EFFECTIVE MANAGEMENT OF TOPICAL NOSOCOMIAL *ASPERGILLUS SPP.* INFECTIONS IN THREE SEVERELY BURNED PATIENTS

TRAITEMENT DE L'ASPERGILLOSE CUTANÉE CHEZ TROIS PATIENTS GRAVEMENT BRÛLÉS

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SUMMARY. Nosocomial opportunistic fungal infections by *Aspergillus* spp. represent increasing morbidity and mortality factors for severely burned patients, who are fragile and immunocompromised. Voriconazole (VRC), a modern antifungal drug, is used as a first-line therapy against systemic mold and yeast infections. Little has been published about the place, relative importance and efficacy of voriconazole in the treatment protocols involving *Aspergillus* spp. in Burn Centers. The objective of the present work was to assess the place and importance of voriconazole for the treatment of burn patients presenting superficial *Aspergillus* spp. infections. We performed a retrospective evaluation of VRC treatment in three severely burned patients with superficial nosocomial *Aspergillus* spp. infections in our Burn Center. Results showed that VRC allowed for control and cure of topical nosocomial *Aspergillus* spp. infections. In two cases, treatment with VRC had to be discontinued because of hepatotoxicity. In two cases, following or during systemic treatment with VRC, a 1% terbinafine cream was applied to resolve the infection in order to continue standard wound management. Overall, VRC has been shown to be an effective antifungal agent and is an alternative to amphotericin B to fight *Aspergillus* spp. infections developing in the wounds of severely burned patients.

Keywords: burns, infection, *Aspergillus*, voriconazole, antifungals

RÉSUMÉ. *La survenue d'une aspergillose chez les patients gravement brûlés, dès lors immunodéprimés, est une cause de morbidité et de mortalité. Le voriconazole (VRC) est un antifongique utilisé en première intention dans le traitement des infections à moisissures. La littérature est pauvre au sujet de son utilisation dans l'aspergillose chez le brûlé. Cette étude a pour but de l 'évaluer dans le traitement de l'aspergillose cutanée chez le brûlé et a consisté en l'évaluation rétrospective de la prise en charge de trois patients de notre CTB, gravement brûlés et victimes d'une aspergillose cutanée. VRC en a permis la guérison, mais a dû être suspendu 2 fois en raison d'une toxicité hépatique. Dans 2 cas, il a été associé à de la crème de terbinafine à 1%. Le traitement habituel a pu être repris après guérison de l'aspergillose. Globalement, VRC semble efficace et représente une alternative à l'amphotéricine B dans le traitement de l'aspergillose cutanée chez les brûlés.*

Mots-clés : *brûlure, infection,* Aspergillus*, voriconazole, antifongiques*

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Introduction

Nosocomial opportunistic fungal infections remain major morbidity and mortality factors negating, hindering, or delaying effective therapeutic management of severely burned patients.[1,2] Large (i.e. >30-60%) total body surface area (TBSA) burns widely expose vulnerable patient organisms to opportunistic infectious attack.[3-7] Successful implementation of classical surgical grafting or novel cell-based therapies prompting burn wound closure remain widely dependent on fragile patient equilibriums. Failure to rapidly and effectively close all open wounds increases the risk of bacterial and/or fungal colonization, potentially enabling life-threatening infection by deep-tissue or systemic invasion.[3] Amphotericin B (AMB) is widely used to fight systemic fungal infections but is difficult to use in burn patients because of its renal toxicity.[8] However, novel antifungal drugs such as voriconazole (VRC) are key in the strategic control and prevention of the burdens brought down by fungal pathogens. This work focused retrospectively on three clinical cases from our Burn Center (Lausanne, Switzerland) implicating *Aspergillus fumigatus* or *Aspergillus flavus* and around the use of VRC for management of *Aspergillus*-complicated wounds. VRC treatment was revealed to be effective and an alternative to AMB treatment to fight nosocomial *Aspergillus* spp. topical infections in the wounds of the considered severely burned patients.

Methods

Clinical data collection

All clinical burn patient data were collected from centralized files in the Lausanne Burn Center. Appropriate informed consent statements were obtained at the time of treatment concerning the use of specific data for research purposes. The data were anonymized and are succinctly presented herein with regard to patient and burn wound characteristics, fungal pathogen identity, and pharmacologic treatment regimens that were initiated. Outcomes of the infection phases and general patient outcomes are reported. Patients were selected based on the severity of the primary trauma (i.e. >50% TBSA burn wounds), presence of *Aspergillus* spp. pathogens, as defined by institutional guidelines and appropriate methods, and use of VRC as a therapeutic agent.

Results

Aspergillus-infected burn patients

Anonymized data are presented for three burn patients treated in the CHUV Burn Center for periods ranging between 2000 and 2019, and who developed nosocomial topical *Aspergillus* spp. fungal colonization or infection (*Fig. 1*). Preliminary identification

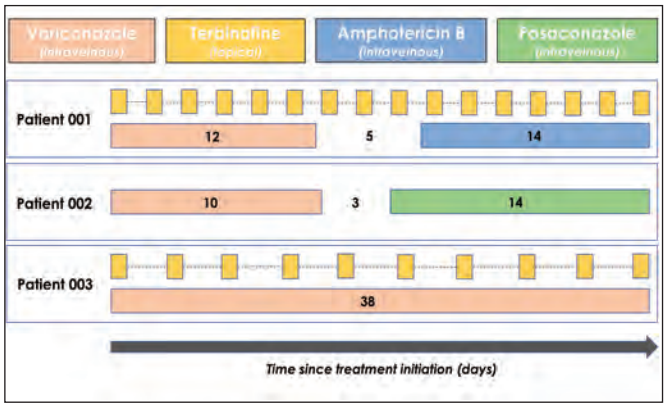

Fig. 1 - Representation of the antifungal therapeutic drug regimens administered to the three patients treated in our Burn Center and included in this study. All clinical cases comprised treatment with at least two different antifungal drugs.

of suspected cutaneous fungal colonization or infection was made by the care team by visual inspection of wounds. Clinical signs of primary cutaneous fungal colonization or infection (e.g. ulcerative lesions, nodules, papules, induration, swelling, nodular necrotic lesions with or without surrounding erythema, and cellulitis) appearing between two to six weeks after admission were specifically investigated using galactomannan titer assays, skin histopathology with periodic acid-Schiff staining, and Grocott-Gomori's methenamine-silver staining. Based on results of such specific assays and therapeutic responses to treatment, as well as negative bacterial histopathology testing, concomitant bacterial colonization or infection was excluded for the burn wounds of interest. Biological samples were analyzed in our Medical Mycology Laboratory after suspicion of fungal pathogen implication

during wound care. Identification and antifungal sensitivity quantification assays were performed by the mycology laboratory to guide clinical decisions in the Burn Center. Diagnostic testing for infections was performed by direct examination (i.e. KOH or Calcofluor reactive), identification in culture assays (i.e. macroscopic and microscopic classification) coupled to antifungigrams, histopathology and repeated galactomannan (GM) detection assays (i.e. PLATELIA™ *Aspergillus*, Bio-Rad®, Basel, Switzerland) monitoring dynamic evolution of serum concentrations.

Patient 001

Patient 001 (*Table I*) was a 17-year-old male (67 kg) who suffered a 64% TBSA second to third degree burn. Significant colonization by *Aspergillus fumigatus* was detected by culture on day 26 after admission to our hospital, with colonized body areas being the neck, ear and thigh. Galactomannans were negative. The infection diagnosis was confirmed by histopathology on day 30. Antifungal therapy was initiated four days later. Voriconazole was administered intravenously (i.e. due to recent digestive surgery) twice *per* day at a total daily dose of 4 mg/kg for 12 days, with adaptation to target a steady-state concentration of 1-5.5 µg/mL.

The voriconazole treatment was then stopped due to hepatotoxicity (i.e. cholestasis and cytolysis signs). Starting five days later, AMB was administered intravenously at a daily dose of 5 mg/kg once *per* day for 14 additional days. Concomitantly with systemic treatments, terbinafine 1% cream was applied on the days the patient was showered and his bandages were changed (i.e. several times *per* week, on average two to four times). The topical preparation (i.e. 1% terbinafine and 0.1% gentamycin) was provided by the Pharmacy Service of our Hospital as a magistral preparation. The pharmacotherapeutic management of the cutaneous infection allowed for effective control thereof. The infection was then considered to be resolved (i.e. negative galactomannan detection assays, surveillance wound biopsies, and wound site clinical observation), therefore standard wound care could continue.

Patient 002

Patient 002 (*Table I*) was a 51-year-old male (97 kg) who suffered a 56% TBSA burn. Infection was detected on day 11 after admission to our hospital, with colonized and infected body areas being the skin of the back, right arm, and shoulder. Culture identification methods revealed *Aspergillus flavus* to be responsible for the infection. No biopsy was performed. Galactomannans were negative. Antifungal therapy was initiated immediately after detection of the infection. Voriconazole was administered intravenously (i.e. due to malabsorption) twice *per* day at a total daily dose of 4 mg/kg for ten days with adaptation to target a steady-state concentration of 1-5.5 µg/mL. Starting three days after the end of treatment with voriconazole, posaconazole was administered at a daily dose of 300mg intravenously for 14 days. The pharmacotherapeutic management of the cutaneous infection allowed for effective control thereof. The infection was then considered to be resolved (i.e. negative galactomannan detection assays, surveillance wound biopsies, and wound site clinical observation), therefore standard wound care could continue.

Patient 003

Patient 003 (*Table I*) was a 29-year-old male (99 kg) who suffered an 81% TBSA burn. Infection was detected six weeks after admission to our hospital, with skin infections covering the whole body. Culture identification methods revealed *Aspergillus flavus* to be responsible for the infection. The biopsy confirmed the infection diagnosis. Galactomannans were negative. Antifungal therapy was started immediately after detection of the infection. Voriconazole was administered intravenously (i.e. due to malabsorption) twice *per* day at a total daily dose of 4 mg/kg for 38 days, with adaptation to target a steady-state concentration of 1-5.5 µg/mL. Terbinafine 1% cream was concomitantly applied during the whole systemic treatment. The pharmacotherapeutic management of the cutaneous infection allowed for effective control thereof. The infection was then considered to be resolved (i.e. negative galactomannan detection assays, surveillance wound biopsies, and wound site clinical observation), therefore standard wound care could continue.

Table I - Summary of the characteristics of three patients treated in our Burn Center who presented nosocomial cases of topical aspergillosis.

	Patient 001	Patient 002	Patient 003
Age [years]	17	51	29
Sex	M	M	M
Weight [kg]	67	97	99
TBSA (degree)	64% (2-3)	56%	81%
Pathogen	*A. fumigatus*	*A. flavus*	*A. flavus*
Area of colonization/infection	Neck, ear, thigh	Back, arm, shoulder	Whole body
Treatment	VRC, AMB, TER	VOR, POS	VOR, TER

AMB = Amphotericin B, **POS** = Posaconazole, **TER** = Terbinafine, **VOR** = Voriconazole

Discussion

VRC is effective for topical aspergillosis

We report three cases of nosocomial superficial aspergillosis caused by *Aspergillus flavus* or *Aspergillus fumigatus* in severely burned patients. We have shown that systemic VRC was an effective antifungal agent to fight these infections. The effect of VRC may be potentiated by local applications of terbinafine. Although the use of voriconazole for *Aspergillus* spp. infections is well described in the relevant literature and guidelines, the specific application to burn patients is sporadically mentioned throughout case-reports and related studies.

Topical aspergillosis in burn victims

Other *Aspergillus* species (e.g. *Aspergillus niger, Aspergillus terreus* and *Aspergillus nidulans*) cause aspergillosis in burned patients, but only a few cases are reported with *A. flavus* in Europe. *A. flavus* and *A. fumigatus* are the main causative agents of invasive aspergillosis in immune-suppressed patients. While in the case of invasive aspergillosis infection occurs through inhalation of spores before developing in the lungs and in other organs, superficial aspergillosis infections in severely burned patients were not mediated by inhalation, but by wound exposure. The severity of fungal infections following colonization in burned patients often correlates with both the extent and degree of primary skin lesions.[5] Primary thermal trauma increases risk factors by altering the activities of phagocytes, T cells, and natural killer cells.[9]

Specificities of VRC

Pharmacologic management of invasive aspergillosis relies on administration of voriconazole, amphotericin B (i.e. deoxycholate or encapsulated forms), or caspofungin in single or combination therapies.[8] The introduction of VRC, which possesses a broad activity spectrum *in vitro* and *in vivo* against commensal and opportunistic fungi, has contributed to renew and broaden the crucial therapeutic armamentarium to be deployed against invasive fungal infections. VRC antimicrobial effects are deployed by indirect disruption of fungal cell membranes by inhibition of the ergosterol biosynthesis pathway (i.e. lanosterol 14α-demethylase inhibition, CYP51A1).[10] The usage of VRC is preferable in burn wound infections because burn injuries negatively impact renal function, and amphotericin B has substantial nephrotoxicity in its non-encapsulated form. Nevertheless, VRC causes visual disturbances, skin affections, and possesses hepatic toxicity (i.e. induction of cholestasis and hepatocytolysis, poorly correlated to plasma drug level), which is a major cause of treatment discontinuation.[11] Minimizing the incidence of hepatotoxicity and related adverse effects may be performed with simultaneous therapeutic drug monitoring of VRC and close monitoring of patient liver functions (e.g. ALP, ASAT, ALAT, GGT, bilirubin), enabling early transition to relatively less hepatotoxic antifungal drugs. Voriconazole is available on the Swiss market in the form of tablets, powders for reconstitution, and suspensions (*Table II*). Notwithstanding the fact that the drug has been on the market for some time (i.e. FDA/EMEA approval in 2002 and generic preparations approved recently), voriconazole preparations remain relatively expensive in comparison to other antifungals used in the clinic (*Table II*).[12]

Table II - Overview of the different commercial therapeutic products containing voriconazole, authorized and available on the Swiss market as of 2020 (www.compendium.ch).

Commercial preparation names	Form, dosage, and countenance	Manufacturers	Retail price range [CHF/box]
VFEND® 50 mg and generics	50 mg coated tablets; 56 tablets/box	Sandoz Pharmaceuticals AG (Rotkreuz, Switzerland) Pfizer PFE Switzerland GmbH (Zurich, Switzerland) Mepha Pharma AG (Aesch, Switzerland)	689.25 857.45
VFEND® 200 mg and generics	200 mg coated tablets; 28 tablets/box	Sandoz Pharmaceuticals AG Pfizer PFE Switzerland GmbH Mepha Pharma AG	1,375.60 1,704.10
VFEND® 200 mg and generics	200 mg of lyophilized preparation/box	Sandoz Pharmaceuticals AG Pfizer PFE Switzerland GmbH Mepha Pharma AG	169.00 207.15
VFEND® 40 mg/mL and generics	Lyophilized preparation for 70 mL of 40 mg/mL reconstituted suspension	Pfizer PFE Switzerland GmbH	771.20 959.95

CHF = Swiss Franc

Previous report of VRC use in a burn victim

A 2015 case report from our Burn Center was the first to describe the efficacious treatment of an invasive *Aspergillus fumigatus* infection in a burn victim using a combination of systemic voriconazole and topical terbinafine.[13] The evolution of this patient was highly illustrative of the difficulties of equilibrium maintenance in severe burn patients. Indeed, infectious complications hindered the wound healing medical process and eventually resulted in the patient's death. This case underlined the limits of voriconazole treatments, as the inherent hepatic toxicity led to multiple treatment discontinuations, although therapeutic drug monitoring showed acceptable therapeutic blood concentrations.[13]

Conclusion

In conclusion, VRC has been shown to be an effective antifungal agent and is an alternative to AMB to fight *Aspergillus* spp. infections in the wounds of severely burned patients. Specific VRC applications in nosocomial aspergillosis in Burn Centers, in combination with appropriate treatments and drug delivery options, are of high interest for clinical use. Continued developments around antimicrobial management in the context of burn centers will in all probability allow for mortality rate reductions in these complex patient populations worldwide.

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