates are used for adjustment.^{19, 20} To overcome the model non-convergence issue, efficient parsimonious models were created by using the propensity score (PS) methodology, which involved two steps:

- Step 1 - a multivariate logistic regression treatment model was used to estimate for each patient his/her predicted probability of being treated with anidulafungin vs caspofungin or micafungin (ie, PS); and
- Step 2- the PS estimate for each patient was entered as an adjusted variable along with the treatment indicator in the multivariate log-binomial regression model for the outcome.

All potential confounders listed in Section 9.4.3 were included as predictors in the multivariate logistic regression model used to estimate the PS, with the exception of drug formulary for echinocandin (because the inclusion in the PS models of variables that have a strong impact on the treatment and no direct effect on the outcome may lead to biased estimates).

Incidence Rate Ratio

The IRR of severe hepatotoxicity between two groups of echinocandin was defined as the ratio of the incidence rates between two echinocandin groups.

Unadjusted IRR and corresponding 95% CIs were estimated using univariate Poisson regression models with a binary indicator of severe hepatotoxicity as dependent variable, offset corresponding the total person-days in each echinocandin group (corresponding to the duration of the observation period for each patient), and a binary indicator for echinocandin treatment (anidulafungin vs caspofungin in one model and anidulafungin vs micafungin in the second model) as independent variable.

Adjusted IRRs and corresponding 95% CIs were estimated for each treatment comparison using multivariate negative binomial regression models (negative binomial models were used instead of Poisson models to account for the over-dispersion observed in the data). Final adjustment variables in the negative binomial model were selected using stepwise selection methodology, with significance level of 0.25 as covariate entry cut-off and 0.10 as covariate retaining cut-off. The final model included the binary indicator of treatment, eight covariates that were forced in the model based on clinical relevance (ie, the seven covariates forced in the model for Objective 2 [highest baseline AST grade, highest baseline ALT grade, highest baseline total bilirubin grade, age at the hospital admission, gender, Cerner vs Humedica dataset, mortality prognosis measured by CCI] and the hospital formulary for echinocandins [hospital formulary was not used in the models for Objective 2 and PS because it had a deterministic impact on treatment]) and any additional variables from the list presented in Section 9.4.3 that were selected by the stepwise statistical criteria.

Control Of Confounding

Multivariate analyses were employed to adjust for confounding in the estimation of RRs and IRRs of severe hepatotoxicity between the different types of echinocandins (anidulafungin vs caspofungin and micafungin, respectively) (Objective 3). Covariates listed in Section 9.4.3 were included in the final corresponding models based on both clinical and statistical significance. The preferred strategy for confounding adjustment was to include in the multivariate regression model both the treatment and the potential confounders as independent variables. This strategy was preferred because it also estimated the effect of other covariates on the absolute risk and incidence rate of severe hepatotoxicity. However, when the multivariate regression models adjusted for covariates did not converge, adjustment for PS was used as an alternative strategy. While PS adjustment may not yield a straightforward interpretation for the effect of specific covariates, it is an effective composite measurement for covariates and its parsimony prevents model non-convergence.

In sensitivity analyses, confounding by the baseline LFT was also addressed by restricting the study sample to the subset of patients with normal (ie, Grade 0) LFT at baseline and to those with normal, mildly or moderately elevated (ie, Grade 0-2) LFT at baseline.

9.9.3. Missing Values

Due to confidentiality concerns, the raw Humedica data did not include the date of hospital discharge/death for the patients who died during a hospitalization, although the in-hospital death status was known. For these patients, a date of hospital discharge/death was imputed, which corresponded to the first day of the first gap of 3 or more consecutive days in the EMR within the hospitalization in which the death occurred. The 3+ day gap cut-off (as opposed to other duration cut-offs) was chosen based on evidence from the Cerner database, where death dates were available.

Missing values were assessed and compared across the three echinocandin groups for all covariates in the descriptive analyses. Given that few covariates had missing values, the observations with missing values were grouped together and modelled as an “Unknown” category for the corresponding covariate (eg, fungal infection severity). The percent of missing values can be found in Table 1 (Baseline demographics and clinical characteristics by echinocandin group) and Table 2 (Known liver aetiology status and concomitant hepatotoxic treatments by echinocandin group).

9.9.4. Sensitivity Analyses

Alternative Samples

Objective 2 analyses were replicated in the subgroup of patients from the main sample who were treated in the year of index admission in hospitals that had both echinocandins of interest (anidulafungin and caspofungin or anidulafungin and micafungin) on their formulary in the year of the index hospital admission (ie, physician had the option to base the selection of treatments on the characteristics of the patient).

Objective 3 analyses were replicated in 12 subgroups of the study main sample and one sensitivity population, as follows:

- Subgroups of the main study sample
 - Stratified by baseline liver function status (ie, use restriction as a method to adjust for confounding due to hepatic impairment in the baseline period)
 - The baseline LFT 0-2 subgroup was defined as the subset of patients with normal, or mildly or moderately elevated (ie, Grade 0-2) LFT at baseline;
 - The baseline LFT 0 subgroup was defined as the subset of patients with normal (ie, Grade 0) LFT at baseline;
 - Stratified by database (ie, to address the overlap between the Cerner and Humedica databases and possible differences between the patients from the two databases)
 - The Cerner subgroup was defined as the subgroup of patients extracted from the Cerner database;

- The Humedica subgroup was defined as the subgroup of patients extracted from the Humedica database;
- Subgroups of Cerner sample
 - Stratified by baseline liver function status
 - The Cerner baseline LFT 0-2 subgroup was defined as the subset of Cerner patients with normal, or mildly or moderately elevated (ie, Grade 0-2) LFT at baseline;
 - The Cerner baseline LFT 0 subgroup was defined as the subset of Cerner patients with normal (ie, Grade 0) LFT at baseline;
 - Stratified by duration of treatment (ie, assess possible dose-effect relationship in the impact of echinocandins on the absolute risks and incidence rates of severe hepatotoxicity)
 - The Cerner treatment duration 1-3 days subgroup was defined as the subset of Cerner patients with short duration of echinocandin treatment (ie, 1-3 days);
 - The Cerner treatment duration 4-7 days subgroup was defined as the subset of Cerner patients with moderate duration of echinocandin treatment (ie, 4-7 days);
 - The Cerner treatment duration 8+ days subgroup was defined as the subset of Cerner patients with long duration of echinocandin treatment (ie, 8+ days) ;
- Subgroups of Humedica sample
 - Stratified by baseline liver function status
 - The Humedica baseline LFT 0-2 subgroup was defined as the subset of Humedica patients with normal, or mildly or moderately elevated (ie, Grade 0-2) LFT at baseline;
 - The Humedica baseline LFT 0 subgroup was defined as the subset of Humedica patients with normal (ie, Grade 0) LFT at baseline; and
 - The Humedica IDN subgroup was defined as the subset of Humedica patients who belonged to an IDN, who had more complete measurements of comorbidities and health resource utilization. In this analysis, patients' comorbidities and health resource utilization were assessed during the 6-month period prior to the index hospitalization. The list of covariates considered in the log binomial regression analysis included the same covariates as considered in the main analysis and four additional binary

indicators for health resource utilization: inpatient visit, outpatient visit, ED visit and other health resource utilization.

- The sensitivity population included patients without baseline LFT and encompassed patients who met all eligibility criteria specified for the main study sample, except for the criterion requiring ≥ 1 LFT in the baseline period; this sensitivity analysis aimed to increase the generalizability of the study by including patients with immunocompromised conditions, who may have necessitated immediate treatments (ie, did not have the opportunity to have LFT before initiating the echinocandins), and healthier patients who had no indication for baseline LFT.

Alternative Outcomes

The study outcome was severe hepatotoxicity defined as the first LFT of Grades 3 or higher in the observation period. In sensitivity analyses, two alternative definitions for the outcome were used:

- First LFT of Grade 4 or higher in the observation period
- First LFT of Grade 5 (death due to hepatic causes) in the observation period (similar to the main analysis; death due to hepatic causes was identified based on the presence of a Grade 4 LFT; this definition was used because neither cause of death nor the primary or secondary discharge diagnoses could be identified in the Cerner and Humedica data). Because this was the most severe outcome group, baseline demographic and clinical characteristics were assessed and compared across echinocandins.

The two alternative study outcomes were analysed for the main study sample and the sensitivity population.

9.9.5. Amendments to the Statistical Analysis Plan

Six additional subgroup analyses were added to the statistical analysis plan based on baseline LFT. Restricting to patients with certain baseline LFT allowed for the assessment of incidence cases of hepatotoxicity and adjustment for confounding due to hepatic impairment in the baseline period. These subgroups included patients with:

- Baseline LFT corresponding to Grade 0 for the combined databases, Cerner only, and Humedica only
- Baseline LFT corresponding to Grades 0-2 for the combined databases, Cerner only, and Humedica only.

Since hospital formulary information was not available in Cerner and Humedica databases, a proxy variable was created to measure the formulary for echinocandin based on all types of echinocandins observed in the data for a given hospital (Cerner) or hospital grouping (Humedica) in the year of the patient's index admission. This proxy variable included the following:

- All three echinocandins (anidulafungin, caspofungin and micafungin)
- Anidulafungin and caspofungin only
- Anidulafungin and micafungin only
- Caspofungin and micafungin only
- Anidulafungin only
- Caspofungin only
- Micafungin only

Additionally, a subgroup analysis was performed for Objective 2 among patients from hospitals or hospital groupings who had both treatments (anidulafungin vs caspofungin and anidulafungin vs micafungin) available in this proxy variable for formulary. This was done to reduce hospital bias in formulary selection.

9.10. Quality Control

Internal audits of all data collection, analytical modelling, and written materials were conducted by the Analysis Group, Inc. Internal audits consisted of a review of all final work product materials and the underlying analysis, including all statistical programs, and supporting source documentation by a team member or another conflict-cleared employee who was not involved in the creation of the original work product. Quality review of all final deliverables were documented and retained by a qualified individual independent of the writing team and incorporated the following steps:

1. Confirmed that the source of the data and/or results was documented and that results and data had been verified against the source.
2. Checked the internal consistency of any data presented in the document.
3. Confirmed that the conclusions were accurate, objective, balanced, and consistent with other published or released results.
4. Confirmed that the format and content of the document were aligned with applicable external requirements.

9.11. Protection of Human Subjects

Subject Information and Consent

Not Applicable

Independent Ethics Committee (IEC)/ Institutional Review Board (IRB)

The final protocol documentation was reviewed and approved by the New England Institutional Review Board(s) (IRB). All data provided were de-identified in compliance with HIPAA regulations

Ethical Conduct Of the Study

The study was conducted in accordance with legal and regulatory requirements, as well as with scientific purpose, value and rigor, and followed generally accepted research practices described in Good Pharmacoepidemiology Practices (GPP) issued by the International Society for Pharmacoepidemiology (ISPE), European Medicines Agency (EMA), European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology, and Food and Drug Administration (FDA) Guidance for Industry: Good Pharmacovigilance and Pharmacoepidemiologic Assessment, FDA Draft Guidance for Industry and FDA Staff: Best Practices for Conducting and Reporting of Pharmacoepidemiologic Safety Studies Using Electronic Healthcare Data Sets.

10. RESULTS

10.1. Participants

Main Study Sample

The study sample selection flowchart is presented in Figure 2. The study sample was extracted from the Cerner and Humedica databases. The raw Cerner data included 13,628 patients with records for at least one dose of echinocandin over the period January 2006 - June 2013, while the raw Humedica data included 11,246 patients with records for at least one dose of echinocandin over the period January 2007 – September 2013. The study eligibility criteria were applied on the combined sample of 24,874 Cerner and Humedica patients. Patients who did not have any hospitalization during the study period (n = 1124), who had hospital admissions but did not have any in-hospital echinocandin use (n = 1648), who used multiple echinocandins during their hospitalizations with echinocandin use (n = 225), or who were <18 years of age at the eligible hospital admission (n = 598) were excluded. In the remaining sample, on average, a patient had 1.07 hospitalizations. Among patients with multiple eligible hospitalizations, the most recent hospitalization was selected for the analysis. Patients without AST, ALT or total bilirubin tests in both the baseline and observation periods (n = 8601) were further excluded, while patients without AST, ALT, or total bilirubin result at baseline only were added back for sensitivity analyses. The remaining 12,678 patients formed the main study sample, of which 1700 (13.4%) patients were in the anidulafungin group, 4431 (35.0%) in the caspofungin group and 6547 (51.6%) in the micafungin group (Figure 2).

Analyses related to Objective 1 were performed in each echinocandin group separately. Analyses related to Objectives 2 and 3 were performed in two analytic samples: one analytic sample including the patients in the anidulafungin and caspofungin groups combined (n = 6131) and another analytic sample including the patients in the anidulafungin and micafungin groups combined (n = 8247).

Subgroups Of the Main Study Sample and Sensitivity Population

The multivariate regression models that estimated the impact of echinocandin treatment on the absolute risk of severe hepatotoxicity (Objective 3) were conducted in the following 12 subgroups of the main study sample and one sensitivity population, as follows:

- Subgroups of the main study sample
 - Stratified by baseline liver function status
 - Baseline LFT 0-2 subgroup (n = 9161),
 - Baseline LFT 0 subgroup (n = 3562),
 - Stratified by database
 - The Cerner subgroup (n = 6930),
 - The Humedica subgroup (n = 5748);
- Subgroups of Cerner sample
 - Stratified by baseline liver function status
 - Cerner baseline LFT 0-2 subgroup (n = 5177),
 - Cerner baseline LFT 0 subgroup (n = 1997),
 - Stratified by duration of treatment
 - Cerner treatment duration 1-3 days subgroup (n = 1512),
 - Cerner treatment duration 4-7 days subgroup (n = 2086),
 - Cerner treatment duration 8+ days subgroup (n = 3060);
- Subgroups of Humedica sample
 - Stratified by baseline liver function status
 - Humedica baseline LFT 0-2 subgroup (n = 3984),
 - Humedica baseline LFT 0 subgroup (n = 1565),
 - Humedica IDN subgroup (n = 5748);
- Sensitivity population including patients without baseline LFT (n = 14,043);

Please see Section 9.9.4 for the definitions of the subgroups and sensitivity population. The flowchart that presents the derivation of the subgroups of the main study sample and the sensitivity population is presented in Figure S1. Similar to the main analysis, two analytic samples were defined for each of the subgroups of the main sample and sensitivity population: one anidulafungin-caspofungin analytic sample and one anidulafungin-micafungin analytic sample.

10.2. Descriptive Data

Comparisons of patient characteristics between the anidulafungin and the caspofungin and micafungin groups are presented in Tables 1 and 2.

Demographics

Compared to patients in the caspofungin and micafungin groups, patients in the anidulafungin group were slightly younger (median age 60 years vs 62 and 61 years, $p < 0.001$ for both comparisons) and included more males (55.7% vs 50.1% and 51.5%, $p < 0.001$ for both comparisons) and fewer Caucasians (58.1% vs 72.3% and 75.0%, $p < 0.001$ for both comparisons) (Table 1).

The median year of index admission was 2009 for anidulafungin and caspofungin groups and 2011 for the micafungin group. Humedica data were over-represented in the anidulafungin group (70.1%), while Cerner data were over-represented in the caspofungin group (70.3%). In contrast, the datasets were equally distributed in the micafungin group (49.5% Humedica and 50.5% Cerner) (Table 1).

Baseline Liver Function

All measurements of baseline liver function pointed to worse LFT results for the anidulafungin group relative to caspofungin and micafungin groups (with all p -values less than < 0.001). Compared to the patients in the caspofungin and micafungin groups, patients in the anidulafungin group had higher AST (median IU/L: 78.0 vs 58.0 and 52.0), higher ALT (median IU/L: 52.0 v. 45.0 and 42.0), and higher total bilirubin (median mg/dl: 1.5 vs 1.3 and 1.1) at baseline, with p -values < 0.001 for all the comparisons. This was reflected in more patients with AST, ALT and total bilirubin tests of Grades 3-4 in the baseline period in the anidulafungin group than in the caspofungin and micafungin groups (AST: 26.6% vs 16.8% and 16.9%; ALT: 17.9% vs 9.8% and 10.9%; bilirubin: 27.7% vs 15.5% and 14.9%; all p -values < 0.001). Consistently, the overall grade of hepatotoxicity, defined based on the combined results of AST, ALT and bilirubin tests in the baseline period, was also higher in the anidulafungin than the caspofungin and micafungin groups (overall baseline LFTs of Grades 3 and 4: 40.4% vs 25.9% and 25.6%, $p < 0.001$ for both comparisons) (Table 1).

Fungal Treatment and Infection

There were no differences between the echinocandin groups with respect to the proportions of having previous hospitalizations with echinocandin use (range 5.2%-6.2%, all p -values were non-significant). At baseline, 42.9% of the anidulafungin patients had used amphotericin B, fluconazole or extended-spectrum azoles versus 44.1% caspofungin patients ($p = 0.390$) and 31.7% micafungin patients ($p < 0.001$) (Table 1).

Type of fungal infection was determined based on the diagnosis code for candidiasis. However, such diagnosis codes were poorly populated in the data, with $> 70\%$ of the patients treated with echinocandins having no diagnosis code for candidiasis. Patients in the anidulafungin group were less likely to miss such fungal infection codes than those in the

caspofungin and micafungin groups (missing codes: 66.2% vs 80.2% and 80.0%, $p < 0.001$). Among patients with non-missing candidiasis codes, the anidulafungin group had more patients with ≥ 3 infection sites (5.9% vs 1.1% and 1.4%, $p < 0.001$), compared to the caspofungin and micafungin groups (Table 1).

Comorbid Conditions

When compared to patients in the caspofungin and micafungin group, patients in the anidulafungin group had higher probability of death based on their comorbidity profiles (CCI score ≥ 3 : 60.4% vs 36.4% and 41.8%) and higher prevalence of CVD (71.1% vs 42.1% and 49.8%), diabetes (28.1% vs 19.1% and 22.7%), endocarditis (9.9% vs 2.0% and 2.8%), oesophageal varices (2.4% vs 0.9% and 1.3%), hypertension (53.7% vs 29.2% and 35.5%), kidney disease (40.2% vs 17.5% and 21.2%), mild liver disease (12.8% vs 7.3% and 9.6%), organ failures (69.4% vs 46.7% and 51.5%), pancreatitis (8.5% vs 5.1% and 5.4%), and sepsis or septic shock (68.5% vs 46.9% and 47.9%), all with p -values < 0.05 . The CKD stage determined based on kidney function lab tests was also higher for anidulafungin than caspofungin patients (CKD stage 5: 24.9% vs 19.9% and 19.4%, $p < 0.001$). However, no differences were observed between the anidulafungin and caspofungin patients in the prevalence of GERD, neutropenia and obesity and no differences were observed between the anidulafungin and micafungin patients in the prevalence of alcohol abuse and neutropenia. Also, the prevalence of alcohol abuse was slightly higher in the caspofungin than anidulafungin patients (1.5% vs 0.9%, $p = 0.048$), while the prevalence of obesity was slightly higher in the micafungin than anidulafungin patients (9.4% vs 7.3%, $p = 0.007$) (Table 1).

Risk Factors For Fungal Infection

When compared to patients in the caspofungin and micafungin groups, patients in the anidulafungin group had more critical care admissions (75.3% vs 52.6% and 48.6%) and surgeries in the baseline period (41.1% vs 33.7% and 27.1%) and used more often central venous catheters (43.8% vs 13.3% and 19.3%) and immunosuppressive therapy (14.6% vs 4.4% and 5.9%), with p -values < 0.001 for all the comparisons. Broad-spectrum antibiotics were widely used in the baseline period by patients in all echinocandin groups ($> 94\%$ in all groups), with no significant differences being observed between the anidulafungin and caspofungin patients ($p = 0.691$) and slightly higher use being observed in the anidulafungin than micafungin patients (95.6% vs 94.3%, $p = 0.042$). Fewer than 20 patients across the full sample had diagnosis or procedure codes for hyperalimentation and catheter removal within 24 hours of admission (Table 1).

Index Hospitalization Characteristics

The index hospitalization length of stay was similar for the anidulafungin and caspofungin patients with the same median length of stay of 28 days ($p = 0.702$), and shorter for the micafungin patients (23 days, $p < 0.001$). The duration of the baseline period was also similar for the anidulafungin and caspofungin patients with the same median duration of 10 days ($p = 0.931$) and shorter for the micafungin patients (7 days, $p < 0.001$) (Table 1).

Of all anidulafungin patients, 35.2% were treated in hospitals that only used anidulafungin treatments in the year of the index admission (proxy for drug formulary); for caspofungin 34.7% were treated in hospitals that only had caspofungin on the drug formulary; for micafungin, 25.3% were treated in hospitals that only had micafungin on the drug formulary (Table 1).

Possible Liver Aetiology Indicators

Possible liver aetiology indicators included acute forms of liver disease and medications that were known to have hepatotoxic effects.

Pre-existing liver diseases were more common among the patients in the anidulafungin group than those in the caspofungin and micafungin groups (26.9% vs 12.5% and 15.9%, $p < 0.001$ for both). In all echinocandin groups, the most common forms of liver disease were liver disease secondary to biliary pathologies, followed by viral hepatitis (Table 2).

Across all echinocandin groups, the median number of distinct hepatotoxic drugs used at the baseline period was 12, with acetaminophen being the most commonly used hepatotoxic drug in all groups, followed by antibiotics and NSAIDs. Acetaminophen and antibiotics were used significantly less by the anidulafungin than the caspofungin and micafungin patients (73.8% vs 81.9% and 82.1%, 46.2% vs 53.8% and 51.3%, respectively; all p -values < 0.001); however, there were no differences between the study groups with respect to the use of NSAIDs (Table 2).

10.3. Outcome Data

Of 12,678 patients in the study sample, 3148 (24.8%) experienced a severe hepatotoxicity event over a median observation period of 11 days (IQR: 4-21 days). Grade 3 events were the first severe hepatotoxicity events described in the observation period for 21.0% of the patients in the study sample while Grade 4 events were the first severe hepatotoxicity events described in the observation period for 3.8% of the patients in the study sample. By definition, hepatotoxicity-related deaths (Grade 5 events) had to be preceded by a LFT of Grade 4, so they could not be the first event experienced by a patient (Tables 3 and 4).

In the anidulafungin, caspofungin and micafungin groups, 37.2%, 22.4% and 23.3% of the patients, respectively, experienced a severe hepatotoxicity event over a median observation period of 9, 12 and 10 days, respectively (Tables 3 and 4). Grade 3 events were the first severe hepatotoxicity events observed in the observation period for 29.6%, 19.6% and 19.7% of patients in anidulafungin, caspofungin and micafungin groups, respectively; Grade 4 events were the first severe hepatotoxicity events observed in the observation period for 7.6%, 2.8% and 3.6% of these patients, respectively.

10.4. Main Results

Unadjusted and Adjusted Estimates Of Absolute Risks and Incidence Rates Of Severe Hepatotoxicity in Hospitalized Patients Treated With Echinocandins (Objective 1)

Absolute Risk Of Severe Hepatotoxicity

The unadjusted absolute risk of severe hepatotoxicity in the study sample of all echinocandins was 24.8% (95% CI: 24.0 - 25.7), and it was 37.2% (95% CI: 34.3 - 40.1), 22.4% (95% CI: 21.0 - 23.8), and 23.3% (95% CI: 22.1 - 24.4) in the anidulafungin, caspofungin and micafungin groups, respectively (Table 3).

By standardizing patient covariates in each echinocandin group to the distribution of covariates in the full study sample, the adjusted absolute risk of severe hepatotoxicity decreased to 25.7% (95% CI: 24.7 - 26.7) in the anidulafungin group, and increased to 24.3% (95% CI: 23.4 - 25.2) and 24.8% (95% CI: 23.9 - 25.6) in the caspofungin and micafungin groups, respectively (Table 3).

Incidence Rate Of Severe Hepatotoxicity

The unadjusted incidence rate of severe hepatotoxicity in the study sample was 0.44 events per 30 person-days (95% CI: 0.43 - 0.46); in the anidulafungin, caspofungin and micafungin groups it was 0.72 (95% CI: 0.66 - 0.77), 0.35 (95% CI: 0.33 - 0.37), and 0.45 (95% CI: 0.42 - 0.47), respectively (Table 4).

By standardizing patients covariates in each echinocandin group to the distribution of covariates in the full study sample, the adjusted incidence rate of severe hepatotoxicity decreased to 0.47 (95% CI: 0.44 - 0.51) in the anidulafungin group, increased to 0.41 (95% CI: 0.38 - 0.44) in the caspofungin group, and remained almost unchanged in the micafungin group (0.45 [95% CI: 0.43 - 0.48]) (Table 4).

Time to First Severe Hepatotoxicity Event

Unadjusted and adjusted Kaplan-Meier curves for time to first severe hepatotoxicity event are presented in Figure 3 for anidulafungin versus caspofungin groups and in Figure 4 for the anidulafungin vs micafungin groups.

Figure 3 shows that most severe hepatotoxicity occurred soon after the index date (median time to severe hepatotoxicity was 1 day [Table 4]), and patients in anidulafungin group had lower event-free survival rates over time than patients in caspofungin group both before and after adjusting the survival curves to the covariate distribution of the anidulafungin-caspofungin analytical sample. While the adjusted log-rank test remained statistically significant ($p = 0.0310$), the two survival curves became closer after covariate adjustment.

Similar results are illustrated in Figure 4 for the anidulafungin versus micafungin comparison, except that the log-rank test became not significant after the adjustment ($p = 0.1870$).

Clinical and Demographic Features Associated With the Type Of Echinocandin Received (Objective 2)

Predictors of treatment with anidulafungin versus caspofungin and with anidulafungin versus micafungin were estimated using logistic regressions with stepwise selection (please see Section 9.9.2 for methodology).

The baseline clinical and demographic features that were found to be associated with a statistically significantly increased probability of receiving anidulafungin treatment, as opposed to caspofungin treatment ($p < 0.05$), included the following (Table 5):

- Being Caucasian and Black or African-American race (relative to other race),
- Having higher grade of baseline bilirubin,
- Using extended-spectrum azoles,
- Having ≥ 2 fungal infection sites,
- Having critical care admission,
- Using central venous catheter,
- Using immunosuppressive therapy,
- Using a larger number of distinct hepatotoxic drugs,
- Using antiretroviral drugs known to have hepatotoxic effects, and
- Having CVD, hypertension, endocarditis, kidney disease, sepsis or septic shock;

The baseline clinical and demographic features that were found to be associated with a statistically significantly decreased probability of receiving anidulafungin treatment ($p < 0.05$), as opposed to caspofungin treatment, were the following (Table 5):

- Having emergency admission to the index hospitalization,
- Having more recent index hospitalization,
- Being Cerner patient,
- Using amphotericin B or fluconazole,
- Using antibiotics and psychotropic drugs known to have hepatotoxic effects, and
- Having diabetes or GERD.

The baseline clinical and demographic features that were found to be associated with a statistically significantly increased probability of receiving anidulafungin treatment ($p < 0.05$), as opposed to micafungin treatment, included the following (Table 5):

- Being Caucasian and Black or African-American,
- Having higher grade of baseline AST and bilirubin tests,
- Having prior use of in-hospital echinocandins,
- Using extended-spectrum azoles,
- Having ≥ 2 fungal infection sites,
- Having critical care admission,
- Using central venous catheter,
- Having surgery,

- Using immunosuppressive therapy,
- Using antiretroviral drugs known to have hepatotoxic effects, and
- Having CVD, hypertension, kidney disease, endocarditis, sepsis or septic shock;

The baseline clinical and demographic features that were found to be associated with a statistically significantly decreased probability of receiving anidulafungin treatment ($p < 0.05$), as opposed to micafungin treatment, were the following (Table 5):

- Having emergency admission to the index hospitalization,
- Having more recent index hospitalization,
- Being Cerner patient,
- Using a larger number of distinct hepatotoxicity drugs,
- Using antibiotics and acetaminophen drugs known to have hepatotoxic effects,
- Having higher mortality prognosis (measured by $CCI \geq 0$),
- Having renal dysfunction, and
- Having mild liver disease or GERD.

Unadjusted and Adjusted Relative Risks and Incidence Rate Ratios Of Severe Hepatotoxicity in Hospitalized Patients Treated With Anidulafungin Versus Caspofungin and Micafungin (Objective 3)

In unadjusted analyses of RR, anidulafungin was associated with higher absolute risk of severe hepatotoxicity in hospitalized patients as compared to caspofungin (RR: 1.66 [95% CI: 1.53 - 1.80]) or micafungin (RR: 1.60 [95% CI: 1.48 - 1.73]). After adjusting for the baseline LFT and other potential confounders by including the PS in the logistic regression models, the RR for the effect of anidulafungin versus caspofungin decreased to 1.07 and was not statistically significant (95% CI: 0.95 - 1.20). Similarly the adjusted RR for the effect of anidulafungin versus caspofungin decreased to 1.03 and was not statistically significant (95% CI: 0.93 - 1.15) (Table 3).

Incidence Rate Ratio

In unadjusted analyses of IRR, anidulafungin was associated with higher rates of severe hepatotoxicity in hospitalized patients as compared to caspofungin (IRR: 2.05 [95% CI: 1.85 - 2.26]) or micafungin (IRR: 1.61 [95% CI: 1.47 - 1.76]). After adjusting for the baseline LFT and other potential confounders in multivariate negative binomial models, the IRR for anidulafungin versus caspofungin decreased to 1.43 (95% CI: 1.14 - 1.79), and the IRR for anidulafungin versus micafungin decreased to 1.19 (95% CI: 0.92 - 1.54) (Table 4). The multivariate regression models from which the adjusted IRRs for echinocandin treatments were obtained are presented in Table A2 and show that, for both models, the strongest predictors of severe hepatotoxicity were baseline abnormal LFT (bilirubin of Grades >0 , baseline AST of Grades >0 , baseline ALT of Grades >2), oesophageal varices, sepsis, and the presence of mild liver disease. For the anidulafungin versus micafungin model, CKD stage 4 and $CCI \geq 3$ were also strong predictors of severe hepatotoxicity.

10.5. Other Analyses

10.5.1. Sensitivity Analyses

Clinical and Demographic Features Associated With the Type Of Echinocandin Received (Objective 2)

When analyses for the predictors of treatment with anidulafungin versus caspofungin were conducted in the subgroup of patients treated in hospitals that used both anidulafungin and caspofungin treatments (ie, patients for whom physicians could choose between the two treatments, n = 3450), the results were very similar to those obtained from the main analysis for Objective 2 (see Table 5 and Table A1 for the results of the main analysis and the sensitivity analysis, respectively). Notable exceptions were as follows:

- Factors that were associated with an increased probability of anidulafungin treatment in the main analysis (Table 5), but not in the sensitivity analysis (Table A1)
 - Black and African-American race,
 - Having higher grade of baseline bilirubin,
 - Using extended-spectrum azoles,
 - Using anti-retroviral drugs known to have hepatotoxic effects, and
 - Having kidney disease.
- Factors that were associated with a decreased probability of anidulafungin treatment in the main analysis (Table 5), but not in the sensitivity analysis (Table A1)
 - The use of psychotropic drugs known to have hepatotoxic effects.
- Factors that became associated with an increased probability of anidulafungin treatment
 - CCI ≥ 4 and
 - Having liver disease secondary to biliary pathologies.

When analyses for the predictors of treatment with anidulafungin versus micafungin were conducted in the subgroup of patients treated in hospitals that used both anidulafungin and micafungin treatments (ie, patients for whom physicians could choose between the two treatments, n = 2721), the results were very similar to those from the main analysis for Objective 2 (see Table 5 and Table A1 for the results of the main analysis and the sensitivity analysis, respectively). Notable exceptions were as follows:

- Factors that were associated with an increased probability of anidulafungin treatment in the main analysis (Table 3), but not in sensitivity analysis (Table A1)
 - Race,
 - Having critical care admission,
 - Using central venous catheter,
 - Using antiretroviral drugs known to have hepatotoxic effects, and
 - The presence of comorbid kidney disease.

- Factors that were associated with a decreased probability of anidulafungin treatment in the main analysis (Table 3), but not in sensitivity analysis (Table A1)
 - Admission through emergency room,
 - Having GERD or mild liver disease,
 - Using acetaminophen drugs known to have hepatotoxic effects, and
 - Having high mortality prognosis (measured by CCI ≥ 0).
- Factors that became associated with an increased probability of anidulafungin treatment
 - Having neutropenia and
 - Admission through emergency room.

Adjusted Relative Risks and Incidence Rate Ratios Of Severe Hepatotoxicity in Hospitalized Patients Treated With Anidulafungin Versus Caspofungin and Micafungin (Objective 3)

Relative Risk

The results of the adjusted analyses of RR conducted in the subgroups of patients from the main study sample and sensitivity population are summarized in Table S1. After adjustment for baseline LFT and other potential confounders, the RR estimates for most subgroup analyses were not statistically significant. For instance, in the main baseline LFT 0-2 subgroups, the adjusted RR was 1.11 (95% CI: 0.88 - 1.41) and 1.08 (95% CI: 0.87 - 1.34) for the anidulafungin versus caspofungin patients, and anidulafungin versus micafungin patients, respectively. Exceptions were observed in Humedica and Humedica IDN subgroups, which yielded higher adjusted absolute risks for the anidulafungin patients compared to the caspofungin patients (RRs: 1.21 [95% CI: 1.00 - 1.46] and 1.29 [95% CI: 1.06 - 1.57], respectively) (Table S1).

Incidence Rate Ratio

The results of the adjusted analyses of IRR conducted in the subgroups of patients from the main study sample and sensitivity population are summarized in Table S2. After adjustment for baseline LFT and other potential confounders, the IRR estimates for most subgroup analyses were not statistically significant. For instance, the IRRs for the main baseline LFT 0-2 subgroups, the adjusted IRRs were 1.46 (95% CI: 0.91 - 2.37) and 1.62 (95% CI: 0.95 - 2.77) for the anidulafungin versus caspofungin patients, and anidulafungin versus micafungin patients, respectively. Exceptions were observed in the Humedica subgroup, which yielded statistically significantly higher adjusted incidence rates for the anidulafungin patients compared to the caspofungin patients (IRR: 1.56 [95% CI: 1.16 - 2.08]), the Humedica IDN subgroup, which yielded statistically significantly higher adjusted incidence rates for the anidulafungin patients compared to the caspofungin patients (IRR: 1.74 [95% CI: 1.31 - 2.31]), and the sensitivity population, which yielded statistically significantly higher adjusted incidence rates for the anidulafungin patients in the anidulafungin-caspofungin analytical sample (IRR: 1.37 [95% CI: 1.09 - 1.71]) (Table S2).

(Alternative Outcomes) Adjusted Relative Risks and Incidence Rate Ratios Of Severe Hepatotoxicity

Relative Risk

When alternative outcome definitions were applied and defined as 1) the first occurrence of a severe hepatotoxicity event of Grade 4 or higher or 2) as the occurrence of a severe hepatotoxicity event of Grade 5, most analyses remained non-statistically significant. Specifically, the adjusted RRs estimated in the main study sample and sensitivity population for the anidulafungin versus caspofungin and anidulafungin versus micafungin comparisons ranged from 0.92 to 1.34 and reached statistical significance only for the anidulafungin versus micafungin comparison in the analysis for Grade 5 severe hepatotoxicity (RRs: 1.34 [95% CI: 1.03 - 1.76] and 1.32 [95% CI: 1.01 - 1.73] for the adjusted Grade 5 risk in anidulafungin vs micafungin patients from the main study sample and sensitivity population, respectively) (Table S3).

Incidence Rate Ratio

The adjusted IRRs estimates in the main study sample and sensitivity population for the anidulafungin versus caspofungin and anidulafungin versus micafungin comparisons ranged from 0.85 to 1.48 and reached statistical significance only for the anidulafungin versus caspofungin comparison in the sensitivity population analysis for Grade 5 severe hepatotoxicity (RR: 1.43 [95% CI: 1.01 - 2.03]). (Table S4).

Comparison Of Baseline Characteristics, Known Liver Aetiology Status, and Concomitant Hepatotoxic Treatments Of Patients With Grade 5 Severe Hepatotoxicity Events in the Anidulafungin, Caspofungin and Micafungin Groups

Of the 517 patients in the study sample who had a Grade 5 hepatotoxicity event, 88.2% had severe LFT (Grades 3 or 4 for the overall LFT) in the baseline period. The proportion of patients with Grades 3 or 4 for the overall LFT in the baseline period was slightly higher for the anidulafungin than caspofungin and micafungin patients (92.6% vs 89.3% and 84.5%, respectively), although the differences were not statistically significant. However, patients with Grade 5 events in the anidulafungin group were significantly more likely than patients in the caspofungin and micafungin group to be admitted to critical care (86.0% vs 59.6% and 58.0%), to have surgeries (61.3% vs 34.0% and 35.4%), to use a central venous catheter (62.7% vs 10.6% and 26.5%), and to have high mortality prognosis overall (78.0% with CCI $\geq$ 4 vs 36.9% and 46.5%) and for comorbidities that are known to be associated with increased risk of death in hospitalized patients (90.7% anidulafungin patients had organ failures vs 66.7% and 71.7% caspofungin and micafungin patients; 92.0% had sepsis or septic shock vs 64.5% and 70.4%; and 82.7% had CVD vs 36.9% and 50.0%) (Table S5; all p-values <0.001). Additionally, among patients with Grade 5 events, patients on anidulafungin were more likely to have liver disease aetiologies (60.0% in anidulafungin vs 27.7% and 39.4% in caspofungin and micafungin, respectively) (Table S6; p-value <0.001).

10.6. Adverse Events / Adverse Reactions

Not applicable.

11. DISCUSSION

11.1. Key Results

In this retrospective cohort study based on EMR data of hospitalized patients treated with echinocandins, the majority of the adjusted RRs and IRRs estimates for the effect of anidulafungin versus caspofungin and micafungin on the occurrence of severe hepatotoxicity events were not statistically different from the null, indicating that anidulafungin was not associated with a statistically significantly higher absolute risk or incidence rate for severe hepatotoxicity compared to caspofungin and micafungin. In the IRR comparison to caspofungin, however, there was a statistically significantly higher incidence rate in anidulafungin in the main study sample, although the statistical significance was not present in the subgroup of baseline LFT Grades 0-2.

In unadjusted analyses, patients treated with anidulafungin had higher absolute risk and incidence rates for severe hepatotoxicity events than patients treated with caspofungin and micafungin. However, confounding by indication likely biased the unadjusted absolute risk and incidence rate estimates, because anidulafungin is the only echinocandin not metabolized by the liver. As a result, anidulafungin tended to be channelled towards patients with worse liver function and higher comorbidity burden prognosis at baseline, as shown in the comparison of baseline clinical characteristics in anidulafungin versus caspofungin and micafungin. Attempts to account for this confounding by indication in the current analyses included adjustment for baseline LFT results, other clinical characteristics and potential confounders. Upon adjustment, the difference in risk between the anidulafungin and the caspofungin/micafungin patients decreased: the RRs associated with anidulafungin versus the other echinocandins decreased below 1.10 for both comparisons and became non-statistically significant, while the IRR decreased to values below 1.50 in both comparisons and remained statistically significant only for the anidulafungin versus caspofungin comparison. Noteworthy, caspofungin patients had more favourable clinical profiles than anidulafungin patients at baseline. Similar contrast was observed between patients on micafungin and anidulafungin, although it was slightly less pronounced. Considering the favourable clinical profile of the caspofungin patients as compared to anidulafungin patients at baseline, it is not surprising that the main results of the study correlated with the baseline profile and that the RRs and IRRs estimates decreased after adjustment for baseline covariates. As documented in epidemiology literature,^{15-17, 21} confounding by indication bias is difficult to adjust for, if not impossible, even when detailed clinical measurements are available. In addition, confounder control was limited by the availability of data in the EMR databases. In an attempt to adjust for residual confounding by baseline LFT, subgroup analyses on patients with normal or mildly or moderately elevated LFT results at baseline were conducted. In these analyses, no evidence was found for differences in the absolute risk and incidence rates for severe hepatotoxicity across echinocandins. Results from other subgroup and sensitivity analyses were generally consistent and supported the same findings.

The unadjusted absolute risk of severe hepatotoxicity in patients receiving echinocandins in the study population was higher than that previously reported in clinical trials and literature reviews based on clinical trial data.⁷ In this study population, 24.8% among all patients using echinocandins experienced severe hepatotoxicity, whereas prior reports from clinical trials reported estimates that ranged from 0.2% in patients on caspofungin, 0.8% in patients using

anidulafungin, and 2.7% in patients using micafungin. Several factors could explain the high absolute risk of severe hepatotoxicity observed in this study. First, real-world patients are usually less healthy than patients enrolled in clinical trials due to the restrictive eligibility criteria for enrolment in most trials.^{22, 23} For instance, patients with elevated levels of hepatic enzymes were excluded from the Phase III trial on anidulafungin versus fluconazole in patients with invasive candidiasis,⁶ whereas in the current study, as many as 40.5% of patients on anidulafungin had Grade 3 or higher LFT results at baseline. Given that elevated LFT in the baseline period was found in the current study to be the strongest predictor of severe hepatotoxicity post-treatment, higher baseline LFT in this study than in clinical trials were likely to translate to higher unadjusted absolute risks. Second, because physicians were likely to order LFTs for patients who were at risk for elevated liver enzymes, patients who did not have LFTs in the baseline period were likely to have lower risk for developing hepatotoxicity. Therefore, by excluding from the study sample the patients who did not have LFTs in the baseline period the absolute risk estimates in each echinocandin group might have been inflated. However, because all echinocandin groups were subject to the same inclusion criteria, the comparison of risk between the echinocandin groups may not be as much affected by the exclusion of patients without LFT in the baseline period.

Relative to patients on caspofungin and micafungin, at the unadjusted level, anidulafungin patients had the highest absolute risk of severe hepatotoxicity, with 37.2% of anidulafungin patients experiencing severe hepatotoxicity, compared to 22.4% in patients on caspofungin and 23.3% in patients on micafungin. Unadjusted RR estimates indicated that patients on anidulafungin had 66% and 60% higher absolute risk of severe hepatotoxicity than patients on caspofungin and micafungin, respectively. The differences in the absolute risk of severe hepatotoxicity between patients on anidulafungin and other echinocandins were mostly attributable to the underlying differences in baseline LFT results, and clinical and demographic characteristics of patients receiving the three echinocandins. Indeed, the adjusted RRs indicated only 7% and 3% higher absolute risks in the anidulafungin group compared to the caspofungin and micafungin groups, respectively, and the RRs were not statistically significantly different from 1. Indeed, patients on anidulafungin were significantly more likely to have elevated LFT at baseline: 40.5% patients on anidulafungin had Grade 3 or above LFT results at baseline, compared to only 25.9% and 25.6% in patients on caspofungin and micafungin, respectively.

Physician preference for treating sicker patients with anidulafungin is likely due to the fact that anidulafungin is the only echinocandin not metabolized by the liver. Furthermore, anidulafungin was found in clinical trials to be well tolerated across patients with all degrees of hepatic impairment, even among those with severe hepatic conditions. As a result, anidulafungin is more likely to be channelled to patients with worse hepatic conditions and who are more at risk for severe hepatotoxicity. Indeed, in assessing predictors of echinocandins (Objective 2), the study found that patients with higher baseline bilirubin and/or AST result, multiple fungal infection sites, immunosuppressive therapy or antiretroviral drugs with known hepatotoxic effect, CVD, endocarditis, hypertension, kidney disease, sepsis, or admission to critical care had significantly higher likelihood of treatment with anidulafungin as opposed to caspofungin and micafungin. Consequently, at the unadjusted level, the absolute risk of severe hepatotoxicity across the three echinocandins was confounded by the non-comparability of these patients. In fact, when adjusted for

baseline LFT and other potential confounding variables, the absolute risks of hepatotoxicity across the three echinocandin cohorts became similar (RRs 1.07 for the anidulafungin vs caspofungin comparison and 1.03 for the anidulafungin vs micafungin comparison, not statistically significant).

The confounding by indication was likely even stronger among patients who experienced Grade 5 severe hepatotoxicity. The differences in baseline characteristics between the anidulafungin and caspofungin/micafungin patients who experienced Grade 5 hepatotoxicity events were more striking than in the full main study sample. In particular, patients on anidulafungin with Grade 5 severe hepatotoxicity had higher rates of baseline comorbidities and other clinical characteristics that are known to be highly associated with mortality; in the anidulafungin group, over 90% patients had organ failures (heart and renal failures) or sepsis and septic shock, and nearly 83% patients had CVD. In comparison, no more than 67% or 72% of patients on caspofungin and micafungin, respectively, had these conditions. Additionally, 86.0% of patients with Grade 5 event and on anidulafungin were admitted to critical care, compared to 58.6% and 58.0% in patients on caspofungin and micafungin. Notably, in the current study, cause of death could not be ascertained because the principal or secondary discharge diagnoses associated with death were either not available (in Humedica) or did not have a date indicator (in Cerner) (see additional details in Section 11.2). As a result, a death was attributed to hepatic causes if there were laboratory LFT results indicating Grade 4 elevations of LFT between the index date and death. In so doing, certain deaths may have been erroneously ascribed to hepatic cases, over-estimating Grade 5 events across all echinocandins. Given that anidulafungin patients with Grade 5 severe hepatotoxicity had higher rates of baseline comorbidities and other clinical characteristics that are known to be highly associated with mortality, it is possible that they were more likely to experience any death during the observation period, potentially contributing to the increased risk of Grade 5 event observed in this group of patients.

In the current study, the underlying differences in prognosis across echinocandins were accounted for in the analysis by adjusting for observable baseline differences in all multivariate analyses. However, the amount of confounding adjustment was limited by data availability, such that only variables that were assessed and sufficiently populated could be adequately adjusted for in the analyses. Inevitably, residual confounding was likely. While it is not possible to quantify the amount of residual confounding, it is nonetheless possible to identify potential sources of unmeasured confounding. For instance, hospitals may have specific formularies for echinocandins, which dictate the prevalence of echinocandins use across hospitals. Given that the variable for hospital formulary was not available in the Cerner and Humedica data, the amount of confounding adjustment resulting from using a proxy measure of the formulary on the outcome models remains unknown. Nonetheless, assuming that the most likely reason for hospital formulary differences is drug costs, confounding by indication bias may be minimal because in hospitals that covered only one echinocandin the treatment choice could not have been driven by the characteristics of the patient. However, if formulary status is associated with other practices that might affect the risk of severe hepatotoxicity (eg, the hospital's quality of care), then the current risk estimates may be under- or over-estimated. For instance, given that previous studies have shown anidulafungin to be more cost-saving than other echinocandins,^{6, 24} specialized hospitals with greater admittance of more severe patients and more incurred costs may be

more likely to have anidulafungin on their formulary. This may result in confounding by indication that biases against anidulafungin. Another potential source of unmeasured confounding is alcohol use, a risk factor for hepatotoxicity that is often underreported in routine care and in EMR data. Because in the study sample from this study only approximately 1.3% of the patients had records indicating alcohol use/abuse, likely an underestimate, the variable was not useful in the confounding adjustment.

In an effort to control for residual confounding, in particular confounding by indication, subgroup analyses were conducted on both patients with normal (ie, Grade 0) LFT results, and patients with normal, or mildly or moderately elevated LFT results (ie, $\leq$ Grade 2) during the baseline period. In these subgroups, the increase in severe hepatotoxicity during hospitalization was more likely attributable to the echinocandin of interest, after adjusting for other covariates and confounding variables. Consistent with expectations, no evidence was found for significant differences between the echinocandin groups in the risk of severe hepatotoxicity events. A similar pattern was observed when the subgroup analyses were conducted on patients with $\leq$ Grade 2 LFT results at baseline among Cerner and Humedica subgroups. Nonetheless, it should be noted that the sample sizes of these subgroups were smaller than that for the main study sample, so reduced power may have also contributed to the non-significance of the effects observed in the subgroup analyses.

While the subgroup results suggest that among patients with more favourable LFT results at baseline the three echinocandins had similar risk of severe hepatotoxicity, it is unclear whether similar results would be observed in patients with worse liver function at baseline. The outcome analyses were not replicated in the subgroup of patients with $\geq$ Grade 3 LFT results at baseline, because the sample sizes were much smaller and did not have sufficient power. Furthermore, these results would have nonetheless remained biased by confounding by indication, even with covariate adjustment, because residual confounding related to baseline liver function is expected to be more pronounced in patients with worse prognosis.

11.2. Limitations

This study had several limitations. Aside from general limitations intrinsic in EMR data, such as possible inaccuracies in coding diagnoses, procedures, or pharmacy orders, the study was subject to several design-related limitations, as detailed below.

As discussed in Section 11.1, the results of this study showed that treating physicians channelled patients predisposed to or at risk of hepatic impairments towards anidulafungin treatment potentially because anidulafungin does not metabolize through liver and is less prone for drug-drug interactions. As such, patients on anidulafungin were at a higher baseline risk of severe hepatotoxicity compared to patients on other echinocandins, resulting in confounding by indication, which is unavoidable in real-world observational data where echinocandin assignment is not random, and is difficult to adjust for.^{15-17, 21} Attempts to account for this confounding by indication within this study included both controlling for baseline LFT and other imbalances of confounders across echinocandin patients in the multivariate analyses, and by conducting subgroup analyses on patients with normal to moderately elevated LFT results at baseline. However, the amount of confounding adjustment was limited by data availability, so it is possible that residual confounding biasing the estimates away from the null remains (ie, bias towards higher risk in patients on

anidulafungin). In addition, given that data used in the study were hospitalization data (except for a subgroup of patients in the Humedica IDN subsample who also had outpatient records), available patient medical history was only restricted to the time between hospital admission and first use of echinocandin. Hence, patients' full medical history was not available for the majority of the patients, and potential confounders, such as patients' prior exposure to echinocandins and prior medical history of liver diseases and other comorbidities, could not have been completely controlled for in the study. However, the RR and IRR estimates from the Humedica sample did not change when the comorbidities were measured using the patient's full medical history in the 6 months prior to the index admission (in the Humedica IDN subsample).

Given some hospitals contribute data to both the Humedica and Cerner databases, there were some duplicate records in the main study sample. The extent of the overlap between Cerner and Humedica databases is unknown. Additionally, these duplicate records could not be removed, as originally proposed in the study protocol, because the key variables needed to identify them (eg, hospital identifier, hospital region) were not available in both databases. As such, the variance in the point estimates for the association between echinocandin and severe hepatotoxicity may be underestimated although the point estimates were not affected by such overlap,²⁵ thereby yielding results that were more likely to be statistically significant. To address the overlap, sensitivity analyses stratified by database were conducted, which yielded results with more accurate standard errors.

In the study, severe hepatotoxicity was defined as having LFT results greater than Grade 3 in the observation period. However, because a requirement for normal baseline LFT results was not imposed, patients who had $\geq$ Grade 3 LFT results in both the baseline and observation periods were considered to have the outcome. Therefore, the outcome definition used in this study did not capture only incidence cases of severe hepatotoxicity, but also prevalent cases. As discussed previously, given that a greater proportion of patients on anidulafungin had $\geq$ Grade 3 LFT results at baseline, the current definition would have captured more prevalent cases in this group, thereby biasing the absolute risk and incidence rate ratios against anidulafungin. In an effort to capture only incidence cases, subgroup analyses were conducted on patients with baseline LFT results of Grade 0, as well as $\leq$ Grade 2, and found that the absolute risk and incidence rate of severe hepatotoxicity were not significantly different across the three echinocandins. In the analyses using alternative outcomes, Grade 5 severe hepatotoxicity was defined in the original protocol based on a primary or secondary discharge diagnosis containing at least one diagnosis code for liver diseases. However, in the Humedica databases, no principal or secondary discharge diagnoses could be identified, while in the Cerner database, diagnosis codes were rolled-up during the full hospitalization episode without date indicators (ie, potential diagnoses associated with death could not be matched by date). In order to identify hepatic deaths (Grade 5 events), the study relied on laboratory LFT results indicating Grade 4 elevations of LFT between the index date and death. However, it is possible that some of the deaths were erroneously ascribed to hepatic causes as a result of the measurement limitations for this variable.

Given that only hospitalization data were studied, the observation period was short (mean: 16.9 days). Hence, it is likely that only acute liver injuries were included in the study.

However, echinocandins are not drugs that are used over long periods of time, so it is possible that their effect is more acute than chronic.

In the US, hospital formularies drive the use of specific drugs, including echinocandins. As discussed previously, the data used in this study did not include direct information on hospital formulary, nor hospital characteristics in the databases to assess the hospital effects on the results, so there is likely misclassification in the measurement of the formulary proxy. If formulary status is associated with hospital characteristics that in turn are associated with the risk of severe hepatotoxicity, then the current risk estimates may be biased.

In the Cerner database, diagnosis codes, including those for baseline comorbidities, predictors of echinocandin, and known aetiologies of severe hepatotoxicity, could only be linked to a specific hospitalization, but not to the exact date of diagnosis. As such, covariates that were assessed based on ICD-9-CM diagnosis codes were measured over the full hospitalization period, making temporality between these diagnoses, exposure to echinocandin, and severe hepatotoxicity impossible (to ensure consistency in the combined Cerner and Humedica data, comorbidities were measured for Humedica patients with the same definition). It is possible that in adjusting for baseline comorbidities in the multivariate analyses, over-adjustment for comorbidities that first occurred in the observation period could have resulted. However, for chronic conditions, which comprise the majority of the diagnoses considered in this study, one can assume that they were likely pre-existing prior to hospitalization rather than newly developed during the short observation period.

As aetiologies for hepatotoxicity may require workups and may not be completely identified during hospitalizations, covariates for known aetiologies were possibly under-documented. The increased number of idiopathic hepatotoxicity cases may be falsely ascribed to the study drugs, given that patients on anidulafungin may have worse liver function and more aetiological conditions for severe hepatotoxicity than patients on other echinocandins. As such, the risk of severe hepatotoxicity due to the use of antifungal medication may be biased against anidulafungin.

Patients' underlying severity of fungal infection may affect the underlying risk of hepatotoxicity. Indeed, in the analyses from this study, it was observed that patients with invasive fungal infection had higher risk of severe hepatotoxicity (IRR: 1.41). Although efforts were made to control for fungal infection severity and progression during the hospitalization, data from microbiology lab results were scant since it is expected that many fungal infection cases may have been treated empirically without labs ordered.

The risk of severe hepatotoxicity may increase with extended exposure to echinocandins. However, in the Humedica database, measurements on drug exposure duration were not available, such that the impact of exposure duration on the outcome could not be assessed in the combined datasets and in the stratified analyses on the Humedica database only. Nonetheless, an exposure-specific variable was created to stratify analyses by the exposure duration by number of days in the Cerner database, and the risk of severe hepatotoxicity did not differ by exposure duration.

As part of the eligibility criteria, patients were selected into the study only if they had at least one LFT result during the observation period. If patients who never received LFTs were at lower risk of severe hepatotoxicity, as their physicians may not deem it necessary to order such lab work due to their perceived risk of liver injury, the study population would have excluded patients with good prognosis of severe hepatotoxicity. As such, the absolute risks and incidence rates observed across the echinocandins would have been higher than expected. This could negatively impact the anidulafungin group if physicians were less likely to order LFTs for these patients because of perceived safer liver profiles. Lastly, in order to extend the generalizability of the study results, a sensitivity analysis was conducted to include all patients receiving echinocandins regardless of LFT results prior to receiving the antifungal therapy, as not all patients may have been tested for LFT before treatment initiation. However, this sensitivity analysis may have introduced bias in the etiological assessment of the role of echinocandin on severe hepatotoxicity given that missing baseline LFT results were not random across the three echinocandin groups (5.2% in anidulafungin, 9.1% in caspofungin, and 8.9% in micafungin). Nonetheless, the sensitivity results yielded incidence rates that were similar to those obtained from the main analyses.

Lastly, given that over 70 comparative analyses were conducted in this study, including main, subgroup and sensitivity analyses combined, the risk of Type I error may have been inflated through multiple testing. It is possible that the statistically significant findings observed in this study may have been due to chance alone. While various procedures such as Bonferroni corrections and sequential adjustments could be applied to adjust for multiple testing, these approaches are subject to high Type II error rate, making it difficult to identify a statistically significant difference in the risk of severe hepatotoxicity between anidulafungin and caspofungin or micafungin, if one exists. As such, the approach of not adjusting for multiple testing used in this study is less likely to overlook at potential statistically significant association.

11.3. Interpretation

Upon adjustment for covariates and confounding in the study main analyses using the combined datasets, the absolute risk of severe hepatotoxicity in patients on anidulafungin was 7% higher than that in patients on caspofungin (RR: 1.07, $p = 0.2663$), and 3% higher than that in patients on micafungin (RR: 1.03, $p = 0.5392$). Similarly, the incidence rate of severe hepatotoxicity in patients on anidulafungin was 43% higher than that in patients on caspofungin (IRR: 1.43, $p = 0.0022$), and 19% higher than that in patients on micafungin (IRR: 1.19, $p = 0.1825$). As discussed, these heightened risks of severe hepatotoxicity, especially the statistically significant IRR observed for anidulafungin vs caspofungin, were likely due to residual confounding in the adjustment for the underlying differences in liver function across these patient cohorts. Furthermore, given that the current definition of severe hepatotoxicity did not account for baseline LFT results, existing (ie, prevalent) cases of severe hepatotoxicity prior to echinocandin initiation could have been captured as outcomes. As patients on anidulafungin had worse liver function at baseline, more prevalent cases may have been captured in the anidulafungin patients compared to other echinocandins, leading to an overestimation of the risk among the anidulafungin patients. Indeed, when subgroup analyses were conducted on patients with LFT results $\leq$ Grade 2 (ie, patients considered to have a better prognosis for severe hepatotoxicity), the trend of increased severe

hepatotoxicity risk disappeared. In addition to addressing the issue of confounding by indication due to the channelling of anidulafungin towards sicker patients, these subgroup analyses further support the fact that among patients with lower risk of liver impairment at baseline, the risk of severe hepatotoxicity after using any of the three echinocandins was not significantly different. Among patients with high risk of liver impairment at baseline, the risk of severe hepatotoxicity due to echinocandins is difficult to separate from the risk of severe hepatotoxicity due to the already impaired liver function. Therefore, a conclusion regarding the risk of severe hepatotoxicity associated with echinocandin use that is generalizable to patients with high baseline LFT results requires further assessments and datasets that have more refined measurements of patient clinical characteristics.

It is noteworthy that all adjusted RRs and IRRs for severe hepatotoxicity between anidulafungin and other echinocandins estimated in this study, in both the main analyses and sensitivity analyses, were less than 2. According to epidemiologic sources mostly targeted for litigation settings, an RR of 2 is required to establish causality. Specifically, an RR of 2 translates to an attributable risk of 50%, suggesting that a person exposed to the therapeutic agent would have a 50% likelihood of developing the disease due to the agent. If the attributable risk is <50%, it is considered that the evidence is not strong enough to shift the conclusion away from the null hypothesis. In the study main analysis, the RRs of 1.07 and 1.03, and the IRRs of 1.43 and 1.19 for anidulafungin versus caspofungin and anidulafungin versus micafungin, respectively, fell well below this cut-off. Similarly, the RRs of 1.31 ($p = 0.0931$) and 1.34 ($p = 0.0302$) and the IRRs of 1.48 ($p = 0.0782$) and 1.02 ($p = 0.9435$) from the Grade 5 severe hepatotoxicity outcome analysis for anidulafungin versus caspofungin and anidulafungin versus micafungin, respectively, were also well below this cut-off.

While the study results suggest that the adjusted risk of severe hepatotoxicity is not different across echinocandins, especially in patients with better hepatic prognosis, the fact that the overall absolute risk of severe hepatotoxicity across all echinocandins was about 25% is noteworthy. This high absolute risk may be largely due to prevalent cases of severe hepatotoxicity being mixed with incident cases post-echinocandin initiation as per the outcome definition. Furthermore, one of the eligibility criteria required patients to have at least one LFT during the observation period. As discussed previously, if not having LFT done is associated with better liver function, it is expected that the patients in the study sample would present a higher absolute risk and incidence rate of severe hepatotoxicity than the general population of hospitalized patients receiving echinocandins.

In assessing clinical significance of this absolute risk, it is important to assess the comparative safety and effectiveness of anidulafungin against the other echinocandins. While no head-to-head studies have been conducted in the real-world or clinical trial settings comparing anidulafungin to other echinocandins, data from literature review and meta-analyses suggested that the three echinocandins have comparable safety profile. Despite the potential of confounding by indication biasing against anidulafungin, the results of this study further support this conclusion. In face of the large disease burden of blood stream fungal infections by *Candida* species., which constitute the majority of invasive fungal infections in intensive care patients with a high mortality rate, the absolute risk of severe hepatotoxicity may be acceptable for anidulafungin and other echinocandins.

11.4. Generalizability

This study employed data from two major US-based hospital databases, Cerner and Humedica. The Humedica database encompasses more than 13 million lives, while Cerner's data base contains patient records of >58 million lives in the US. Together, the two databases represent a large proportion of the US inpatient population, thereby yielding hospital records for over 24,000 patients on at least one echinocandin for the current study. With such large sample sizes, it is likely that the current results are highly generalizable to the US population.

Furthermore, in order to capture a more representative real-world safety profile of anidulafungin and other echinocandins in the current study population, the eligibility criteria for the patient selection was not as restrictive as those common in clinical trials. Specifically, no criterion was imposed for specific indications for the use of echinocandins. As a result, the overall risk of severe hepatotoxicity was assessed across various fungal infections, including not only infections due to *Candida* species, but also *Aspergillus* species. By doing so, the analyses performed in this study were able to capture the incidence of severe hepatotoxicity among all users. Likewise, while the study required that patients had at least one LFT result at baseline in order to adjust for baseline LFT in the analyses, some patients, may have immunocompromised conditions that may necessitate immediate treatments, before LFT can be conducted. Hence, excluding such patients from the analyses would hamper the external validity of the study. Therefore, a sensitivity analysis was conducted including all patients (ie, regardless of the availability of baseline LFT results) to ensure that the study main conclusions would also apply to this broader population.

Despite the differences between the healthcare system in Europe and in the US, findings from the current study should still apply to European populations; all three echinocandins are approved for use in Europe, and they differ in availability by hospital drug formulary, as in the US.^{26, 27} Additionally, the health status of the European and the US populations are not expected to be different. One notable difference is that most European countries (eg, France, Germany, and Italy) have universal healthcare coverage, while the US has a payer-, insurance-based healthcare structure. In the US, drug costs are largely dictated by the negotiated rates between payers and drug manufacturers, and may vary across hospitals that are paid by different payers. As mentioned previously, if formularies are associated with other practices within hospitals that can affect the outcome, a certain degree of channelling bias is expected. Beyond this notion, the general healthcare practice in the US vs Europe should not have substantial influence on the current results, which are likely to be generalizable to the European populations.

12. OTHER INFORMATION

Not applicable.

13. CONCLUSIONS

Based on real-world hospital practice data, the analyses overall, suggest that anidulafungin is not associated with a statistically significantly higher absolute risk or incidence rate for severe hepatotoxicity, as compared to caspofungin and micafungin. The main analyses indicated that the adjusted relative risks did not reach statistical significance in the

comparisons between anidulafungin and caspofungin or micafungin. The adjusted incidence rate ratio analyses showed no statistically significant difference between anidulafungin and micafungin, but the incidence rate was significantly higher in anidulafungin versus caspofungin. In the main subgroup analyses on patients with normal to moderately elevated baseline LFT, no evidence was found to indicate differences in the risk of severe hepatotoxicity between anidulafungin and caspofungin /micafungin patients. In the sensitivity population, the inclusion of patients without baseline LFT values yielded consistent results as the main analyses. Anidulafungin tended to have higher risk for severe hepatotoxicity in the Humedica subsample and higher risk for Grade 5 hepatotoxicity.

It is important to note that the baseline data demonstrated the clear channelling of anidulafungin treatment towards patients with impaired liver function and worse overall mortality prognosis. In particular, significantly more anidulafungin patients had AST, ALT, and total bilirubin tests of Grades 3-4 at baseline, higher CCI, and higher prevalence of CVD, organ failures, and sepsis or septic shock among other comorbidities. This channelling bias is especially notable among patients with Grade 5 hepatotoxicity events, among whom 90% anidulafungin patients had organ failures or sepsis and septic shock, while no more than 67% or 72% of patients on caspofungin and micafungin, respectively. Notably, the majority of anidulafungin patients were admitted to critical care, compared to only about half of the caspofungin and micafungin patients did so. Attempts to control for differences in the severity profile of patients were limited to the information available in the databases. Thus, residual confounding due to unobserved factors is possible. In subgroup analyses on patients with normal or mildly/moderately elevated LFT at baseline, which used restriction as a method to homogenize the baseline LFT risk across the treatment groups, no evidence was found to indicate significant differences in the risk of severe hepatotoxicity between patients treated with anidulafungin and patients treated with caspofungin or micafungin.

14. REFERENCES

1. Chen SC, Slavin MA, Sorrell TC. Echinocandin antifungal drugs in fungal infections: a comparison. *Drugs*. 2011;71(1):11-41.
2. Glockner A. Treatment and prophylaxis of invasive candidiasis with anidulafungin, caspofungin and micafungin: review of the literature. *European journal of medical research*. 2011;16(4):167-79.
3. Astellas Pharma US I. Micafungin: manufacturer's summary of product characteristics. Astellas Pharma US, Inc: SmPC 2012.
4. Merck & Co. I. Caspofungin: manufacturer's summary of product characteristics. Merck & Co, Inc: SmPC 2011.
5. Pfizer. Anidulafungin: manufacturer's summary of product characteristics. Pfizer: SmPC 2012.
6. Reboli AC, Rotstein C, Pappas PG, et al. Anidulafungin versus fluconazole for invasive candidiasis. *N Engl J Med*. 2007;356(24):2472-82.
7. Wang JL, Chang CH, Young-Xu Y, et al. Systematic review and meta-analysis of the tolerability and hepatotoxicity of antifungals in empirical and definitive therapy for invasive fungal infection. *Antimicrob Agents Chemother*. 2010;54(6):2409-19.
8. Terminology criteria for adverse events (TCAE) in trials of adult pancreatic islet transplantation 2007 May 27, 2013; Version 4. Available from: <http://www.isletstudy.org/CITDocs/CITTCAE%20V4.pdf>.
9. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 [updated 14 June 2010]. Available from: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf.
10. Quan H, Sundararajan V, Halfon P, et al. Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. *Medical care*. 2005;43(11):1130-9.
11. Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *Journal of chronic diseases*. 1987;40(5):373-83.
12. Carroll LE. The Stages of Chronic Kidney Disease and the Estimated Glomerular Filtration Rate. *The Journal of Lancaster General Hospital*. 2006;1(2):64-9.
13. Chen M, Vijay V, Shi Q, et al. FDA-approved drug labeling for the study of drug-induced liver injury. *Drug Discov Today*. 2011;16(15-16):697-703.

14. Suzuki A, Andrade RJ, Bjornsson E, et al. Drugs associated with hepatotoxicity and their reporting frequency of liver adverse events in Vigibase: unified list based on international collaborative work. *Drug Saf.* 2010;33(6):503-22.
15. Strom BL MK. The use of pharmacoepidemiology to study beneficial drug effects. *Pharmacoepidemiology*. Chichester: Wiley; 1994. p. 449-67.
16. Greenland S, Neutra R. Control of confounding in the assessment of medical technology. *International journal of epidemiology*. 1980;9(4):361-7.
17. Miettinen OS. The need for randomization in the study of intended effects. *Statistics in medicine*. 1983;2(2):267-71.
18. NCSS. PASS Power Analysis and Sample Size Software. 2015.
19. Williamson T, Eliasziw M, Fick GH. Log-binomial models: exploring failed convergence. *Emerging themes in epidemiology*. 2013;10(1):14.
20. J F. Using SAS Procedures FREQ, GENMOD, LOGISTIC, and PHREG to Estimate Adjusted Relative Risks - A Case Study. *Statistics and Data Analysis SAS Global Forum 2011*. 2011.
21. Joffe MM. Confounding by indication: the case of calcium channel blockers. *Pharmacoepidemiology and drug safety*. 2000;9(1):37-41.
22. Rothwell PM. External validity of randomised controlled trials: "to whom do the results of this trial apply?". *Lancet*. 2005;365(9453):82-93.
23. Steg PG, Lopez-Sendon J, Lopez de Sa E, et al. External validity of clinical trials in acute myocardial infarction. *Archives of internal medicine*. 2007;167(1):68-73.
24. García Vargas M MB, Ferro B. Budget impact analysis of three candins in the treatment of invasive candidiasis in adult non-neutropenic patients in Spain. *ISPOR 12th Annual European Congress 2009*.
25. Williams RL. A note on robust variance estimation for cluster-correlated data. *Biometrics*. 2000;56(2):645-6.
26. Fijn R, ESAG, Brouwers JRB, et al. Dutch Hospital Drug Formularies: pharmacotherapeutic variation and conservatism, but concurrence with national pharmacotherapeutic guidelines. *Br J Clin Pharmacol*. 2000;49(3):254-63.
27. Gallini A, Juillard-Condât B, SMC, et al. Drug selection in French university hospitals: analysis of formularies for nine competitive pharmacological classes. *Br J Clin Pharmacol*. 2011;72(5):823-31.

15. LIST OF SOURCE TABLES AND FIGURES

Please see attached pdf for all tables and figures referenced in this study report.