TESE DE DOUTORAMENTO

TOWARDS PERSONALIZED MEDICINE IN ANTIFUNGAL TREATMENT: COMBINATION OF PHARMACOGENETICS AND PHARMACOKINETICS

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Dna. Sara Blanco Dorado

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Resumen

En los últimos años la incidencia de infecciones fúngicas invasivas (IFIs) se ha visto incrementada, a pesar de la mejora en los métodos diagnósticos y la introducción de nuevos fármacos antifúngicos. Además de este aumento en la incidencia de IFIs, se han producido cambios significativos en su epidemiología que han condicionado modificaciones en el manejo terapéutico de estas infecciones.

Este reciente aumento de la incidencia de infecciones fúngicas es debido a múltiples causas. Entre ellas destacan el aumento del uso de antibióticos de amplio espectro, el mayor número de pacientes inmunodeprimidos, el aumento en el uso de dispositivos invasivos de acceso vascular y el uso de nuevas terapias dirigidas al sistema inmunitario. En este sentido, es importante destacar que cada vez son más los pacientes que por alguna razón se encuentran inmunodeprimidos. En los últimos años se ha incrementado el número de pacientes que reciben un trasplante, bien sea de órgano sólido o de progenitores hematopoyéticos, y que por tanto, precisan tratamiento inmunosupresor. Pero además, recientemente, han aparecido nuevas terapias dirigidas al sistema inmunitario, que pueden alterar la respuesta inmune frente a los microorganismos, y ha supuesto la aparición de un nuevo espectro de pacientes con riesgo de desarrollar IFIs. Dentro de estas nuevas terapias se incluyen principalmente fármacos empleados en el tratamiento de enfermedades onco-hematológicas, desde pequeñas moléculas que actúan inhibiendo tirosina-cinasas (TC) , anticuerpos monoclonales o incluso la terapia con células T modificadas con receptor de antígeno quimérico (CAR-T).

Las infecciones causadas por *Candida* spp. continúan siendo las infecciones fúngicas más frecuentes y presentan un elevado impacto sanitario debido a su elevada prevalencia. Estas infecciones constituyen la tercera causa de infección más frecuente en pacientes críticos, y son la cuarta causa de infección nosocomial en España. Aunque la especie más frecuente continúa siendo *C.albicans*, en las últimas décadas se ha visto incrementada la incidencia de las infecciones producidas por especies de *Candida* non-*albicans*, como *C. glabrata*, *C. krusei* y *C. Parapsilosis*, que a menudo presentan mayor resistencia a los fármacos antifúngicos. Por otro lado, las infecciones causadas por *Aspergillus* spp., pese ser poco prevalente, destacan por su elevada mortalidad, especialmente en los pacientes hematológicos sometidos a un trasplante de progenitores hematopoyéticos. En estos pacientes la incidencia de aspergilosis invasiva (AI) puede llegar a ser mucho mayor que en el resto de la población con una mortalidad cercana al 40%. Por último, existen hongos emergentes como *Fusarium* o *Scedosporium*, capaces de originar infecciones fúngicas invasivas multiresistentes en pacientes inmunodeprimidos. A pesar de que estás infecciones son raras, su baja sensibilidad y alta mortalidad hace que también deban ser consideradas.

El número de fármacos disponibles para el tratamiento de las infecciones fúngicas ha aumentado en los últimos años con la aprobación de nuevas moléculas. En la actualidad disponemos de cuatros grupos de antifúngicos que se distinguen por su mecanismo de acción: antimetabolitos, polienos, azoles y equinocandinas. Además, se encuentran en desarrollo clínico nuevas moléculas con actividad antifúngica. Algunas de estas moléculas en investigación presentan mecanismos de acción novedosos y serán interesantes para minimizar la aparición de resistencia. Otros moléculas buscan mejorar la administración, posibilitando la administración oral o subcutánea del

antifúngico para facilitar la administración extrahospitalaria y favorecer la adherencia. Sin embargo, pese a la existencia de investigación en el campo de los fármacos antifúngicos, optimizar el uso de los fármacos ya existentes sigue siendo fundamental. Los fármacos que tenemos hoy en día disponibles son la primera opción de tratamiento en infecciones de elevada mortalidad, donde el tratamiento inadecuado puede suponer un elevado riesgo para el paciente.

El voriconazol es un fármaco antifúngico de amplio espectro perteneciente al grupo de los triazoles. Su efecto antifúngico se debe a la inhibición de la desmetilación del 14 alfa-lanosterol mediado por el citocromo P-450 fúngico, que constituye un paso esencial en la biosíntesis de ergosterol fúngico. Destaca por su amplio espectro siendo activo frente a hongos de los géneros *Candida*, *Aspergillus* y *Cryptococcus*. Además, presenta actividad fungicida frente a patógenos emergentes incluyendo *Scedosporium* y *Fusarium* de sensibilidad limitada a otros antifúngicos. En la actualidad las guías clínicas lo posicionan como el fármaco de primera elección en el tratamiento de la AI. Sin embargo, su elevada variabilidad farmacocinética y su estrecho margen terapéutico hace complicado el manejo de este fármaco.

El voriconazol es metabolizado en el hígado a través del complejo de enzimas hepáticas citocromo P450 (CYP450) y más concretamente a través de las isoenzimas CYP2C19, CYP2C9 y CYP3A4. La evidencia actual apunta al CYP2C19 como la enzima más importante involucrada en este proceso metabólico trasformando al voriconazol en metabolitos inactivos. Pero además de participar en el metabolismo de voriconazol, las enzimas CYP2C19, CYP3A4 y CYP2C9 están implicadas en el metabolismo de muchos otros fármacos por lo que existe un riesgo potencial elevado de interacción farmacocinética entre voriconazol y otros fármacos de uso frecuente.

Por otro lado, el gen que codifica la isoenzima CYP2C19 es altamente polimórfico, identificándose más de 34 variantes alélicas del mismo. Así, se han identificados alelos cuya expresión origina un enzima con distinta actividad. El alelo salvaje, alelo **1*, confiere actividad normal al enzima, pero se han descrito en la bibliografía alelos con pérdida de función o alelos nulos *(*2, *3, *4, *5, *6, *8*) y más recientemente, el alelo **17*, asociado a incrementos en la actividad enzimática. La combinación de estos alelos origina grupos de individuos en función de su capacidad metabolizadora: metabolizadores pobres (PM), intermedios (IM), metabolizadores normales o extensivos (EM) y metabolizdores rápidos (RM) o ultrarápidos (UM). La incidencia de los polimorfismos varia en los distintos grupos raciales. Así, entre la población asiática son frecuentes los alelos **2* y **3*, originado un enzima de menor actividad o sin actividad, según tengan una o dos copias del alelo mutado. En estos individuos es frecuente encontrar concentraciones plasmáticas de voriconazol elevadas con la dosis estándar y que pueden desencadenar en muchos casos toxicidad. Por el contario, en la población caucásica es frecuente el alelo **17* que conlleva una metabolismo acelerado del fármaco y puede condicionar con frecuencia concentraciones subterapeuticas con el potencial riesgo de fracaso terapéutico asociado. La existencia de estos polimorfismos genéticos es una de las principales causas de la variabilidad farmacocinética de voriconazol. Pero existen otros factores que también pueden influir como las interacciones farmacológicas, la edad, la disfunción hepática o el estado inflamatorio del paciente.

Las características del voriconazol lo convierten en un candidato ideal para la realización de monitorización farmacocinética (TDM, por sus siglas en inglés: "*therapeutic drug monitoring*"). Diversos estudios han demostrado la relación entre las concentraciones plasmáticas de voriconazol y los resultados clínicos en términos de eficacia y toxicidad. Por otro lado, varios trabajos han demostrado los beneficios

de la realización de TDM de voriconazol obteniéndose mejores tasas de respuesta en aquellos pacientes en los que la dosis es ajustada en función de la concentraciones plasmática de fármaco comparado con el uso de la dosis estándar. Sin embargo la monitorización farmacocinética de voriconazol todavía no está completamente estandarizada en la práctica clínica. En este sentido es necesario determinar el intervalo de concentraciones plasmáticas ideal que debe emlplearse como objetivo en la TDM de voriconazol. Además se necesita disponer de un método validado para realizar la determinación analítica de voriconazol en muestras plasmáticas de los pacientes. Finalmente, por otro lado, el valor ideal de otro parámetro de creciente interés, el cociente farmacocinético/farmacodinámico (PK/PD) también debe ser dilucidado. Este cociente pretende relacionar las características farmacocinéticas del fármaco a través de un parámetro farmacocinético como el área bajo la curva (AUC) o la concentración mínima (C_{min}) y un parámetro farmacodinámico, que en el caso de los antiinfecciosos viene definido por la Concentración Mínima Inhibitoria (CMI) que define la susceptibilidad del hongos al fármaco.

La medicina personalizada o a la carta constituye uno de los pilares de la medicina del futuro. Su objetivo principal es la optimización de estrategias de prevención y tratamiento de las enfermedades centradas en las características del paciente, incluyendo factores genéticos y ambientales, entre otros. La farmacogenética es una herramienta de la medicina personalizada que considera las características genéticas del paciente para optimizar una terapia farmacológica garantizando la selección del mejor fármaco y la mejor dosis para cada paciente. Las disciplinas en las que más se ha implementado la farmacogenética han sido la oncología y la psiquiatría, áreas en las que se disponen ya de marcadores genéticos recogidos en las fichas técnicas de los medicamentos que ayudan a la selección de una terapia u otra o a la selección de la mejor dosis. Sin

embargo, esta herramienta puede ser aplicada a muchas otras áreas más allá de la oncología o la psiqiatría, como es el caso de las enfermedades infecciosas. Una de las patologías en las que se ha demostrado la utilidad de la genética es el campo de los fármacos antifúngicos. Aunque se han analizado diferentes marcadores genéticos, los más investigados han sido los polimorfismos en los genes que afectan al metabolismo de los fármacos. En el caso concreto de los azoles, y especialmente el voriconazol, la determinación de los polimorfismos genéticos de *CYP2C19* se ha correlacionado con la concentración plasmática de voriconazol en varios trabajos. Sin embargo, el impacto clínico de la farmacogenética en la terapia antifúngica de voriconazol no ha sido suficientemente demostrado como para implementar su uso rutinario en la práctica clínica real.

El objetivo principal de este trabajo ha sido evaluar el uso de voriconazol en la práctica clínica real y estudiar los factores que afectan a su variabilidad farmacocinética, para poder diseñar estrategias basadas en la combinación de farmacogenética y farmacocinética que permitan optimizar el tratamiento antifúngico.

Para ello se realizó en primer lugar un estudio retrospectivo de uso de voriconazol en práctica clínica, a continuación se desarrolló un método analítico para la determinación de voriconazol en muestras plasmáticas y finalmente se investigaron los factores que afectan a la cinética de voriconazol a través de un estudio multicéntrico prospectivo prestando especial atención a la influencia de los polimorfismos genéticos de *CYP2C19* y a las interacciones farmacológicas a nivel del metabolismo hepático.

El estudio retrospectivo observacional inicial sirvió como punto de partida de esta tesis e incluyó pacientes a tratamiento con voriconazol en los que no se realizaba TDM. El objetivo fue conocer el uso de voriconazol en la práctica clínica real. Para ello se analizaron

las características de los pacientes, del tratamiento antifúngico, y los resultados clínicos en términos de eficacia y toxicidad. Los resultados obtenidos mostraron que los pacientes tratados con voriconazol eran complejos y adicionalmente se demostró una gran variabilidad en las características del tratamiento. En primer lugar, se observaron diferentes comorbilidades en los pacientes tratados con voriconazol, destacando un alto porcentaje de pacientes con enfermedad pulmonar obstructiva crónica (EPOC) como único factor de riesgo de IFI. Por otro lado, se observó que a pesar de que el voriconazol se dosificaba según ficha técnica, un porcentaje de pacientes importante no había recibido la dosis de carga el primer día de tratamiento antifúngico. Además se observó una baja tasa de respuestas y un elevado porcentaje de efectos adversos. Y, adicionalmente, se demostró la existencia de un alto porcentaje de pacientes tratados concomitantemente con voriconazol y fármacos susceptibles de interaccionar como los glucocorticoides y los inhibidores de la bomba de protones (IBPs) lo cual podría justificar una elevada variabilidad farmacocinética asociada a riesgo de fracaso terapéutico y/o toxicidad. Los resultados de este estudio retrospectivo pusieron de manifiesto la necesidad de optimizar el uso de este fármaco cosiderando la elevada mortalidad de las IFIs. Esta optimización se plantea con dos estrategias posibles. Por un lado, la necesidad de implementar la monitorización farmacocinética de voriconazol para garantizar concentraciones plasmáticas de voriconazol en rango. Y por otro lado, se sugiere la implementación de programas de uso adecuado de antifúngicos (*Antifungal Stewardhip Programmes*) en los que el farmacéutico debe tener un papel clave.

Posteriormente, en una segunda parte del trabajo, se desarrolló un método analítico de determinación farmacocinética de voriconazol. El método elaborado consistió en una cromatografía líquida de alta resolución (HPLC) que empleó un método isocrático constituido por

una fase móvil a base de por agua y acetonitrilo. El método cumplió todos los requerimientos de las agencias reguladores FDA y EMA y resultó ser simple, rápido, sensible y específico. El método fue aplicado eficazmente al análisis de voriconazol en muestras de pacientes. Además, por su gran sensibilidad, este método permitió determinar concentraciones plasmáticas de voriconazol inferiores al intervalo terapéutico pudiendo ser empleado para realizar TDM cuando el voriconazol es empleado como terapia profiláctica. Además, el método se comparó con otra técnica empleada en otros laboratorios de farmacocinética clínica para la determinación de voriconazol, el inmunoensayo, y se obtuvo una correlación lineal entre ambos.

Finalmente, se estudiaron los factores que afectan a la farmacocinética de voriconazol a través de un estudio multicéntrico, prospectivo y observacional. En este trabajo, se incluyeron pacientes adultos a tratamiento con voriconazol oral o intravenoso para diferentes indicaciones terapéuticas. Se registró la información demográfica de los pacientes (edad, sexo, peso y raza) así como la información relativa a tratamiento (indicación, dosis, vía de administración y duración)e información referida al tratamiento concomitante de los pacientes. Se le realizaron determinaciones farmacocinéticas y farmacogenéticas a todos los pacientes incluidos en el estudio. La determinación farmacocinética se realizó obteniendo una muestra previa a la dosis de voriconazol (predosis) y la concentración se determinó mediante el método cromatográfico previamente desarrollado. Para la determinación farmacogenética se realizó la técnica de PCR en tiempo real en una muestra de sangre total. Se realizó genotipado de *CYP2C19*, *CYP2C9*, *CYP3A4* y *CYP3A5* y los pacientes fueron clasificados según su fenotipo correspondiente.

Este estudio se centró fundamentalmente en evaluar el impacto de los polimorfismos de *CYP2C19* y de las interacciones

farmacológicas en las concentraciones plasmáticas de voriconazol. En primer lugar, en este estudio, se observó un porcentaje muy elevado de pacientes con concentraciones de voriconazol fuera del intervalo terapéutico, la mayoría subterapéuticas. Este resultado se consideró preocupante dada la relación existente entre fracaso terapéutico y concentraciones bajas así como la elevada mortalidad de las IFIs. En segundo lugar, se detectó una alta tasa de pacientes portadores del alelo **17,* relacionado con una actividad acelerada del enzima. Estos datos son concordantes con resultados previos referentes a población caucásica y ponen de manifiesto el riesgo concentraciones subterapéuticas en estos pacientes. En este trabajo, la presencia de este alelo se correlacionó con concentraciones plasmáticas más bajas de voriconazol y con un mayor porcentaje de pacientes con niveles infraterapéuticos, confirmando resultados de estudios previos.

Este trabajo destaca por ser el estudio prospectivo que incluyó mayor número de pacientes de raza caucásica y que evaluó la relación entre polimorfismos *CYP2C19* y concentración plasmática de voriconazol. Los resultados obtenidos apuntan al uso de la farmacogenética de voriconazol como complemento a la monitorización farmacocinética de voriconazol. Esta herramienta es especialmente útil al inicio de la terapia permitiendo la selección de dosis mayores en pacientes portadores del alelo **17* que posteriormente serán ajustadas en función de la determinación cinética. Podría ser interesante disponer con antelación de esta información en pacientes con alta probabilidad de recibir tratamiento antifúngico con voriconazol, como los pacientes sometidos a un trasplante hematopoyético o pacientes hematológicos candidatos a altas dosis de quimioterapia o terapias dirifgidas al sistema inmune. Además, también podría ser muy útil en pacientes con infecciones en localizaciones de difícil acceso como el sistema nervioso central,

donde alcanzar concentraciones terapéuticas las primeras horas de la terapia es clave para garantizar el éxito terapéutico

Finalmente, otro aspecto de gran interés son las interacciones farmacológicas. La interacción entre voriconazol e inhibidores o inductores potentes como ritonavir, fenitoína, rifampicina o hierba de San Juan es bien conocida y su administración está específicamente contraindicada en la fecha técnica. Sin embargo, existen otros fármacos de uso frecuente con efectos inductores o inhibidores de CYP450 pero donde la relevancia clínico de esta interacción es controvertida. Este es el caso de dos grupos de fármacos de uso muy común, como son lo glucocorticoides y los inhibidores de la bomba de protones (IBPs).

Con respecto a los glucocorticoides, diversos estudios han demostrado su efecto inductor enzimático de CYP2C19 y CYP3A4. Sin embargo, se desconoce la relevancia clínica de esta interacción. En nuestro trabajo, no se observó una relación significativa entre el uso de glucocorticoides y las concentraciones plasmáticas de voriconazol. Sin embargo, debe destacarse que había una gran variedad en cuanto al tipo de glucocorticoides empleado y dosis. En este sentido, existen estudios que han demostrado diferente efecto inductor dependiendo tanto de la molécula como de la dosis. Por tanto, son necesarios más estudios para conocer el verdadero impacto clínico de la interacción. Además, pese a no encontrar diferencias estadísticamente significativas en la concentraciones plasmática de voriconazol en el conjunto de la población del estudio, sí que se observó un efecto clínicamente significativo de esta interacción en una paciente tratada con ambos fármacos concomitantemente. Fue el caso de una paciente que estando a tratamiento con voriconazol, la discontinuación del tratamiento esteroideo ocasionó a una hepatotoxicidad severa asociada al aumento de concentración plasmática de antifúngico al cesar el efecto inductor

enzimático del corticoide. Este caso clínico se presenta también como parte de esta tesis como revisión de la interacción entre voriconazol y glucocorticoides.

Otro grupo de fármacos estudiados fueron los IBPs. Estos fármacos son de uso muy habitual en los pacientes tratados con voriconazol y diversos trabajos han evidenciado un efecto inhibidor enzimático competitivo a nivel de CYP2C19. Sin embargo se desconoce la relevancia clínica de esta interacción. Por ello en nuestro trabajo se comparó la concentración plasmática de voriconazol entre los pacientes tratados con omeprazol o pantoprazol. Los resultados obtenidos demostraron un efecto inhibidor enzimático significativamente mayor de omeprazol comparado con pantoprazol. Estos resultados concuerdan con estudios *in vitro* sugestivos de un menor efecto inhibidor de pantoprazol de dicho isoenzima comparado con otros IBPs. Los resultados obtenidos en nuestro trabajo resultan de especial interés para su aplicación en la práctica clínica habitual a la hora de seleccionar el mejor IBPs en un paciente a tratamiento antifúngico con voriconazol.

En conclusión, el voriconazol continua siendo el fármaco de elección en el tratamiento de infecciones de elevada mortalidad como la aspergilosis invasiva por lo que optimizar su tratamiento es fundamental. Los farmacéuticos clínicos como expertos en medicamentos debemos tener un papel clave en los equipos para el uso adecuado de antifúngicos en los hospitales y debemos liderar la implantación de la medicina personalizada, en particular aplicada a la terapia antifúngica. En este sentido, la combinación de estrategias basadas en farmacocinética, farmacogenética así como una cuidadosa selección del tratamiento concomitante de los pacientes a tratamiento con voriconazol, puede ser una solución para contribuir al éxito terapéutico en la terapia antifúngica.

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Contents

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List of abbreviations

ABDC:	amphotericin-B deoxycholate
ABCL:	amphotericin-B lipid complex
ABPA:	allergic bronchopulmonary aspergillosis
ADME:	absorption, distribution, metabolism and excretion
AFS:	antifungal stewardship
ALT:	alanine aminotransferase
AmB-L:	liposomal amphotericin-B
AmB:	amphotericin B
AMS:	antimicrobial stewardship
AP:	alkaline phosphatase
AST:	aminotransferase
AUC:	area under the curve
BCL-2:	B-cell lymphoma 2 receptor
BCR:	B-cell receptor
CAN:	and chronic necrotizing aspergillosis
CAR-T:	modified T cells with chimeric antigen receptor
CCPA:	chronic cavitated pulmonary aspergillosis
CF:	cystic fibrosis
CFPA:	chronic fibrosing pulmonary aspergillosis
CLSI:	clinical and laboratory standard institute
Cl/F:	clearance
$\mathbf{C_{min}}$:	minimum plasma concentration
$\mathbf{C_{max}}$:	maximum plasma concentrations
CNS:	central nervous system

COPD: chronic obstructive pulmonary disease
CPA: chronic pulmonary aspergillosis
CRP: plasma C-reactive protein
CRRT: continuous renal replacement techniques
CSF: cerebrospinal fluid
CYP450: cytochrome P450
CYP2C19: isoform 2C19 of cytochrome P450
CYP2C9: isoform 2C9 of cytochrome P450
CYP34: isoform 3A4 of cytochrome P450
DIPS: drug interaction probability scale
DRESS: drug reaction with eosinophilia and systemic symptoms
EIA: enzyme immunoassays
EM: extensive metabolizer
EMA: european medicines agency
EORTC/MSG: european organization for research and treatment of cancer/nycoses study group
EPINE: study of prevalence of nosocomial infection in spain
ERG: electroretinogram
EU: european union
EUCAST: european committee on antimicrobial susceptibility testing
FA: fast acetylator
FCZ: fluconazole
FDA: U.S. food and drug administration
GSI: glucan synthase inhibitor
HLA: human leucocyte antigen
HPBCD: hydroxypropyl betadex cyclodextrin
HPLC: high-performance liquid chromatography
HSCT: hematopoietic stem cell transplant
IA: invasive aspergilosis

IC:	confidence interval
ICU:	intensive care unit
IFIs:	invasive fungal infections
IL28-B:	interleukin 28-B
IM:	intermediate metabolizer
ISA:	isavuconazole
IV:	intravenous
ITK:	tyrosine kinase inhibitors
ITCZ:	itraconazole
LC-MS:	liquid chromatography-mass spectrometry
LC-MS/MS:	liquid chromatography-tandem mass spectrometry
LOQ:	lower limit of quiantification
MDR1:	multi-drug resistance-1
MIC:	minimum inhibitory concentration
MPP:	precision personalized medicine
OATP1B1:	organic anion transporter protein 1B1
OR:	odds ratio
PD:	pharmacodynamics
P-GP:	p-glycoprotein
PJP:	pneumocystis jiroveci pneumonia
PK:	pharmacokinetics
PK/PD:	pharmacokinetics/pharmacodynamics
PM:	poor metabolizer
POS:	posaconazole
PPIs:	proton pump inhibitors
PRAN:	spanish national plan for antibiotic resistance
RM:	rapid metabolizer
ROS:	reactive oxygen species
SA:	slow acetylator
SBED:	sulfobutyl ether beta-cyclodextrin

SJS:	stevens-johnson syndrome
SNPs:	single nucleotide polymorphisms
SOT:	solid organ transplant
TDM:	therapeutic drug monitoring
TEN:	toxic epidermal necrolysis
T_{max}:	time to maximum plasma concentration
$T_{1/2}$:	half-life
UM:	ultrarapid metabolizer
UPLC:	ultra-high performance liquid chromatography
VCZ:	voriconazole
WHO:	world health organization

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Personalized medicine in infectious diseases

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1 PERSONALIZED MEDICINE IN INFECTIOUS DISEASES

Personalized Medicine or Precision Personalized Medicine (MPP) is an emerging medicine approach that use scientific insights into the genetic and molecular basis of health and disease to guide decisions regard to the prediction, prevention, diagnosis and treatment of disease (1). Therefore, personalized medicine should ensure that patients get the right treatment at the right dose and at the right time, with minimum ill consequences and maximum efficacy. In this sense, personalised medicine can allow healthcare professionals be able to:

- Determine if a person has a higher risk of developing a disease and, consequently, apply prevention strategies.
- Diagnose a disease earlier after onset, thereby allowing more effective treatment options.
- Improve therapeutic efficacy by ensuring that the most appropriate medicine is used and that the dosage is the most suitable according to the patient's genetic makeup.
- To avoid and reduce drug toxicity by taking into account patient characteristics including genetics (1).

The continue evolution of technology and the development in molecular medicine and genomics analysis have contributed to the understanding and interpretation of the human genome and exome. New tools harnessed by personalised medicine include '*-omics*' technologies such as metabolomics (study of the metabolites in an organism), pharmacogenomics (interaction between drug and

individual genetic characteristics), transcriptomics (study of the complete set of RNA transcripts produced by the genome at any one time) and proteomics (analysis of the entire collection of proteins in an organism) (2). Personalized medicine has meant also a paradigm shift in the way of providing health care and it is changing how medicine is practiced and how health care is delivered and financed (3). The possibility to predict and to intervene before damage has transformed the heath system to a "*prospective health care*" system, focused on prevention and treatment in relationship with a personalized risk (2).

Pharmacogenetics is a subset of personalized medicine that focuses on knowing how genetic variation affects on an individual response to a particular drug (4). The term pharmacogenetics is related to studies in which single genes are associated with variations in drug metabolism. In contrast, pharmacogenomic can also concern on how the genome as a whole influences the drug effects in the organism. And, finally, the study of the association between genetics and drug toxicity is known as toxicogenomics (5).

All human genes are subject to extensive genetic polymorphism, with many of this polymorphism resulting in functionally significant clinical effects. A genetic polymorphism is defined as the occurrence together in the same population of more than one allele or genetic marker at the same locus with the least frequent allele or marker occurring more frequently than can be accounted for by mutation alone (6). Although there are a number of different types of polymorphic markers, most attention recently has focused on single nucleotide polymorphisms (SNPs), the most common type of polymorphism found in the human genome. SNPs are random alterations in the nucleotide bases that generate changes in amino acid residues of protein sequences and the are believe to be he main reason of genetic variation among the individuals (7). The best-studied

polymorphisms are those in genes relevant to drug disposition, especially drug metabolism. In this respect, isoenzymes of cytochromes P450 (CYP450) are the most important enzymes involved in drug metabolism. Therefore, genetic polymorphism in this genes may have relevant clinical effects (6).

In essence, pharmacogenetics analyzed how changes in SNPs are correlated with the pharmacokinetics or pharmacodynamics of a drug (8). This discipline can potentially guide health care providers to stratify which patient groups will potentially benefit from a therapy with minimal to no risk of adverse events or to guide drug dossing. In this context, the benefits of the application of pharmacogenetics are especially interesting for medications with high interindividual variability, narrow therapeutic index and nonlinear pharmacokinetics (4).

Despite the implementation of pharmacogenetic has been slowed down by the institutions, over the last several years, the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have been incorporated a number of genetic markers with clinical utility into the drug labels (9,10). In fact, research in the field of pharmacogenetics has allowed agencies to incorporate precision medicine in the labelling of already-marketed drugs (e.g., carbamazepine, abacavir, clopidogrel) years after their approval. In addition to that, in recent years, identification of genetic biomarkers has been incorporating as a study parameter in clinical trials of many new drugs (e.g., enasidenib, venetoclax, vemurafenib, dabrafenib, trametinib). These drugs have already contained genomic and other biomarker information in labelling at the time of initial approval (11). In this respect, the number of new medicinal products authorised each year containing genetic biomarkers in their product label has steadily increased last years. Related to that, almost 15% of medicinal products evaluated according to the EMA centralised procedure between 1995 and 2014 contain pharmacogenomics information in their label that

directly influence patient treatment. Oncology and psychiatry are the main fields in witch pharmacogenetics has been greater implement (12) but it is also being developed in many other pathologies.

In the specific field of infectious diseases, genetics can also have really significant impact. However, in this area, the application of pharmacogenomics is complex because requires consideration of genomes of both the pathogen and the host (13,14) (*Figure 1*). The pathogen genome may be use for antigen identification and to determine antimicrobial resistance, and this information can therefore be used to choose an appropriate antibiotic therapy. In addition, polymorphisms in genes of the host are also important since it have been associated with susceptibility to infections and response to treatment (15). Main genes related to response to antimicrobial drugs as whether if this information is included in the drug's data label are summarized in *Table 1*.

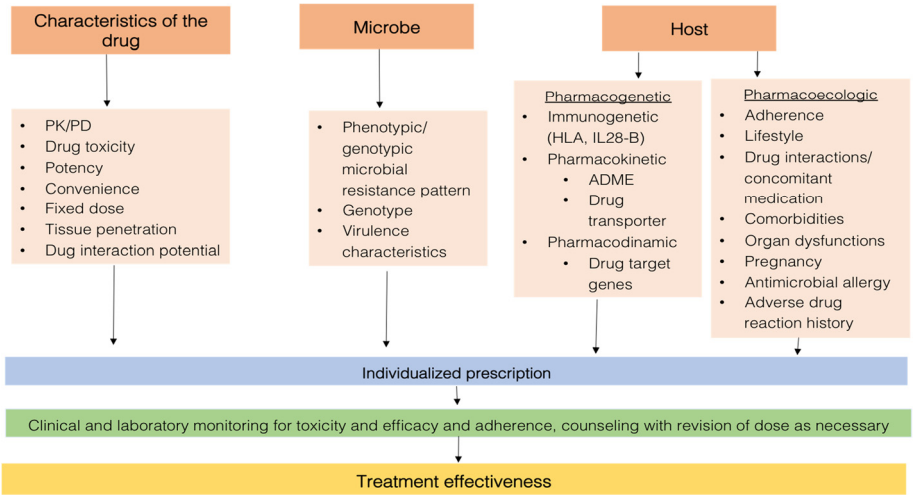

Figure 1. Complex relationships between drug, pathogen and host factors affecting antimicrobial treatment outcome. PK (Pharmacokinetics), PD (Pharmacodynamics), HLA (human leucocyte antigen), IL28-B (interleukin 28-B), ADME (absorption, distribution, metabolism and excretion). Modified from *Aung AK et al., 2014 (16)*.

Within anti-infective drugs, pharmacogenetics has a growing interest in the group of antifungal drugs. Numerous studies have demonstrated wide inter-patient variability in the pharmacokinetics of several antifungal agents. These variations can leads to either lack of efficacy as well as drug toxicity. In this context, genetic polymorphism can explain a significant proportion of this variability (17). The polymorphisms with the greatest impact in the antifungal pharmacokinetics are the genetic polymorphisms that affect some of the processes of drug disposition: absorption, distribution, metabolism or excretion (5,18). Genetic alterations affecting the response of main types of antifungals available have been recently investigated. In this sense, polymorphism in the *MDR1* (multi-drug resistance-1) gene could affect amphotericin B pharmacokinetics. This gen codifies the p-glycoprotein (P-gp) transporter, involved in the amphotericin B metabolism. Some data suggest a possible interaction between *MDR1* polymorphisms and amphotericin B through P-gp expression (19). Regarding echinocandins, genetic polymorphisms related to the organic anion transporter protein 1B1 (OATP1B1) may affect the efficiency of uptake of caspofungin into the liver (20). However, the greater evidence of the clinical impact of pharmacogenetics on antifungal drugs comes from the triazole antifungals. These drugs are substrates of various human cytochromes enzymes like CYP2C19, CYP2C9 and CYP34 and polymorphisms in this genes have bee related to important changes on drug metabolism of azoles (5).

Despite of all the advantages of personalized medicine and pharmacogenetics, its implementation remains restricted to a small proportion of patients. One of the limitations to routine use of personalized medicine is the management of big-data. In this context, in the near future it would be necessary to upgrade the genetic information into electronically medical records and this information must be managed by bioinformatics. In addition, another limitation is

the lack of enough scientific evidence. Much of the results presented so far in the literature are partly contradictory and, in many cases, based on, for example, too small patient populations or patient cohorts that are not homogeneous with respect to the drug treatment studies. There is thus a need for larger randomised prospective studies in the future that further confirms the usefulness of these biomarkers in routine clinical care. In this sense, cost-economic studies are also necessary. Finally advanced training in genetics is required for health professionals to be able to understand and interpret pharmacogenetic information (2).

Apart from pharmacogenetics, mention should also be made of therapeutic drug monitoring (TDM) and pharmacokinetics/ pharmacodynamics (PK/PD) analysis. TDM is the clinical practice of measuring specific drugs at designated intervals to maintain a constant concentration in a patient's bloodstream, thereby optimizing individual dosage regimens (21). . Originally, pharmacokinetics constituted the key conceptual basis of personalized medicine, which is now evolving to include pharmacogenomic approaches as previously commented. However, pharmacokinetics continues to be an attractive option of personalized medicine.

TDM is recommended in those medications with a clear relationship between drug concentrations and clinical outcomes, provided a validated assay is available for determination. It is useful in drugs with non-linear pharmacokinetics and with high pharmacokinetic variability. A field in which the application of TDM is particularly valuable is the area of infectious diseases. The application of pharmacokinetics approaches is really helpful to optimize the prescription of antimicrobials. In this sense, TDM could improve clinical outcome from infections, reduce the development of antimicrobial resistance, and minimise the risk of toxicity. In addition,

TDM is also important in critically ill patients with unpredictable pharmacokinetics and pathophysiological conditions known to alter pharmacokinetics. In these patients, higher doses are usually be necessary based on their pharmacokinetic behaviour (22).

Among antimicrobials drugs, antifungal are a group of drugs in which TDM has also been shown to be very useful. In this context, TDM has proven to be useful in drugs with a narrow therapeutic margin and great pharmacokinetic variability such as the azole voriconazole, itraconazole and posaconazole. These drugs are metabolized by CYP450 isoenzymes and therefore are susceptible to interact with other drugs, which also justifies the use of TDM. And, lastly, in many cases, fungal infections occur in critical patients with variations in the pharmacokinetic parameters, making TDM a very valuable tool (23,24).

In addition, a tool of recent interest in the optimization of antimicrobial treatment is the PK/PD analysis. PK/PD try to define the relationship between drug pharmacokinetics and the success or failure of a therapy, with the aim of defining a simple parameter that helps us to predict therapeutic outcomes. While pharmacokinetics quantifies the changes that occur in the concentration of a particular drug in different biological fluids, pharmacodynamics describes the relationship between drug exposure and pharmacological and/or toxicological effects. In antifungal drugs, pharmacodynamics is described through the Minimum Inhibitory Concentration (MIC) that reflects the antifungal susceptibility (23).

One way to ensure the implementation of all the above strategies to improve the use of anti-infective drugs is the development of Antimicrobial Stewardship (AMS) programmes. The AMS programmes have emerged in response to the alarming increase in antimicrobial resistance as an opportunity to control this problem

(25). In fact, antimicrobial resistance is a serious public health considered by the World Health Organization (WHO) to be one of the three greatest threats to human health (26). The development of resistance is associated with the overuse of antimicrobial drugs. Therefore, the purpose of the AMS programmes is to improve the antimicrobial use in an attempt to facilitate better clinical outcomes and to stem the tide of antimicrobial resistance (27).

The concern about resistance is not exclusive to antibiotics, as antifungal resistance is also a major and emerging problem, mainly for the azole in *Candida* spp. and *Aspergillus* spp. Azole resistance rates of 11.9-14% were reported for *Candida glabrata* and over 2.3% for *Aspergillus fumigatus* (28). As demonstrated in the case of antibiotics, the inappropriate use of antifungal drugs is associated with increased resistance, mortality, morbidity and costs (29). Like AMS, the goals of the Antifungal Stewardship (AFS) programmes are to optimize patient outcomes with an appropriate selection of antifungal drugs based on patient profiles, appropriate doses, route of administration, and duration of treatment, while limiting the consequences of inappropriate use, including the emergence of resistant fungal strains and adverse drug reactions (*Table 2*)(27,30).

While AMS programmes are extensively implemented, little implementation of AFS programmes has been reported in the literature. In 2018, A meta-analysis published including 14 studies that analysed the impact of the implementation of an AFS programme was published (31). The first aspect to be discussed in the results of this work was the composition of the AFS team. According to the recommendations of the guidelines of international scientific societies, the AFS team should include an infectious diseases physician, a clinical pharmacist with infectious diseases training and a clinical microbiologist. In addition, it is optimal that the team includes an

information system specialist and a infection control professional or and hospital epidemiologist (32). However, among the 14 studies including in the review, only five reported a complete AFS team. Interestingly, the number of prescriptions reviewed and performance measures collected were greater if the AFS team was complete, which suggests more extensive investigations is performed. In addition, recent works has suggested that the absence of a pharmacist is associated with an increased rate of inappropriate antimicrobial use and an increased duration of treatment (33). Among the 14 articles included in the present review, the most frequent intervention was the formulation of recommendations to change treatment and the most frequent measure collected was antifungal consumption using. A reduction in antifungal consumption related to the AFS implement was observed in most of the works. With respect to the cost, a reduction of antifungal cost was found in half of the studies (7 of 14). Finally, regarding to fungal resistance, few works evaluated *Candida* resistance, reporting a decrease after the AFS programme implementation in one of these studies (34)

In brief, a coordinated effort is required from all health professionals. It requires the commitment of health leaders, specialists in microbiology/infectious diseases and hospital pharmacists to improve the antifungal management and to achieve the objectives summarised in *Table 2*.

Combating antibiotic resistance is also a priority for the European Union (EU), which has established a common strategy on this issue. In November 2011, the Parliament European published the Action Plan against the rising threats from Antimicrobial Resistance ("the Action Plan") (2011-2016)(35), which stimulated the implementation of national plans in 13 countries. Among these countries was Spain, which in 2014 approved its National Plan for

Antibiotic Resistance (PRAN). This programme has been continued over the following years, and the new PRAN 2019-2021 has been launched, which continues to build on the work previously carried out and with the general objective of reducing or at least halting the growth of antibiotic resistance and its impact on the human health (36).

In conclusion, hospital pharmacists as drug experts must lead the application of personalized medicine to the antifungal treatment. Being part of the antifungal stewardship team will promote to achieve this goal. Moreover, hospital pharmacists must lead the combination of pharmacokinetic, pharmacodynamic and pharmacogenetic approaches as a way to optimize antifungal treatment. In this manner, hospital pharmacists will have a key role in the development of antifungal personalized medicine in the coming years.

Genes	*Drugs*	*Clinical impact*	*Genetic information in drug labelling*
CYP2A6	**Artesunate**	Increased treatment failure with *CYP2A6* PM and possible contribution to apparent artemisinin resistance in Asia with *CYP2A6* PM genotypes	No
CYP2B6	**Efavirenz**	Increased plasma exposure and drug toxicity with *CYP2B6* PM genotypes	Yes
	Nevirapina	Increased plasma exposure and drug toxicity with *CYP2B6* PM genotypes	No
	Artemisina	Increased treatment failure with *CYP2B6* PM genotypes	No
CYP2C8	**Chloroquine**	Increased resistance with *CYP2C8*2* and *CYP2C8*3*	No
CYP2C19	**Omeprazol and lansoprazol**	Increased *Helicobater pylori* eradication with *CYP2C19* PM genotypes	Yes
	Voriconazol	Increased plasma exposure and drug toxicity with *CYP2C19* PM genotypes. Decreased efficacy with *CYP2C19* RM and UM	Yes
	Etravirina	Increased plasma exposure with *CYP2C19* PM genotypes	No
CYP2E1	**Isoniazid**	Increased hepatotoxicity with *CYP2E1*1A/*1A* and *CYP2El*1A-*6-*1D*	No
NAT1	**Sulfamethoxazole**	Decreased hypersensitivity reactions in HIV-infected patients with *NAT1* FA genotypes	No
NAT2	**Isoniazid**	Increased hepatotoxicity with *NAT2* SA genotypes Increased tuberculosis treatment failure with *NAT2* FA genotypes	Yes
	Sulfamethoxazole	Increased hypersensitivity reactions in HIV-infected patients with *NAT2* SA genotypes	No
GSTM1	**Isoniazid**	Increased hepatotoxicity with *GSTM1* null genotype	No
GCLC	**Sulfamethoxazole**	Increased hypersensitivity in patients with rs761142T>G allele	No

Genes	*Drugs*	*Clinical impact*	*Genetic information in drug labelling*
UGT1A1	**Atazanavir**	Increased unconjugated hyperbilirubinemia with *UGT1A1* SM genotypes Increased drug discontinuation *UGT1A1* SM genotypes	Yes
	Dolutegravir	Decreased clearance and increased AUC	Yes
	Raltegravir	Decreased clearance and increased AUC	Yes
	Indinavir	Increased unconjugated hyperbilirubinemia with *UGT1A1* SM genotypes	No
G6PD	**Dapsone**	Increased hemolytic anemia	Yes
	Choroquine	Increased hemolytic anemia	Yes
	Hydroxycholorquine	Increased hemolytic anemia	Yes
	Primaquine	Increased hemolytic anemia	Yes
	Nitrofurantoin	Increased hemolytic anemia	Yes
	Sulfadiazine	Increased hemolytic anemia	Yes
	Sulfamethoxazole and trimoethoprim	Increased hemolytic anemia	Yes
OAT1, 0AT3, ABCC2, ABCC4	**Tenofovir**	Increased renal tubulopathy	No
ITPA	**Ribavirin**	Decreased anemia with hepatitis C treatment with rs1127354 A and rs7270101 C genotypes	No
PDE6	**Voriconazole**	Increased visual toxic effects in PM.	No
HLA-B	**Abacavir**	High risk of hypersensivity reactions	Yes

Table 1. Pharmacogenetic biomarkers of anti-infective drugs. EM (Extensive metabolizer), FA (Fast acetylator), SA (Slow acetylator), PM (Slow metabolizer), UM (Ultrarapid metabolizer), AUC (area under the curve)
Table modified from *Aung AK et al., 2014 (16)*

1.	Restrict antifungal prophylaxis to patients who really need it.
2.	Try to implement new diagnostic techniques to reduce the gap between empirical and targeted antifungal treatment.
3.	Start prompt 'early' antifungal treatment based on risk factors in critically ill patients.
4.	Select the most adequate antifungal drug according to the clinical and the underlying condition of the patient.
5.	Achieve adequate source control.
6.	Use an adequate dose: low dose is associated with resistance. Perform TDM to all patients receiving voriconazole and posaconazole.
7.	Perform biomarkers to confirm or to exclude the diagnosis and to monitor clinical evolution of the disease (galactomannan, Beta-d-glucan…).
8.	Stop 'early' inappropriate therapy at 72–96 hours.
9.	De-escalate whenever possible.
10.	Check duration of therapy.

Table 2. Summary of challenges included in the antifungal stewardship programme. TDM (therapeutic drug monitoring). Adapted from Bassetti *et al.; 2019(27)*.

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Invasive fungal infections

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2 INVASIVE FUNGAL INFECTIONS

The incidence of invasive fungal infections (IFIs) has been increasing over the last years despite the improvement in diagnostic methods and the introduction of new antifungal therapies. In addition to that, significant changes in the epidemiology of fungal infections have occurred, and this has led to important changes in therapeutic management of these infections (37,38).

The rising incidence of IFIs is due to multiple causes. These include the increase in the use of broad-spectrum antibiotics, the greater number of immunosuppressed patients, the use of invasive vascular access devices and also the use of new therapies aimed at the immune system (37,39–43). In this sense, it is important to note the increment in the volume of transplant patients over the last years. Patients who receive a solid organ transplant (SOT) as well as an hematopoietic stem cell transplant (HSCT) will receive immunosuppressive therapy for a longer period of time, so they form a larger group of patients at high risk of developing an IFI. But in addition, recently, new target therapies with immunological effects have appeared. These novel agents can alter the immune response against microorganisms, including moulds, and has led to the emergence of a new spectrum of patients at risk of developing invasive fungal infections. This group of therapies include small tyrosine kinase inhibitor molecules (ITK), monoclonal antibodies or

even therapy with modified T cells with chimeric antigen receptor (CAR-T) (44–46).

2.1 CLINICAL IMPACT AND EPIDEMIOLOGY OF FUNGAL INFECTIONS

2.1.1 *Candida* spp.

Infections due to *Candida* spp. are relevant causes of mortality and morbidity in humans, resulting in very different clinical presentations including superficial and mucosal infections to invasive diseases. Candidemia is one of the most common healthcare-associated bloodstream infections (47). In fact, *Candida* spp. infections account for 90% of fungal infections and continue to be the most frequent cause of fungal infection nowadays. They are the third cause of infection in critical patients, after Gram positive and Gram negative bacteria witch hold the first and second position respectively (48). Yeast infections are also the fourth cause of nosocomial infection in Spain according to data from the 2018 Study of Prevalence of Nosocomial Infection (EPINE) assuming 4.95% of the total microorganisms isolated (49).

There are at least 15 different *Candida* species that cause human disease but *C. albicans*, remains the most frequent specie isolated, causing between 40 and 60% of the candidiasis. However, in recent decades the incidence of *Candida albicans* infection has been decreasing and, on the contrary, the relative incidence of infections caused by *Candida non-albicans* species, such as *C. glabrata*, *C. krusei* and *C. parapsilosis* has risen considerably (40).

The heath impact of invasive fungal infections caused by *Candida* spp. is really significant, especially in critically ill patients admitted to

Intensive Care Units (ICU). In this critical patients, candidiasis have a cumulative incidence of 7.07 episodes per 1,000 admissions and a crude 30-day mortality of 42% according to the data European project EUCANDICU (50).

2.1.2 *Aspergillus* spp.

Fungal infections caused by *Aspergillus* spp. are much less frequent than *Candida* spp. infections, but no less important. Infections caused by *Aspergillu*s spp. represent between 0.3% and 6.9% of the total infections in critical patients admitted to the ICU causing 7% of the total fungal infections in these patients (51). Despite the lower incidence of invasive aspergillosis (IA), this infection is associated with high mortality, which may reach as much as 80% in patients with a confirmed IA.

A special group of patients at high risk for IFI caused by filamentous fungi are patients with malignant haematological diseases as the case of patients with leukaemia treated with high dose of chemotherapy. In these patients, the incidence of IA can be increased to 11% and account for 50% of the total fungal infections (52). In fact, according to the data from the TRANSNET study (53), in patients undergoing HSCT, IA accounted for 43% of IFIs. Similar values were obtained in the SEIFEM study witch included patients with different malignant haematological diseases (54). In this work, 58% of IFIs were IA. The mortality of IA was of around 40% in both studies (TRANSNET and SEIFEM).

Historically, acute leukemia and allogeneic transplantation of hematopoietic progenitors represented the main pathologies that predisposed patients to suffer an IA. However, in the last decade the pattern of haematological patients at risk of developing IFIs is changing with an increase in the incidence of these infections in

patients with conditions other than acute leukemia or HSCT. This is the case of patients with chronic myeloproliferative syndromes witch have been lately associated with an increased incidence of IFIs. This phenomenon is directly related to the use of new therapies aimed at the immune system, such as B-cell receptor inhibitors (BCR), ibrutinib and idelalisib, and selective B-cell lymphoma 2 (BCL 2) protein inhibitors like venetoclax. These drugs have the ability to modulate the immune response by increasing susceptibility to different pathogens, including filamentous moulds. In addition, immunotherapy with monoclonal antibodies check-point inhibitors or with CAR-T cells can also increase the risk of fungal infection in a remarkable way (45).

Another interest aspect is the appearance of new risk factors for aspergillosis. In fact, an increase in the number of patients with IA without any of the traditional risk factors has been reported recently. This observation leads to investigate the profile of these immunocompetent patients. These studies found new relevant factors related to a higher rate of aspergillosis. These new risk factors include the presence of cirrhosis, chronic obstructive pulmonary disease (COPD) (55,56) and previous Influenza virus infection (57,58). Therefore, in assessing the risk for aspergillosis we must consider different aspects including the underlying disease, comorbidities (including COPD and cirrhosis), neutropenia and the use of concurrent immunosuppressive drugs and new-targeted therapies.

Finally, it should be noted that the diagnosis of an invasive fungal infection is challenging. *Aspergillus* species and other filamentous fungi are ubiquitous in the environment, so its detection in a respiratory sample does not have to be synonymous with infection, as a negative culture does not exclude the possibility of aspergillosis either. To facilitate the diagnosis of IA and subsequently to allow

assessment of efficacy, the Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) published standard definitions for invasive fungal infections (59). These definitions were developed to facilitate the identification of reasonably homogeneous groups of patients for clinical and epidemiologic research, to help design clinical trials to evaluate new drugs and management strategies. The definitions assigned 3 levels of probability to the diagnosis of invasive fungal infection that develops in immunocompromised patients with cancer and in hematopoietic stem cell transplant recipients—namely, "proven," "probable," and "possible" invasive fungal infection. The criteria includes host factors and clinical and mycological findings (*Table 3*).

2.1.3 New emerging fungal pathogens

Although *Candida* spp. and *Aspergillus* spp. are the frequent genus involved in the development of IFIs, other emerging pathogens, which include yeasts, hyaline fungi, dematiaceous and mucoral fungi, are increasingly isolated. Infections caused by *Fusarium*, *Scedosporium* and *Mucorales,* despite being less frequent, tend to be more virulent and difficult to treat due to their resistance to most of the available antifungals. In addition, they usually affect patients with an important state of immunosuppression, such as patients undergoing a solid transplantation or stem cell transplantation (60). Therefore, despite being rare diseases, we must always keep them in consideration.

Host factors	-	Recent history of neutropenia (<0.5 × 10^9 neutrophils/L for >10 days).
	-	Receipt of an allogeneic stem cell transplant.
	-	Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for >3 weeks.
	-	Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-*a* blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days.
	-	Inherited severe immunodeficiency
Clinical criteria	-	Lower respiratory tract fungal disease. With 1 of the following 3 signs: o Dense, well-circumscribed lesions with or without a halo sign. o Air-crescent sign o Cavity
	-	Tracheobronchitis
	-	Sinonasal infection. Imaging showing sinusitis plus at least 1 of the following 3 signs: o Acute localized pain (including pain radiating to the eye). o Nasal ulcer with black eschar. o Extension from the paranasal sinus across bony barriers, including into the orbit.
	-	CNS infection wIth focal lesions on imaging or meningeal enhancement.
	-	Disseminated candidiasis. At least 1 of the following 2 entities after an episode of candidemia within the previous 2 weeks: o Small, target-like abscesses (bull's-eye lesions) in liver or spleen. o Progressive retinal exudates on ophthalmologic examination
Mycological criteria	-	Direct test (cytology, direct microscopy, or culture).
	-	Indirect tests (detection of antigen or cell-wall constituents): galatomannan antigen.

Table 3. EORTC/MSG criteria. Adapted from De Pauw *et al.*,2008 (59). Proven infection: required only that a fungus be detected by histological analysis or culture of a specimen of tissue taken from a site of disease. Probable infection: requires the presence of a host factor, clinical criteria and mycological criteria. Possible infection: requires a host factor and clinical criteria.

2.2 SOCIO-ECONOMIC IMPACT OF INVASIVE FUNGAL INFECTIONS

The socio-economic impact of fungal infections is remarkable. Different studies have evaluated the economical burden of invasive fungal diseases in Europe. These studies reported greater costs in patients with IFIs versus patients without infection. In an observational study conducted in 269 haematological patients undergoing induction chemotherapy, the mean total cost per patient was €57,750 with no IA, €68,280 with possible IA and €83,300 with probable or proven IA. The additional IA cost burden ranged from €10,530 to €25,550, and was statistically significantly greater across all areas of expenditure in patients with possible, probable or proven IA versus patients without IA. In addition, hospital stay was also longer in these patients versus patients without IA. These data were corroborated by another study conducted in 50 patients with acute myeloid leukemia and with probable or proven IFIs where an increase in the treatment cost of € 51,033 was calculated, being 70.5% due to the cost associated with antifungal treatment (61).

The economic impact of IFIs can be assessed from the perspective of the hospital, the payer or from the point of view of society. The societal perspective also includes all direct medical and non-medical costs (e.g. lost productivity), as well as indirect costs (e.g. future lost productivity). Unfortunately, there are very few studies that measure the social impact of IFIs. Only two studies reported costs from the societal perspective of a 12-week voriconazole treatment (62,63). Both studies used a Markov model and compared voriconazole with conventional amphotericin B for IA. The mean treatment costs for voriconazole treatment ranged from €25,353 to €30,026. As would be expected, taking into account the previous results, (morbidity and totality and economic data), the social impact of the IFIs is considerable.

The recent rebound in IFIs, changes in both epidemiology and risk factors, their high morbidity and mortality and socioeconomic impact make the management of these infections a real therapeutic challenge. Therefore, the development of new antifungal drugs, more effective and less toxic, is essential, but it is also essential to optimize the treatment of the existing ones.

3

Antifungal drugs

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3 ANTIFUNGAL DRUGS

The number of drugs available for the treatment of fungal infections has recently increased with the approval and commercialization of new molecules (38,64,65) (*Figure 2*).

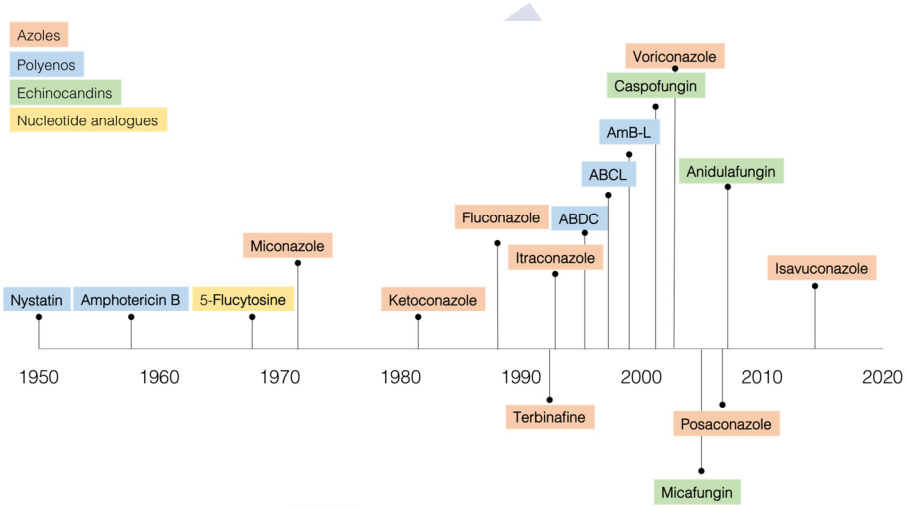

Figure 2. Timeline of antifungal therapy. ABDC (amphotericin-B deoxycholate), ABCL (amphotericin-B lipid complex), AmB-L (liposomal amphotericin-B).

Until the end of the 20th century, there were not many therapeutic alternatives for the treatment of fungal infections. Amphotericin B was the reference drug for the treatment of systemic mycoses up to 1990. This drug was marketed in 1957 and its fungicidal activity together with its wide antifungal spectrum made it the main antifungal drug. A few years later, in 1964, flucytosine was marketed. This antifungal drug is only active against yeast and has a

mechanism of action that favours the development of resistance. For these reasons, fluorcytosine was soon relegated to a second place.

At the beginning of the 1970s, a new group of anti-fungal drugs appeared: the azoles. The first approved azoles were ketoconazole and miconazole, active against yeast and some species of mycelial fungi. These drugs contributed little to the existing drugs, since they presented unfavourable pharmacokinetic characteristics and a deficient efficacy and safety profile (66).

It was in the early 1990s that a real revolution took place. On the one hand, the lipid formulations of amphotericin B were developed. This new formulation of amphotericin maintains the antifungal effect and spectrum of activity but reduces the nephrotoxic effect by formulating the drug in liposomes. On the other hand, in those same years, two new antifungal drugs from the azoles group were introduced to the market: fluconazole and itraconazole. These drugs were the first azole belonging to the triazole family, effective in the treatment of systemic candidiasis and other fungal infections.

In the first decade of the 21st century, two new triazoles for systemic use were approved: voriconazole and posaconazole. In addition, a new class of antifungals was developed: the echinocandins (anidulafungin, caspofungin and micafungin) with a novel mechanism of action and better safety profile (66) Finally, isavuconazol, the lattes drug approved to date, was commercialized in 2016. Isavuconazole is similar to voriconazole; but with activity against *Mucor* and with a better safety profile (67).

In short, 4 classes of antifungal agents are available on market at present: polyenos, nucleotide analogues, azoles and echinocandins (68). *Table 4* and *Figure 3* show the mechanism of action and the main mould targets of these four groups of antifungal drugs

In addition to the drugs already on the market, there are also a large number of molecules in clinical research. The search for new antifungal drugs responds to several requirements. First, there is a need of new antifungals with novel mode of action to avoid cross-resistance and/or cross-toxicity. In addition, the availability of oral alternatives is another objective of antifungal development. Oral administration would enable ambulatory treatment resulting in an improved patient's comfort and therapy adherence. And finally, there is an interest to develop new antifungals with no pharmacokinetic interactions with other drugs (69). In this context, new molecules are currently being investigated in different phases of clinical trials (*Table 5*). Rezafungin, currently in phase III, is a new long-acting echinocandin with an improved stability and active against different *Candida* spp. and *Aspergillus* spp. This molecule seems to be safe and is effective in front-loading dose regimen of a once weekly administration, also enabling outpatient therapy (70,71). Another molecule in phase III clinical trial is SCY-078, the first member of a new glucan synthase inhibitor (GSI) class, which can be administered both orally and intravenously. This molecule doesn't seem to have clinically relevant interactions with CYP450-substrates and has a wide activity against *Candida* and *Aspergillus* species(72). Finally, also in a phase III clinical trial, olorofim, another new antifungal is being investigated. It belongs to the new orotomide antifungal class, which targets the pyrimidine synthesis. It can be administered both IV (intravenous) and orally and has a broad spectrum, including *Aspergillus* spp. and *Scedosporium* spp. A major advantage of this molecule is that due to the novel mechanism of action, there seems to be no cross-resistance with other antifungal classes (73).

Antifungal drug family	*Approved drugs*	*Mechanism of action*	*Biological effect*
Polyenes	Amphotericinn B Nistatine	Formation of pores in the fungal cell membrane	Increased membrane permeability and accumulation of toxic ROS
Pyrimidine analogs	Flucytosine	Interfiring with the fungal RNA and DNA metabolism	Impairment of the fungal RNA and DNA synthesis
Azoles	Ketoconazole Fluconazole Itraconazole Voriconazole Posaconazole Isavuconazole	Inhibition of the fungal enzyme Erg11	Block of the lanosterol to ergosterol conversion
Echinocandins	Caspofungin Anidulafungin Micafungin	Inhibition of the fungal enzyme beta1,3-glucan synthase	Block of the cell wall beta1,3-glucan synthesis

Table 4. Available antifungal drugs: Mechanism of action and biological effect. ROS (reactive oxygen species). Adapted from *Di Mambro et al.*, 2019(68).

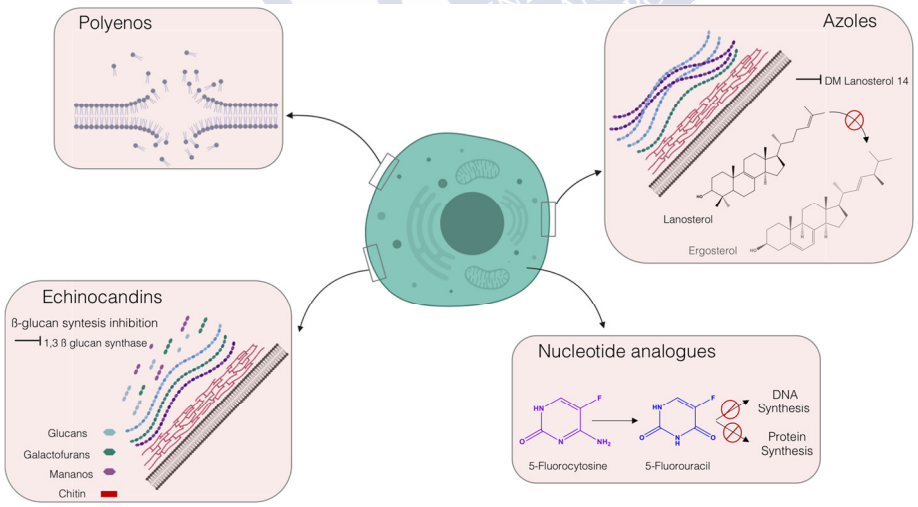

Figure 3. Biological targets of the four classes of antifungals. Adapted from *Di Mambro et al.*, 2019 (68).

Despite the current therapeutic antifungal arsenal available, the treatment of fungal infections still remains unsuccessful. There are several factors that determine the evolution of patients with an invasive fungal infection. Some of the most relevant are: the patient immunological status, the pathogen susceptibility to antifungals, the time between the establishment of the infection until the diagnosis, as well as the safe and effective use of the drug (74). Some of these factors are independent of the patient and affect in a dramatically way to the IFI-related mortality, like the inadequate antifungal selection, the delay in the start of treatment or the use of insufficient doses to achieve effective therapeutic plasma concentrations. Incorrect therapeutic decisions can have fatal consequences for both the individual patient and the global population. In addition to the therapeutic failure, inadequate antifungal treatment can lead to the emergence of multidrug-resistant microorganisms, with the terrible consequences that this entails for society (75). Therefore, research to develop new antifungal drugs should be an objective for the next years alongside with the improvement in the use of the already approved antifungal drugs.

Agent	*Phase*	*Mechanism of action*	*Advantage*	*Disvantage*	*Potential use*
Rezafungin	III	Inhibition of the fungal enzyme beta1,3-glucan synthase	- Improved stability - Long half-life - Safety profile - Minimal drug interactions	- No oral formulation	Broad antifungal spectrum, also activity against *Candida auris* and PJP
SCY-078	III	Inhibition of the fungal enzyme beta1,3-glucan synthase	- Oral and IV formulation - Activity against resistant strains	-	*Candida* and *Aspergillus* spp. *Scedosporium*
Olorofim	III	Inhibition of dihydroorotate dehydrogenase	- Oral and IV formulation - No evidence of cross-resistance - Activity against *Lomentospora prolificans*	- Little or no ativity against *Candida* spp., *Mucorales* spp. and *Crytococcus neoformans*	*Aspergillus* spp. *Scedosporium* spp.

Table 5. Summary of the main antifungal drugs undergoing clinical evaluation. PJP (Pneumocystis jiroveci pneumonia), IV (intravenous). Modified from Van Daele *et al*., 2019. (69)

4

Voriconazole

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4 VORICONAZOLE

4.1 CHEMICAL STRUCTURE AND MECHANISM OF ACTION

Voriconazole was discovered in the late 1980s with the aim of extend the spectrum of the previously marketed fluconazole. Both voriconazole and fluconazole are included in the antifungal class of "azoles". Structurally, this compound belongs to "triazoles" group, since it features three nitrogen molecules in the imidazole ring(76) (*Figure 4*). Voriconazole was finally approved and marketed in 2002.

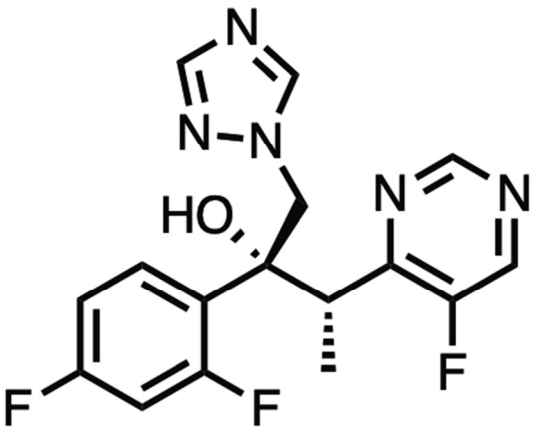

Figure 4. Voriconazole chemical structure.

The antifungal effect of voriconazole is due to the inhibition of the 14 alpha-lanosterol demethylation, which constitutes a key point in the biosynthesis of fungal ergosterol. The conversion of lanosterol in

ergosterol is mediated by the fungal CYP450 dependent enzyme 14 alpha-sterol demethylase and the inhibition of this pathway leads to a disruption of the fungal cell wall (*Figure 3*)(77).

4.2 ANTIFUNGAL SPECTRUM

Voriconazole is a broad-spectrum antifungal, effective for the treatment of *Candida* spp., *Aspergillus* spp., and *Cryptococcus* spp. infections. Moreover, it shows antifungal activity against emerging pathogens like *Scedosporium* and *Fusarium,* which have exhibited limited susceptibility to another antifungal drugs. However, voriconazole has limited activity against fungi of zygomycetes family including *Rhizopos*, *Mucor* and *Absidia (38,78). Figure 5* summarizes the *in vitro* antifungal susceptibility of the main moulds to the available antifungals.

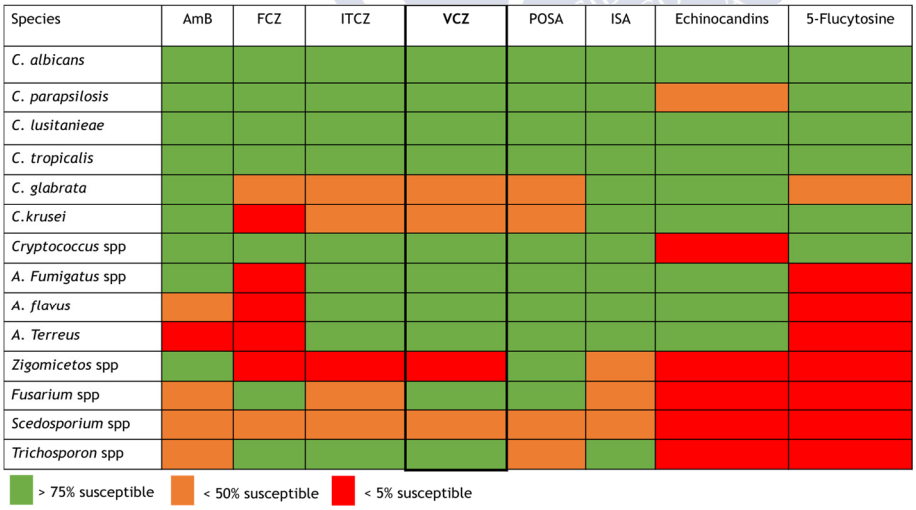

Figure 5. *In vitro* susceptibility to the most relevant antifungals.
AmB (Amphotericin B) FCZ (Fluconazole), ITCZ (Itraconazole), VCZ (Voriconazole), POSA (Posaconazole), ISA (Isavuconazole).

Voriconazole antifungal activity against species from *Aspergillus* is reasonably uniform including different species: *A. fumigatus, A. flavus, A. terreus and A. niger*. However, some cases of acquired resistance from *Aspergillus* spp. have been reported (even in triazole naive patients) (79), hence routine susceptibility testing is of utmost importance. Drug fungal susceptibility is expressed through the MIC value. The interpretation of this value is complex and it is strongly recommended to follow the criteria of the two main organisms in this field, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standard Institute (CLSI)(80,81).

In the case of *Aspergillus fumigatus*, the EUCAST organization establishes a MIC breakpoint of 1 µg/mL to define the susceptibility to voriconazole. In addition, another parameter that has become much discussed is the PK/PD ratio. This parameter in the case of voriconazole is defined by the ratio between a representative parameter of voriconazole pharmacokinetics as the voriconazole AUC (the 24 hours area under the concentration-time curve) and the MIC, which indicates the voriconazole pharmacodynamics. Different studies have investigated the relationship between this parameter and clinical results in order to define a suitable cut-off point. In this sense, in a dynamic *in vitro* model of invasive pulmonary aspergilosis, the total drug AUC/MIC ratio of voriconazole associated with near maximum effect was 32.1 for *Apergillus fumigatus* (82). Another *in vitro* PK/PD model showed that a ratio AUC/MIC of 25.28 was related with 50% of maximal antifungal effect (83). Similarly, a murine model of disseminate aspergillosis showed a relationship between the ratio AUC/MIC and survival witch maximum effect reached at values around 80 to 100 (84).

Regarding *Candida* spp., as discussed above, there are at least 15 distinct *Candida* species that cause human disease, but > 90% of invasive disease is caused by the most common 5 pathogens, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*. The *in vitro* activity of voriconazole against *Candida* spp. is not uniform with different susceptibilities observed depending on the specie concerned. Specifically, C. *glabrata* and *C. krusei* show a voriconazole MIC 10-fold higher than C. *albicans*. In addition, fluconazole resistant *Candida* spp. has voriconazole MICs proportionately higher than those fluconazole susceptible. Therefore, every effort should be made to identify *Candida* to species level (85). Also in this case, investigations have been developed to know the value of the parameter PK/PD ratio that best relates to the efficacy of voriconazole in the different *Candida* species. Regarding to that, one study revealed that a ratio more than 25 for AUC/MIC predicted voriconazole efficacy in a murine model of candidiasis (86). In addition, an observational study showed that patients with higher ratio C_{min} (minimum plasma concentration)/MIC had greater probability of success (87). Similarly, an exploratory PK/PD study in 15 patients with IFI showed that clinical response was associated with an AUC/MIC ratio higher that 25 and a C_{min}/MIC higher than 1. Both ratios (AUC/MIC and C_{min}/MIC) may be useful as predictors of clinical outcomes because a good correlation was observed between voriconazole C_{min} and AUC (88). This is really interesting because a frequent blood sampling is required to estimate AUC and it can be an inconvenient in daily clinical practices.

4.3 THERAPEUTIC INDICATIONS

Voriconazole is indicated in adults and children aged 2 years and above as follows (77):

- Treatment of invasive aspergillosis.

- Treatment of candidemia in non-neutropenic patients.
- Treatment of invasive *Candida* infections (including *C. krusei*) resistant to fluconazole.
- Treatment of severe fungal infections caused by *Scedosporium* spp. and *Fusarium* spp.
- Prophylaxis of invasisve fungal infections in high risk-receptors of an allogeneic hematopoietic stem cell transplantation (HSCT).

4.3.1 Invasive pulmonary aspergilosis

Voriconazole is the current antifungal drug of choice for the treatment of IA as set out by consensus in the main clinical practice guidelines of this disease (89–92).

The most relevant study demonstrating the efficacy of voriconazole for the treatment of IA was the study of Herbrecht *et al*. This randomized clinical trial compared voriconazole with amphotericin B deoxycholate (ABDC)(93). In this study, 277 patients with definite or probable IA where randomized to receive voriconazole (loading dose of intravenous (IV) of 6 mg/kg every 12 hours the day one followed by 4 mg/kg IV every 12 hours for at least seven days) o ABDC (1-1.5 mg/kg IV once daily). At week 12, there were successful outcomes in 52.8% of the patients in the voriconazole group versus 32.6% of those in the amphotericin B group. The survival rate at 12 weeks was also better in the voriconazole group (70.8% versus 57.9%, p=0.02). In addition, less severe drug-related adverse events were reported in the voriconazole group (12% versus 24%, p=0.008). Most of the patients included in this study presented a haematological underlying condition, specialty HSCT and acute leukemia. The outcome of this clinical trial led to the commercialization of voriconazole in 2002. Similar results were

obtained in an open, nonblinded, noncomparative trial published by Denning *et al*. in the same year. In that trial, 48% of the patients receiving voriconazole met the primary efficacy end point of a good response (defined as complete or partial response) (94). A similar trial performed later involved 36 patients with subacute invasive and chronic pulmonary aspergillosis who were receiving voriconazole as primary or salvage therapy (95). The rates of therapeutic response and toxic effects were comparable to those observed by Denning *et al* (96). In parallel, other cohort studies have confirmed the efficacy of voriconazole as a primary treatment for AI in onco-haematological patients (94,97).

A new triazolic antifungal, isavuconazole, has recently been approved for the treatment of IA. This new azole has demonstrated non-inferiority for the primary treatment of IA compared to voriconazole in a phase 3 multicentre, double-blind, randomized clinical trial involving 532 patients (98). Isavuconazole was well tolerated compared with voriconazole, with fewer study-drug-related adverse events (especially liver toxicity and fewer eye disorders and psychiatric disorders compared with voriconazole). However, in this study in the voriconazole arm no TDM was performed so these differences could have been avoided if the dose had been adjusted according to plasma concentration. Lastly, due to the linear pharmacokinetics of isavuconazole it does not seem necessary TDM, although the data are preliminary and this should be corroborated with more pharmacokinetic studies in the coming years.

There are no randomized clinical trials comparing the efficacy and safety of the liposomal formulation of amphotericin B (L-AmB) versus voriconazole in the primary treatment of IA. However, there are clinical trials that have evaluated the efficacy of different doses of L-AmB in the treatment of IA and have demonstrated overall

responses of about 50% and a survival at 12 weeks similar to that reported in the pivotal study of voriconazole (99,100).

Finally, echinocandins have fungistatic activity against *Aspergillus* spp. and the evidence to recommend them as primary treatment for IA is limited (101,102) therefore they are only indicated when voriconazole or amphotericin B are contraindicated. There are also no sufficiently well-contrasted studies to recommend posaconazole as a first-line treatment for IA (89–92).

With regard to combination therapy of antifungal drugs with different mechanisms of action in the primary treatment of IA, the results are controversial. It should be noted the results of a randomized trial compared outcomes of voriconazole monotherapy to combination therapy with voriconazole plus anidulafungin. The trial enrolled 454 patients with hematologic malignancy to evaluate hypothesized superiority in 6-week survival in combination therapy recipients. Mortality at 6 weeks was 19.3% for combination recipients and 27.5% for monotherapy recipients (p =0.087)(103). In post hoc analyses of the dominant subgroup of patients who were diagnosed as having "probable" aspergilosis, the difference in mortality was most notable (15.7% combination versus 27.3% monotherapy; p= 0.037). This study suggest potential benefits for combination therapy with voriconazole and an echinocandin and, based in these results, guidelines recommend considered the voriconazole and echinocandin combination therapy in the primary treatment of severe IA, especially in haematological patients and those with deep neutropenia as well as refractory IA (92).

In addition to use as the primary treatment for AI, voriconazole may also be an appropriate option in rescue treatment if it has not been used previously (92).

4.3.2 Other clinical forms of aspergilosis

Voriconazole is also recommended as the first line treatment in extrapulmonary aspergillosis infections, as is the case of CNS (Central Nervous System) aspergillosis, endophtalmitis, aspergillosis of the paranasal sinuses and other less common forms of non-pulmonar presentations of aspergillosis (endocarditis, pericarditis, myocarditis, osteomyelitis, peritonitis, esophagitis and renal or hepatic aspergillosis, among others) (92).

Furthermore, in addition to invasive aspergillosis, there are two types of non-invasive pulmonary presentations: chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA) in which voriconazole also plays an important role (104).

Chronic pulmonary aspergillosis includes several clinical manifestations including single aspergillomas, chronic cavitated pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA) and chronic necrotizing aspergillosis (CNA). These clinical presentations are more frequent in patients with tuberculosis, ABPA, resolved lung cancer, COPD or pulmonary cavitary sarcoidosis. The prognosis of CCPA or CFPA in the absence of treatment leads to a 3 and 7 year mortality of 25 and 70%, respectively (89,104). Regarding the antifungal treatment, it is recommended in patients where surgery is not an option and there is clinical or radiological progression. In these patients, oral therapy with voriconazole or itraconazole is considered the first line of therapy. Posaconazole can be used as an alternative but not as a first option because of the lack of clinical data in this pathology and the high cost of very longs periods (105). Antifungal treatment should be continued for a minimum of 6 months, and if it is well-tolerated and the clinical response is good, it may be continued for years(106).

ABPA occurs when the bronchial tree is colonized by *Aspergillus* spp. and leads to episodes of bronchial obstruction and inflammation. It usually occurs in patients with asthma or cystic fibrosis (CF) (104). The optimal management of ABPA depends on patient response, drug adverse events and antifungal susceptibility. ABPA treatment combines two approaches: controlling the immune response, and decreasing the burden of organisms. Despite oral corticosteroids is the basis of the ABPA treatment, they are associated with many adverse effects. In this sense, antifungal therapy with itraconazole or voriconazole may be helpful for many patients to minimize the steroids use (107,108).

4.3.3 Fungal prophylaxis

With regard to prophylaxis of filamentous fungi in haematological patients undergoing allogeneic transplantation of haematopoietic progenitors, the drug of choice is posaconazole. Although voriconazole has shown in a randomized clinical trial lower rate of *Aspergillus* infections compared to fluconazole, it did not demonstrate significant changes in overall survival (109).

4.3.4 Candidemia

Voriconazole is also used the treatment of candidemia in non-neutropenic patients, especially in infections caused by species of *Candida* resistant to fluconazole. This triazole has demonstrated effectiveness for both mucosa *Candida* infections and also in invasive candidiasis (110,111). Because of the existence of other effective and safe drugs in the treatment of candidiasis like echinocandins, voriconazole has been relegated for the step-down oral therapy in patients with infections due to *C. krusei* and fluconazole-resistant, voriconazole susceptible *C. glabrata*. At last, another situation in

witch voriconazole has an interest role is in the treatment of Candida infections in “difficult to access” tissues as the case of cerebrospinal fluid (CSF) or the ocular location. In this sites, voriconazole has been shown to reach concentrations greater than 50% of the serum concentrations (112–114).

4.3.5 Fusarosis and scedosporosis

The efficacy of voriconazole in the treatment of rare invasive fungal infections has been documented in small observational studies due to the low incidence of these infections. Perfect *el al.* conducted a multicentre observational study to evaluate voriconazole outcomes in this type of fungal infections. They observed a global response of 45.5% for fusariosis and 30% for scedosporosis (115).

4.4 POSOLOGY AND ADMINISTRATION ROUTE

There are currently three available formulations of this antifungal: 200 mg vials for intravenous administration and two type of oral presentations: oral tablets (50 and 200 mg) and a 40 mg/ml oral suspension (77).

Therapy must be initiated with an oral or intravenous loading dose to achieve plasma concentrations on Day 1 that are close to steady state. Then, treatment should be continued with an oral or intravenous maintenance dose as necessary according to the therapeutic indication, the patient’s clinical evolution and the mycological response. On the basis of the high oral bioavailability, switching between intravenous and oral administration is appropriate when clinically indicated.

The recommended oral and intravenous dosing regimen is summarized in the *Table 6* and *7* (77). In young adolescents with low body weight (12 to 14 years and <50 kg) voriconazole should be dosed as children. The reason is that young adolescents may metabolize voriconazole more similarly to children than to adults (77).

	Intravenous	**Oral**	
		Patients 40 kg and above*	Patients less than 40 kg*
Loading dose (first 24 hours)	6 mg/kg every 12 hour	400 mg every 12 hours	200 mg every 12 hours
Maintenance dose (after first 24 hours)	4 mg/kg every 12 hour	200 mg every 12 hours	100 mg every 12 hours

Table 6. Posology of voriconazole in adults
*This also applies to patients aged 15 years and older.

	Intravenous	**Oral**
Loading Dose (first 24 hours)	9 mg/kg every 12 hours	Not recommended
Maintenance Dose (after first 24 hours)	8 mg/kg every 12 hours	9 mg/kg every 12 hours (a maximum dose of 350 mg every 12 hours)

Table 7. Posology of voriconazole in children and young adolescents with a low body weight.

4.5 TOXIC EFFECTS

Voriconazole is a generally well-tolerated drug. The most frequent adverse caused by this drug are liver disorders, rash, visual disturbances and neurotoxicity. In addition, azole therapy is associated with prolongation of the QTc interval, so it should be used with caution in patients with other risk factors for cardiac arrhythmias as

the case of patients who receive concomitantly drugs that prolong the QTc interval (93).

4.5.1 Hepatic toxicity

The overall incidence of transaminase increases >3 xULN (Upper limit of normal) in the voriconazole clinical programme was 18.0% (319/1,768) in adults and 25.8% (73/283) in paediatric subjects who received voriconazole for either therapeutic and prophylaxis use. Liver function test abnormalities could be associated with higher plasma concentrations of voriconazole. The most of the abnormal liver function tests either resolved during treatment without dose adjustment or following dose adjustment, including discontinuation of therapy. Voriconazole has also been associated rarely with cases of fatal hepatic toxicity in patients with other serious underlying conditions including cases of fulminant hepatic failure leading to death (77).

4.5.2 Dermatological reactions

Dermatological reactions were very common in patients treated with voriconazole in clinical trials, but the majority of these dermatological reactions were of mild to moderate severity. Patients have developed severe cutaneous adverse reactions in extremely rare cases, including Stevens-Johnson syndrome (SJS) (uncommon), toxic epidermal necrolysis (TEN) (rare), drug reaction with eosinophilia and systemic symptoms (DRESS) (rare) and erythema multiforme (rare) during treatment with voriconazole (93).

4.5.3 Visual impairments

In clinical trials, visual impairments (including blurred vision, photophobia, eye disorder, halo vision, photopsia, scintillating

scotoma, visual acuity reduced, visual brightness, visual field defect among others) with voriconazole were very common. These visual impairments were transient and reversible, with the most of the cases spontaneously resolving in the first minutes and without clinically significant long-term visual effects (77,93). The visual impairments were generally mild, rarely resulted in discontinuation. Visual impairments may be associated with higher plasma concentrations of voriconazole. The mechanism of action is unknown, although the site of action is most likely to be within the retina. In a study in healthy volunteers investigating the impact of voriconazole on retinal function, voriconazole caused a decrease in the electroretinogram (ERG) waveform amplitude. The ERG measures electrical currents in the retina. The ERG changes did not progress over 29 days of treatment and were fully reversible on withdrawal of voriconazole (77).

4.5.4 Long-term side effects

In addition to the known side effects described in the voriconazole data sheet, the use of voriconazole in prolonged (weeks to months) periods of time (such as prophylactic therapy in solid organ or hematopoietic stem cell transplant patients or in patients with a confirmed aspergillosis) has been associated with less rare but potentially serious adverse effects. Some of these adverse effects are common to the other azole like hepatotoxicity and hormone-related effects, including gynecomastia, alopecia, decreased libido, oligospermia, azoospermia, impotence, hypokalemia, hyponatremia, and adrenal insufficiency. Others, like peripheral neuropathies, pancreatitis, periostitis, phototoxic reactions, and squamous cell carcinoma have been specifically linked to voriconazole (116).

The pathophysiological mechanism of these side effects is not known exactly t is thought that they are more related to cumulative doses due to prolonged treatments than to high plasma concentrations of antifungal. Furthermore, due to the non-specificity of these side effects and taking into account that patients treated with voriconazole usually receive many multiple medications simultaneously, establishing causality is in most cases complicated. However, we should not forget the possibility of their occurrence, especially, as previously mentioned, in long-term antifungal treatments (116).

4.6 PHARMACOKINETIC PROPERTIES

4.6.1 Absorption

Voriconazole is rapidly absorbed following oral administration. The time to maximum plasma concentration (*Tmax*) ranges for 1-2 hours after dosing with maximum plasma concentrations (*Cmax)* of 1.88 to 5.27 µg/mL. The absolute bioavailability of voriconazole after oral administration is estimated to be 96% and it is not affected by changes in gastric pH (77).

4.6.2 Distribution

The volume of distribution at steady state for voriconazole is estimated to be 4.6 L/kg, suggesting extensive distribution into tissues. Plasma protein binding is estimated to be 58%. With respect to the ability to reach tissues that are difficult to access, such as the central nervous system, the available pharmacokinetic studies demonstrated a high penetration capacity at this level, reaching CSF greater than 50% of the drug concentration in plasma (113). It is also distributed extensively to vitreous fluid, aqueous humour and bones (117).

4.6.3 Metabolism

Voriconazole is metabolized in the liver by the cytochrome P450 (CYP450) liver enzyme complex, comprising CYP2C19, CYP2C9 and CYP3A4/5 enzymes subfamilies. CYP2C19 isoenzyme is responsible mainly for the conversion of voriconazole into its principal inactive metabolite, voriconazole N-oxide, witch accounts for the 72% of the total plasma metabolites of voriconazole. CYP3A4 and CYP2C9 play a relative minor role in voriconazole hepatic transformation, with a lower affinity for this drug (118). Because these isoenzymes are involved in the metabolic pathway of many other drugs, it should be noted the high number of potential drug interactions that can occur, including inhibition and induction or voriconazol by other drugs. Moreover, voriconazol is a strong inhibitor of CYP3A4 thereby affecting the metabolism of other drugs (38,119–121).

The gene encoding the CYP2C19 isoenzyme is highly polymorphic with more than 34 allelic variants identified (122). Specific alleles have been identified and have been related to altered enzyme activity. Thus, there is the wild type allele**1*, witch is the normal function allele. In addition, alleles with loss-of-function or null alleles have been described in the literature *(*2, *3, *4, *5, *6, *8*) and more recently, a gain-of-function allele have been reported *(*17*). Based on the polymorphisms of *CYP2C19*, patients could be classified into five different phenotypes: poor metabolizers (PM), intermediate metabolizers (IM), extensive o normal metabolizers (EM), rapid metabolizers (RM) and ultra-rapid metabolizers (UM)(17).

The incidence of polymorphisms varies in the ethnic groups as shown in *Table 8*. Thus, among the Asian population the alleles **2* and **3* are frequent, originating an enzyme of lower activity or null activity, depending on whether they have one or two copies of the

mutated allele. In these individuals it is frequent to find high voriconazole plasma concentrations with the normal dose and this fact can lead to serious toxicity associated with the drug. On the other hand, in the Caucasian population is much more frequent the allele **17* that explain an accelerated metabolism of the drug and can often be associated with subtherapeutic voriconazole plasma concentrations and increase the risk of therapeutic failure (123).

Patients with a slow metabolizing phenotype may show much higher concentrations compared to a fast or ultra-fast metabolizer. Thus, the condition of being a carrier of a certain genetic variant of the *CYP2C19* gene may imply a different exposure to the drug in the organism.

***CYP2C19* Genotype**	**Phenotype**	**Caucasian**	**African-American**	**Hispanic**	**Asian**
***1/*17 *17/*17**	Rapid metabolizers (RM) Ultrarapid metabolizers (UM)	31,2%	33,3%	18,3%	1%
***1/*1**	Extensive metabolizers (EM)	42%	39%	58%	35-43%
***1/*2, *1/*3 *2/*17 *3/*17**	Intermediate metabolizers (IM)	19%	15%	20%	43-46%
***2/*2 *2/*3 *3/*3**	Poor metabolizers (PM)	2,8%	6,7%	0,87%	14-19%

Table 8. *CYP2C19* Genotypes and their corresponding Phenotypes for Various Racial-Ethnic Groups. Adapted for Owusu Obeng A et *al.,* 2014 (17)

4.6.4 Elimination

Voriconazole is eliminated by liver metabolism, with less than 2% of the dose eliminated unchanged in the urine. 80% of the drug's metabolites are eliminated in urine and the remainder is excreted

through the faecal route. The terminal half-life is estimated at between 6 and 12 hours but since pharmacokinetics is non-linear, the terminal elimination half-life is not useful in predicting voriconazole accumulation or elimination (77).

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Therapeutic drug monitoring of voriconazole

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5 THERAPEUTIC DRUG MONITORING OF VORICONAZOLE

TDM has been demonstrated to be an excellent tool for the optimization of the therapy with several drugs. Susceptible drugs for the application of TDM should have certain characteristics including narrow therapeutic index, wide inter-individual pharmacokinetic variability and finally a proven correlation between at least one pharmacokinetic parameter (plasma concentration, area under the plasma concentration-time curve, etc.) and the response in terms of efficacy and/or toxicity (74).

As mentioned previously, voriconazole has non-linear pharmacokinetics as a result of its saturable hepatic clearance. Moreover, it has a narrow therapeutic index and high intra- and inter-individual variability. In this context, numerous studies have demonstrated the clinical utility of TDM of voriconazole as way to improve clinical efficacy and minimize toxic effects. Indeed, the main international clinical guidelines of the management of fungal infections recommend routinely TDM of voriconazole to enhance treatment outcomes (89–92).

Different studies have shown a positive impact of voriconazole TDM on therapeutic results. In 2012, Park *et al.* conducted a randomized controlled clinical trial to evaluate the clinical utility of TDM in 110 patients treated with voriconazole for IFIs. In this study, patients were randomized into two groups: patients undergoing TDM

and patients not receiving TDM. These authors used the range of 1-5.5 µg/mL as the target therapeutic index in the TDM group. Authors observed a significant greater number of complete or partial responses in the TDM group versus those patients not receiving TDM (81% vs 57%). In addition, a lower rate of discontinuations due to adverse effects associated was observed in the TDM group (124). Later, twenty-four studies were analysed in the meta-analysis conducted by Luong *et al.* to assess the relationship between voriconazole serum concentration and clinical outcomes. Pooled analysis for efficacy endpoint demonstrated that patients with therapeutic voriconazole serum concentrations (1.0-2.2 µg/mL) were more likely to have successful outcomes compared with those with subtherapeutic voriconazole serum concentrations (Odds ratio, OR: 2.30; 95% Confidence Interval, CI 1.39-3.81). Furthermore, pooled analysis for toxicity endpoint demonstrated that patients with supratherapeutic voriconazole serum concentrations (4.0-6.0 µg/mL) were at increased risk of toxicity (OR 4.17; 95% CI 2.08-8.36) (125). *Table 9* summarizes the main studies assessing the relationship between voriconazole serum concentration and clinical outcomes.

5.1 THE THERAPEUTIC RANGE OF VORICONAZOLE

An important challenge to the optimal use of TDM is the selection of an adequate therapeutic index. Although some studies suggest a possible correlation between plasma concentration of voriconazole and efficacy, no break points have been formally established. The suggested lower threshold for the therapeutic range varies from 0.50 to 2 µg/mL and the upper threshold to avoid toxicity varies from 4 to 6 µg/mL in different investigations (126).

In the randomized controlled study conducted by Park *et al.* a range of 1-5.5 µg/mL was used to perform the dose adjustments in the

TDM group. This therapeutic index was subsequently used in most observational studies. However, the fact is that this range is not yet fully established, and indeed, antifungal management guidelines still do not recommend an explicit optimum range of trough concentrations.

Regarding to that, *in vitro* studies based on the voriconazole MIC of *Aspergillus* spp. suggest that a lower cut-off point of 0.50 µg/mL would be sufficient to optimize prophylactic treatment with voriconazole (127). On the other hand, *Pascual et al.* observed a greater rate of treatment failure with plasma voriconazole concentrations below 1 µg/mL (128). In addition, in the multicentre study conducted by Dolton *et al.,* voriconazole plasma concentrations below 1.7 µg/mL increased the incidence of treatment failure (119). More recent data suggest that higher concentrations may be necessary for the treatment of infections caused by more resistant pathogens. Some authors propose a voriconazole plasma concentration greater than 2 µg/mL, especially in infections caused by *Aspergillus* spp. to achieve therapeutic success (129–131). Voriconazole toxicity has also been correlated with voriconazole plasma concentration. Plasma concentrations above 4.5-5.5 µg/mL have been associated with adverse effects such as visual disturbance, rash and hepatotoxicity (128,132,133).

5.2 IMPACT OF TDM IN CLINICAL OUTCOMES

5.2.1 Relation between plasma concentration and efficacy

As previously mentioned, different studies have been carried out to investigate the effect of plasma concentrations of voriconazole on the effectiveness of the therapy (131-140). However, it is important to note that many of these studies are retrospective, small and with a

great heterogeneity in terms of the type of patients included as well as in the method used to measure the results of antifungal treatment efficacy.

An observational analysis of 9 phase II or III studies involving 825 patients treated with voriconazole is noteworthy. These authors analysed the relationship between voriconazole concentration and response, yielding a non-linear relationship between concentration and response that was significant ($p<0.003$). In addition, the best therapeutic results were observed with concentrations between 0.5 and 5 µg/mL, while patients with extreme concentrations (less than 0.5 µg/mL or more than 5 µg/mL) had worse outcomes (87). Comparable results were obtained in other observational study of 52 patients treated with voriconazole. In this study, investigators found that voriconazole trough concentration is a predictor of response, with a higher lack of response in patients with concentrations lower than 1 µg/mL versus patients with higher concentration (46% versus 12%, $p=0.02$) (128). In another prospective study in 29 Japanese patients, voriconazole trough concentrations less than 1.2 µg/mL were related with worse outcomes (132). Similar findings were reported in pediatric patients in the study of Neely *et al*. These authors found a significant relationship between voriconazole trough concentrations less than 2 µg/mL and mortality (134).

5.2.2 Relation between plasma concentration and toxicity

Similar to the evaluation of the relationship between voriconazole concentration and efficacy, the relationship between concentration and toxicity has also been assessed. The most frequently observed voriconazole adverse effects are mild, such as elevated liver function test, visual disturbances and neurological alterations. Some of these adverse events have been associated with greater voriconazole

trough concentrations. Regarding to date, a retrospective study analysed the results of 10 phase II and III voriconazole trials including a total of 2053 patients. The results showed a relationship between voriconazole plasma concentrations and visual impairments (p=0.011) and also a significant relationship between concentration and elevated liver function values of aspartate aminotransferase (AST), alkaline phosphatase (AP) and bilirubin but not alanine aminotransferase (ALT) (141). Comparable findings were published by Trifilio *et al.* who found a correlation between voriconazole trough concentrations and levels of AST and AP but no with ALT or bilirubin (142).

Another adverse effect that has been associated with higher concentrations of voriconazole has been neurological toxicity. The study of Pascual *et al.* involving 96 patients treated with voriconazole demonstrated a relationship between voriconazole concentrations higher than 5.5 µg/mL and neurological adverse events (including confusion, agitation, patterns of toxic encephalopathy on electroencephalography, extrapyramidal signs, myoclonus and visual and auditory hallucinations) (128). Similar to that, a small retrospective study showed that most od the patients with neurologic adverse events had trough concentrations of voriconazole greater than 4 µg/mL being this correlation significant (143).

Authors (year)	Study design	Nº of patients	Nº of serum samples analysed	Population	Indication of therapy	Demostrated voriconazole concentration-response relationship	C_{min} target or therapeutic index proposed (µg/mL)	Ref
Smith *et al.*(2006)	Retrospective	28	28	A,P	T	Yes, relationship between Cmin and efficacy	>2.05	131
Trifilio *et al.* (2007)	Retrospective	71	71	A	P	Yes, relationship between Cmin and efficacy	>2	136
Pascual *et al.*(2008)	Prospective	52	52	A	T, E	Yes, relationship between Cmin and efficacy and toxicity	1-5.5	128
Okuda *et al.* (2008)	Retrospective	23	28	A	T, E	Yes, relationship between Cmin and efficacy and toxicity	1-5	203
Ueda *et al.* (2009)	Prospective	34	49	A	T, E	Yes, relationship between Cmin and efficacy and toxicity	2-6	130
Matsumoto et al. (2009)	Prospective	29	29	A	T,E	Yes, relationship between Cmin and efficacy and toxicity	1.2 -4	132
Neely *et al.* (2010)	Retrospective	46	108	P	T	Yes, relationship between Cmin and efficacy	>1	134
Miyakis *et al.* (2010)	Retrospective	25	147	A	T	Yes, relationship between Cmin and efficacy	>2.2	129
Troke *et al.* (2011)	Retrospective	825	3052	A	T, E	Yes, relationship between Cmin and efficacy	>1	87

Authors (year)	Study design	Nº of patients	Nº of serum samples analysed	Population	Indication of therapy	Demostrated voriconazole concentration-response relationship	C_{min} target or therapeutic index proposed (µg/mL)	Ref
Dolton *et al.* (2012)	Retrospective	163	783	A	T, E	Yes, relationship between Cmin and efficacy and toxicity	1.7 -5	119
Park *et al.*(2012)	Prospective, randomized controlled trial	55	67	A	T	Yes, relationship between Cmin and efficacy and toxicity	2-5.5	124
Soler-Palacin *et al.* (2012)	Prospective	15	68	P	T	Yes, relationship between Cmin and efficacy	1-5.5	135
Gómez-López *et al.* (2012)	Retrospective	14	58	P, A	T	Yes, relationship between Cmin and efficacy	>1	136
Mitsani *et al.* (2012)	Retrospective	93	157	A	P	Yes, relationship between Cmin and efficacy and toxicity	1.5-4	137
Chu *et al.* (2013)	Retrospective	46	46	A	T	Yes, relationship between Cmin and efficacy and toxicity	1 -5.5	138
Choi *et al.*(2013)	Retrospective	20	104	P	T	Yes, relationship between Cmin and efficacy	>1	139
Lee *et al.*(2013)	Retrospective	52	52	A	T	Inconclusive findings	>1	140

Table 9. Summary of studies evaluating the relationship between voriconazole trough concentrations and clinical Efficacy.
A (adults), P (pediatrics), T (treatment), E (empiric), P (prophylactic)

5.3 VORICONAZOLE MESUREMENT IN HUMAN PLASMA

For the pharmacokinetic determination of voriconazole in human samples, there are two important aspects to be discussed: the sample collection process and the analytical method of voriconazole measurement.

5.3.1 Sampling

We must know the precise moment in which the extraction of the biological sample from the patient should be carried out. In addition, we must also know how often this sampling should be performed.

With respect to the first aspect mentioned, the initial sampling should only be performed if the voriconazole steady state has been achieved. Otherwise, the value obtained cannot be properly interpreted. The time required to achieve the voriconazole steady state is variable, depending mainly on the administration of a loading dose. When the patient receives the corresponding loading dose on the first day of treatment, the steady state can be reached in the first 48-72 hours (77). Because of this, clinical guidelines recommend waiting 2-5 days from the start of treatment to perform the first blood extraction (89). This period of time is more than enough when a loading dose of voriconazole is given on the first day as commented, but could be prolonged to 6 days if this is not the case (77). Hence, this explains the need to give the loading dose to all patients.

Subsequently, following determinations should be made until the voriconazole plasma is within the therapeutic range. This usually involves a weekly plasma determination but sometimes more frequent measurements are needed, such as in the case of changes in the

patient's concomitant medication or physiological alterations in the patient (increase in the volume of distribution, changes in drug clearance...), or when toxicity or lack of efficacy is suspected (74,89).

Finally, it should be noted that the ideal time for taking the sample is just before the drug dose ("*pre-dose*"), which in pharmacokinetics is known as C_{min}. This value is best correlated with the AUC, the pharmacokinetic parameter that has been best related to therapeutic response (74).

5.3.2 Drug assay

Using TDM in routine clinical practice irrevocably implies that a validated assay has to be readily available for the measurement of voriconazole plasma levels (58). Different analytical methods have been developed to quantify voriconazole concentration in human plasma or serum including agar well diffusion bioassays, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS) and enzyme immunoassays (EIA)(74). Microbiological bioassays, despite their low cost, are not used in clinical practice due to their low sensitivity and specificity (74). *Table 10* summarizes the main advantages and disadvantages of HPLC, LC-MS and EIA, which are currently the three most common used methods for voriconazole measurement in human samples.

An ideal analytical assay must be sensitive, in order to determine subtherapeutic concentrations of voriconazole and at the same time it must be specific, to avoid interference with other concomitantly drugs. It should also be rapid; the analytical result can be available as soon as possible so that the patient's dosage regimen can be changed if necessary. And finally, it must be simple and affordable. Considering the burden of work in clinical

pharmacokinetic laboratories, having an analytical method that is easily performed is particularly helpful.

	HPLC	LC-MS	EIA
Advantages	- Sensitive and specific - Possibility of simultaneous analysis of multiple drugs - Less expensive equipment than LC-MS	- Highly sensitive and specific - Possibility of simultaneous analysis of multiple drugs - Small sample volume	- Fast results - No need for specific equipment - No need a sample preparation step
Disvantages	- Large sample volume to ensure sensitivity - Subject to interference from multiple substances - Requires a sample preparation step	- Expensive equipment - Requires a high degree od specialty and experience - Not widely available - Requires a sample preparation step	- Low specificity - High cost per determination
References	(144–149)	(150–155)	(156,157)

Table 10. Analytical methods for voriconazole determination in human plasma or serum.

Currently, there is no consensus on the analytical method to be used in the daily practice of clinical pharmacokinetic laboratories. HPLC with a mass spectrometer detector is rarely used, since this equipment is expensive and is not usually available in clinical pharmacokinetic laboratories. Most of these laboratories use HPLC or EIA methods, depending on the availability of chromatographic equipment and the formation of the staff(158).

Finally, when interpreting the analytical result, the type of assay used must be considered and the method must also have been adequately validated following the recommendations established in the European Medicines Agency (EMA) and FDA guidelines for the validation of bioanalytical methods (159,160). To establish a correlation between the different analytical methods is very

interesting, taking into account the great variability in terms of the methodology used depending on the laboratory, as mentioned above.

5.4 FACTORS AFFECTING VORICONAZOLE PHARMACOKINETICS

Numerous interpersonal and intrapersonal factors can influence the voriconazole pharmacokinetics. Among this factors, polymorphisms *CYP2C19* gene are considered by many researchers as the main cause of voriconazole pharmacokinetic variability leading to 30-50% of this variability. But apart from the voriconazole pharmacogenetics (which will be discussed thoroughly in section 6) there are many other factors that can explain the voriconazole pharmacokinetic variability. These factors includes age, drug interactions, renal and hepatic impairment and the patient´s inflammatory status (123).

5.4.1 Age

The influence of age on voriconazole pharmacokinetics has been evaluated in different studies. Differences in plasma concentrations of voriconazole have been observed depending on the age of the patients.

A pharmacokinetic work that analysed data from 10 studies involving a total of 553 patients showed trough plasma concentrations 80-90% higher in elderly patients compared to younger patients, regardless of the type of voriconazole formulation. These data suggest special caution in patients over 65 years of age, monitoring of possible adverse effects and dose adjustments according to TDM if necessary in this population of patients (161). On the contrary, an opposite trend of alteration in plasma voriconazole concentrations seems to apply to the pediatric patients. In this group of patients the elimination of voriconazole is accelerated compared with adults.

Therefore, higher weigh-based doses are needed in pediatric patients to achieve optimal plasma concentrations (162–165). Finally, the case of adolescents is also special. While most adolescents aged 12-17 years have plasma voriconazole concentrations similar to adults, young adolescents aged 12-14 years and with low body weight (<40 kg) behave similarly to children; therefore they should be dosed as pediatric patients (162).

5.4.2 Drug interactions

As we mentioned previously, voriconazole metabolism undergo by the enzymatic isoforms of CYP450, more mainly by CYP2C19 with a lower contribution of CYP2C9 and CYP3A4/5. Since these metabolic pathways are involved in the metabolism of many commonly used drugs, the risk of potential pharmacokinetic interactions is really high.

The strong enzyme-inducing effect of drugs such as phenytoin (112) or rifampicin (119), is well known. The simultaneous administration of this drugs with voriconazole leads to a drastic decrease in plasma drug concentrations and its concomitant use is specifically contraindicated in the data sheet (77).

In addition, when we mention pharmacokinetic drug interactions, we cannot forget the enzyme-inducing effect of certain medicinal plants such as St. John's worth used as a remedy for the treatment of depressive disorders. More specifically, the hyperforin present in this herb has been shown to act as a powerful inducer of voriconazole metabolism and have been found to decrease the AUC by more than 50%. This reduction in voriconazole exposure has clinical relevance, as plasma concentrations of voriconazole could decrease to subtherpaeutic values, and this could lead to the

development of fungal resistance as well as clinical ineffectiveness (166).

In addition inductor drugs, the effect of enzyme inhibitors is also relevant. These drugs, when administered simultaneously with voriconazole, can increase plasma concentrations and cause toxicity at standard doses of the drug. In this sense, the simultaneous administration of potent inhibitors such as ritonavir is contraindicated (167). Special caution is also recommended when performing sequential voriconazole treatment after fluconazole, because of the persisting enzymatic inhibitory effect of the azole during voriconazole treatment. Monitoring of possible adverse effects and voriconazole dose adjustment according to TDM is recommended in these cases (77).

5.4.2.1 Glucocorticoids

Glucocorticoids are widely used drugs that have been shown to affect voriconazole liver metabolism. However, their clinical repercussion is less well known. An *in vitro* study has identified glucocorticoid binding sites in the promoter region of the *CYP2C19* gene and demonstrated up-regulation of *CYP2C19*, supporting and inductive effect of glucocorticoids on *CYP2C19* (168,169). However, the results of *in vivo* studies are contradictory. In a multicentre pharmacokinetic study involving 201 patients, the concomitant administration of dexamethasone was associated with a significant decrease in plasma concentrations of voriconazole (119). These results were reproduced by other authors demonstrating the enzymatic induction of dexamethasone and also methylprednisolone although the last-mentioned drug appears to have a less potent enzyme-inducing effect (170).The enzyme-inducing effect of glucocorticoids has also been observed by discontinuing this treatment in patients treated with voriconazole, resulting in high concentrations of antifungal drug as a

consequence of the withdrawal of the steroid. This effect has been described in various case reports. In both cases, authors conclude that special caution should be taken when starting or stopping steroid treatment in patients on voriconazole treatment and highlight the importance of TDM to avoid toxicity or inefficacy (43,171). However, perhaps due to the heterogeneity of doses and types of glucocorticoids, as mentioned above, there are also studies that have not demonstrated differences in plasma voriconazole concentrations in patients treated with glucocorticoids (120,123).

5.4.2.2 Proton pump inhibitors

A very common group of drugs with an enzyme inhibitory effect that needs to be discussed are proton pump inhibitors (PPIs). PPIs such as omeprazole, pantoprazole, esomeprazole or lanzoprazole are a group of drugs widely used in the prevention and treatment of acid-related gastrointestinal disorders (peptic ulcer, eradication of *Helicobacter pylori*, gastroesophageal reflux disease, etc.) (172). These drugs undergo liver metabolism through CYP2C19 as well as voriconazole, so there is a potential risk of pharmacokinetic interaction at this stage. Several studies have shown an increase in pharmacokinetic exposure to voriconazole in patients simultaneously treated with PPIs due to an enzymatic inhibitory effect of these drugs (119,120,130,170,173–176). In addition, *in vitro* studies have shown differences in the enzymatic inhibitory effect according to the PPI used, suggesting a lesser inhibitory effect of pantoprazole than the other PPIs (177,178). The differences between the enzyme inhibitory effect of pantoprazole and omeprazole have also been evaluated in a study with 78 patients, with significantly higher plasma voriconazole concentrations observed in patients treated with omeprazole than in those treated with pantoprazole (179), highlighting the importance of PPI selection in patients treated with the antifungal voriconazole.

Finally, we must not ignore the fact that voriconazole is a strong inhibitor of CYP3A4 and a weak inhibitor of CYP2C19 and CYP2C19, and that it can increase the serum concentrations of drugs metabolized by these enzymes, such as immunosuppressive drugs (tacrolimus, sirolimus, cyclosporine), macrolide antibiotics, opiates, anticonvulsants like phenytoin, or PPIs, among others (180).

5.4.3 Patient´s inflammatory status

Another factor that has been associated with changes in voriconazole pharmacokinetics is the patient's inflammatory status. In a prospective study, 489 voriconazole plasma samples were analysed and correlated with the inflammatory status of the patient (determined by plasma C-reactive protein (CRP) levels). In this study, higher voriconazole concentrations were observed in patients with a inflammatory response, reflected by high CRP levels, suggesting that voriconazole metabolism decreases as a result of the patient's inflammatory status (181). The same conclusion was reached in another study, this time retrospective, in which the relationship between CRP levels and voriconazole concentrations in 139 plasma samples was analysed (182). Therefore, in patients with a severe inflammatory condition, voriconazole TDM should be performed routinely to optimize treatment.

5.4.4 Renal and/or hepatic impairment

5.4.4.1 Renal insufficiency

Voriconazole pharmacokinetics is not affected by the presence of kidney failure (183,184). However, the intravenous formulation of voriconazole is solubilized in sulfobutyl ether beta-cyclodextrin (SBECD) or hydroxypropyl betadex cyclodextrin (HPBCD). The cyclodextrin vehicle present in this formulation may accumulate in

patients with kidney failure and studies in animals indicate that accumulation of SBECD can result in renal toxicities as a result of massive cytoplasmic vacuolation (185). However, the data available on humans are controversial. Studies have shown accumulation of this excipient in patients with renal failure and in patients undergoing renal replacement techniques but without increasing drug toxicity (183,186,187). Nevertheless, a pharmacokinetic study in critical patients undergoing continuous renal replacement techniques (CRRT) found no accumulation of SBECD (188).

Given the uncertainty in its safety, intravenous voriconazole should be avoided when creatinine clearance is less than 50 ml/min unless an assessment of the risk-benefit balance justifies the intravenous use of voriconazole. In these patients, serum creatinine levels should be carefully monitored and, if an increase occurs, a switch to oral treatment should be considered (77).

5.4.4.2 Hepatic dysfunction

In contrast to renal impairment, the elimination of voriconazole decreases in patients with liver dysfunction (161). It is recommended to use the usual loading dose, but to reducing by half the maintenance dose in patients with mild to moderate liver cirrhosis (Child Pugh A and B). It has not been studied in patients with severe chronic liver cirrhosis (Child-Pugh C), so its use is not recommended in these patients (77).

6

Voriconazole pharmacogenetics

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6 VORICONAZOLE PHARMACOGENETICS

6.1 *CYP2C19* POLYMORPHISM AND THEIR INFLUENCE ON VORICONAZOLE PLASMA CONCENTRATIONS

As previously mentioned, the CYP2C19 isoenzyme is the main responsible for voriconazole metabolism and the *CYP2C19* gene is highly polymorphic. The presence of polymorphisms in *CYP2C19* is the main cause for the pharmacokinetic variability of voriconazole. Therefore, the effect of *CYP2C19* polymorphisms on plasma concentrations of voriconazole has been the subject of several studies (*Table 11*).

The relationship between the *CYP2C19* genotype and voriconazole pharmacokinetics was initially demonstrated in studies conducted in healthy volunteers. In these studies, with a merely pharmacokinetic design, significant differences were already demonstrated in the pharmacokinetic parameters of voriconazole, including clearance (Cl/F), half-life ($t_{1/2}$), C_{min}, and AUC of voriconazole according to the *CYP2C19* genotype (189–192).

Subsequently, the influence of genetics on voriconazole pharmacokinetics was also demonstrated in patients with fungal infections treated with voriconazole (. In a study of 24 Caucasian patients who received voriconazole as prophylaxis or antifungal treatment in the context of a lung transplant, it was shown that carriers of the **17* allele needed higher doses of voriconazole and took longer

to reach concentrations in the therapeutic range compared to normal metabolizing patients (**1/*1*) (193). This study was also the first to demonstrate the impact of pharmacogenetics on the high percentage of patients with subtherapeutic plasma voriconazole concentrations in carries of the **17* allele. Despite the modest number of patients, the results of this study indicate the potential usefulness of the programmed determination of *CYP2C19* polymorphisms in patients undergoing lung transplantation. With a much larger number of patients, Hassan *et al*. analysed in a retrospective study the relationship between plasma voriconazole concentrations and the *CYP2C19* genotype in 335 patients. A high rate of patients with subtherapeutic concentrations in the subgroup of patients carrying the allele *17 were observed (194).

Another group of patients where the initial determination of the genotype not only of *CYP2C19* but also of *CYP3A4* could be interesting are hematological patients undergoing an allogeneic transplant. A retrospective study carried out in France calculated a genetic score based on the polymorphisms of various CYP is enzymes including *CYP2C19*, *CYP3A4* and *CYP3A5*. These authors propose that the combined genetic score could be used to selection of the drug dosage on an individual basis (120).

All these studies are retrospective observational studies and, to date, only two prospective studies analysing the impact of the *CYP2C19* genotype on voriconazole C_{min} in patients with IFI have been published. The first study, published in 2017, analyzed voriconazole C_{min} in 70 adult patients undergoing voriconazole treatment for a proven or probable IFI. They observed a high inter-individual variability in plasma concentrations with a high rate of patients with concentrations outside the therapeutic range. When comparing C_{min} according to the phenotype of *CYP2C19,* they

obtained significantly lower values in RM and UM patients compared to the rest. They found also higher percentage of patients with subtherapeutic concentrations in RM and UM patients (195). Similarly, the most recent study, published in 2019, is a multicenter study that analyzed the influence of genetics in 78 adult patients treated with voriconazole for different causes. A higher percentage of patients with subtherapeutic concentrations in the allele **17* carriers were observed (123).

Comparable results to those discussed for adults similar results have been reported in the pediatric population. In these patients achieving plasma voriconazole concentrations in the therapeutic range is usually a challenge, and requires significantly higher weight-based doses than the adult population (196,199). This group of patients is, therefore, another that would potentially benefit from early or anticipated determination of *CYP2C19* polymorphisms

Authors (year) and reference	Study design	Nº of patients	Population	Relationship between *CYP2C19* and PK	Conclusions	Ref
Wang *et al.* (2009)	Controlled, open-label	20	Healthy Chinese male volunteers	Yes	T1/2, AUC, Cl/F higher in PMs compared with the rest patients ($p<0.005$)	190
Weiss *et al.* (2009)	Controlled, open-label	35	35 healthy, drug- free individuals (32 Caucasian, 2 Asiatic and 1 American)	Yes	AUC differed significantly between CYP2C19 phenotype groups: 3 times greater in PMs vs. EMs ($p < 0.01$); *CYP2C19* genotype accounts for 49% of voriconazole AUC variability after multiple regression analysis ($p<0.0001$).	191
Lee *et al.* (2012)	Controlled, open-label	18	Healthy Korean male	Yes	Mean AUC of IMs and PMs was 1.5 and 3.4 times higher than EMs respectively ($p = 0.002$); mean troughs were 2.8 times higher in IMs than in EMs ($p = 0.005$) and 5.1 times higher in PMs than in EMs ($p = 0.008$)	192
Berge *et al.* (2011)	Retrospective	24	> 15 years Caucasian lung transplant recipients with cystic fibrosis	Yes	Daily doses were significantly higher in **17* carriers (35% more; 14.1 ± 3.9 mg/kg) and EMs (29.6% more; 13.6 ± 3.2 mg/kg) vs. IMs (9.5 ± 1.7 mg/kg) ($p < 0.05$); time to achieve therapeutic range was significantly longer in carriers of *2 and **17*compared with Ems ($p=0.012$). *CYP2C19* polymorphisms accounted for 38% of maintenance dose variability according to multivariate analysis; authors recommended *CYP2C19* genotyping prior to voriconazole therapy initiation to help determine initial dose to promptly achieve therapeutic plasma concentrations without out-of-range troughs	193
Hassan *et al.* (2011)	Retrospective	335	Caucasian patients	Yes	TDM group with low voriconazole concentrations had significantly higher frequency of UMs compared with the control group ($p=0.01$)	194

Authors (year) and reference	Study design	Nº of patients	Population	Relationship between *CYP2C19* and PK	Conclusions	Ref
Narita *et al.*(2013)	Retrospective	37	Japanese pediatric patients	Yes	All patients with troughs > 5 µg/mL were PMs or IMs; troughs were also higher in PMs and IMs compared with EMs and UMs (p=0.004); two UMs had very low concentrations (0.09 and 0.12 µg/mL. Voriconazole plasma concentration in children is significantly correlated with *CYP2C19* phenotype.	199
Wang *et al.* (2014)	Retrospective	144	Adult Asian patients with proven or probable IFI	Yes	Cmin and Cmin/dose were higher in PMs compared with IMs y EMs	200
Gautier *et al.* (2015)	Retrospective	33	Adult hematological Caucasian patients with IFI	Yes	Developed a combined genetic score based on *CYP2C19* and *CYP3A4* as an independent predictor of Cmin (p=0.004)	214
Lamoureux *et al.* (2015)	Retrospective	35	Adult Caucasian patients with proven or probable IFI	Yes	Lower Cmin in patients carrying the *17 allele compared to *1/* 1 (p<0.001). Cmin higher in patients *2 allele compared with *1/* 1 (p<0.001).	212
Chawla *el al.* (2015)	Retrospective	72	Adult Asian patients with IFI	Yes	Cmin higher in patients with the 2 allele compared with the rest	201
Hamadeh *et al.* (2017)	Prospective	70	Adult Caucasian patients with proven or probable IFI	Yes	Cmin significantly lower in UMs and RMs (p=0.0093) and a higher percentage of subtherapeutic concentrations in this group of patients (52% vs 16%, p=0.0028)	195
Blanco-Dorado *et al.* (2019)	Prospective multicentric	78	Adult Caucasian patients with proven or probable IFI	Yes	Cmin lower in RMs and UMs compared with the rest and a higher percentage of subtherapeutic concentrations in this patients	123

Table 11. Summary of the main studies evaluating the association between polymorphisms in *CYP2C19* and plasma concentrations of voriconazole. PK: pharmacokinetics, T1/2 (half-life), AUC(area under curve), Cl/F (clearance), PMs (poor metabolizers), EMs (extensive metabolizers), IMs (intermediate metablizers), RMs (rapid metabolizers), UMs (ultrarapid metabolizers), TDM (therapeutic drug monitoring).

6.2 INFLUENCE OF *CYP2C19* POLYMORPHISMS ON CLINICAL OUTCOMES

Regardless of the existence of a correlation between genetic polymorphisms and plasma concentrations, it is of particular interest to know the influence of voriconazole pharmacogenetics on clinical outcomes, i.e. on efficacy and toxicity. In this regard, we currently have little scientific evidence on this matter and the available results are contradictory.

An observational study conducted in an Asian population observed that despite differences in voriconazole C_{min} between different phenotypes of *CYP2C19*, there were no differences in mortality or voriconazole toxicity not being able to demonstrate the clinical impact of voriconazole pharmacogenetics (197). However, in a subsequent meta-analysis differences in response to treatment were observed, with better responses in PM patients compared to EM (198). This meta-analysis showed that the poor metabolizing patients also had more voriconazole-associated side effects than the rest, although the difference was not significant. The impact of pharmacogenetics on voriconazole toxicity has also not been demonstrated, with contradictory results and without statistically significant results.

In short, the *CYP2C19* genotype has been demonstrated to be a good predictor of voriconazole trough plasma concentrations but there is currently not enough scientific information about the genotype-clinical outcomes relationship. Actually, analyzing the clinical impact of pharmacogenetics, in terms of efficacy and toxicity, is really complex. The first difficulty we meet is the complexity of evaluating clinical efficacy. Voriconazole is a drug with a wide number of indications, including antifungal prophylaxis and empirical treatment of IFI. Efficacy can only be adequately assessed in patients with a

proven or probable diagnosis of IFIs. This definition of IFI is based on criteria published by the European Organisation for Research and Treatment of Cancer (EORTC), which are very strict and are specifically validate for haematological patients (59). Currently there are no published criteria that include new groups of patients at risk of developing IFIs, such as patients with COPD, or patients treated with new-targeted immunomodulatory drugs. With a review and update of these criteria, in the future it will be possible to evaluate properly the efficacy of treatment with voriconazole and therefore be able to assess the real clinical impact of the pharmacogenetics of voriconazole.

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Ethical Considerations

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7 ETHICAL CONSIDERATIONS

This thesis was carried out in the context of a study was approved by the ***Galician Autonomous Committee of Research Ethics (CAEIG)*** on 14 June 2016 with registration code ***2016/319***.

Written informed consent has been obtained from all patients included in the study.

The study has also been approved by the ***Spanish Medicines and Healthcare Products Agency (AEMPS)*** where the work was categorized as a *Prospective Monitoring Post-Authorization Study (EPA-SP)* (study protocol code: **MJL-VOR-2016-01**).

The doctoral student declares ***not to have any conflict of interest*** in relation to the doctoral thesis.

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DICTAMEN DEL COMITÉ AUTONÓMICO DE ÉTICA DE LA INVESTIGACIÓN DE GALICIA

Paula M. López Vázquez, Secretaria del Comité Autonómico de Ética de la Investigación de Galicia

CERTIFICA:

Que este Comité evaluó en su reunión del día 14/06/2016:

> **Título:**Evaluación de eficacia y toxicidad de la terapia antifúngica con voriconazol en base a estudios farmacogenéticos y análisis farmacocinéticosfarmacodinámicos.
> **Promotor**: María Jesús Lamas Díaz
> **Código de Registro:** 2016/319

Y, tomando en consideración las siguientes cuestiones:

- La pertinencia del estudio, teniendo en cuenta el conocimiento disponible, así como los requisitos legales aplicables, y en particular la Ley 14/2007, de investigación biomédica, el Real Decreto 1716/2011, de 18 de noviembre, por el que se establecen los requisitos básicos de autorización y funcionamiento de los biobancos con fines de investigación biomédica y del tratamiento de las muestras biológicas de origen humano, y se regula el funcionamiento y organización del Registro Nacional de Biobancos para investigación biomédica, la ORDEN SAS/3470/2009, de 16 de diciembre, por la que se publican las Directrices sobre estudios Posautorización de Tipo Observacional para medicamentos de uso humano, y la Circular nº 07 / 2004, investigaciones clínicas con productos sanitarios.
- La idoneidad del protocolo en relación con los objetivos del estudio, justificación de los riesgos y molestias previsibles para el sujeto, así como los beneficios esperados.
- Los principios éticos de la Declaración de Helsinki vigente.
- Los Procedimientos Normalizados de Trabajo del CEIC de Galicia

Emite un **INFORME FAVORABLE** para la realización del estudio por el/la investigador/a del centro:

Centros	Investigadores Principales
C.H. Universitario de Santiago	María Jesús Lamas Díaz

NOTA: remitir la clasificación de la AEMPS en cuanto la tengan disponible.

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Y HACE CONSTAR QUE:

1 El CAEIG cumple los requisitos legales vigentes (R.D 1090/2015 y la Ley 14/2007)
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Susana María Romero Yuste. Médico Especialista en Reumatología.
Mª Asunción Verdejo González. Médico Especialista en Farmacología Clínica.

En Santiago de Compostela, a 16 de junio de 2016

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Contra la presente resolución que pone fin a la vía administrativa podrá interponerse Recurso Potestativo de Reposición, ante la Directora de la Agencia, en el plazo de un mes a contar desde el día siguiente a aquel en que tenga lugar la notificación de la presente resolución. [5]

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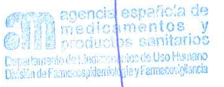

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[1] Son de aplicación al presente procedimiento la Ley 30/1992, de 26 de noviembre, de Régimen Jurídico de las Administraciones Públicas y del Procedimiento Administrativo Común; la Ley 14/2000, de 29 de diciembre, de medidas fiscales, administrativas y de orden social; Real Decreto Legislativo 1/2015, de 24 de julio, por el que se aprueba el texto refundido de la Ley de garantías y uso racional de los medicamentos y productos sanitarios; Real Decreto 1090/2015, de 4 de diciembre, por el que se regulan los ensayos clínicos con medicamentos, los Comités de Ética de la Investigación con medicamentos y el Registro Español de Estudios Clínicos; el Real Decreto 1275/2011, de 16 de septiembre, por el que se crea la Agencia estatal "Agencia Española de Medicamentos y Productos Sanitarios" y se aprueba su estatuto; el Real Decreto 577/2013, de 26 de julio, por el que se regula la farmacovigilancia de medicamentos de uso humano y la Orden SAS/3470/2009, de 16 de diciembre, por la que se publican las directrices sobre estudios posautorización de tipo observacional para medicamentos de uso humano.

[2] De acuerdo con la Orden SAS/3470/2009, de 16 de diciembre.

[3] Directorio disponible en la página web de la AEMPS (http://www.aemps.es/actividad/invClinica/estudiosPostautorizacion.htm)

[4] En el caso de que el promotor no sea quien presente la documentación, se deberá incluir en la misma un documento que indique las responsabilidades delegadas por el promotor a la persona o empresa que actúa en su nombre.

[5] De conformidad con lo dispuesto en los artículos 116 y 117 de la Ley 30/1992, de 26 de noviembre, o Recurso Contencioso-Administrativo ante el Juzgado Central de lo Contencioso-Administrativo de Madrid, en el plazo de dos meses contados desde el día siguiente al de la notificación de la presente resolución, de conformidad con la Ley 29/1998, de 13 de Julio, reguladora de la Jurisdicción Contencioso-Administrativa, sin perjuicio de poder ejercitar cualquier otro recurso que se estime oportuno. En caso de interponerse recurso de reposición no podrá interponerse recurso contencioso-administrativo hasta la resolución expresa o presunta del primero.

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Fecha: 6 de junio de 2016

REFERENCIA: ESTUDIO FUNGUTOX-VORI

ASUNTO: NOTIFICACIÓN DE RESOLUCION DE CLASIFICACIÓN DE ESTUDIO CLÍNICO O EPIDEMIOLÓGICO

Adjunto se remite resolución de clasificación sobre el estudio titulado **"Evaluación de eficacia y toxicidad de la terapia antifúngica con voriconazol en base a estudios farmacogenéticos y análisis farmacocinéticos-farmacodinámicos."** con código **MJL-VOR-2016-01**

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ASUNTO: **RESOLUCIÓN DEL PROCEDIMIENTO DE CLASIFICACIÓN DE ESTUDIO CLÍNICO O EPIDEMIOLÓGICO**

DESTINATARIO: **Dª MARÍA JESÚS LAMAS DÍAZ**

Vista la solicitud-propuesta formulada con fecha **3 de junio de 2016**, por **Dª MARÍA JESÚS LAMAS DÍAZ,** para la clasificación del estudio titulado **"Evaluación de eficacia y toxicidad de la terapia antifúngica con voriconazol en base a estudios farmacogenéticos y análisis farmacocinéticos-farmacodinámicos."** con código **MJL-VOR-2016-01** y cuyo promotor es **Dª MARÍA JESÚS LAMAS DÍAZ.,** se emite resolución.

El Departamento de Medicamentos de Uso Humano de la Agencia Española de Medicamentos y Productos Sanitarios (AEMPS), de conformidad con los preceptos aplicables, (1) **RESUELVE** clasificar el estudio citado anteriormente como ***"Estudio Posautorización de seguimiento prospectivo*** (abreviado como EPA-SP).

El promotor del estudio deberá remitir solicitud de autorización del mismo (2) a todas aquellas Comunidades Autónomas en las que se pretenda llevar a cabo, incluyendo la siguiente documentación (una copia en papel y otra en formato electrónico) y enviando una copia de la misma (papel y formato electrónico) a la AEMPS en el momento de la primera solicitud de autorización:

- Carta de presentación dirigida a los responsables de esta materia en la Comunidad Autónoma(3) en la que se solicite la autorización del estudio e indique la dirección y contacto del solicitante y la relación de documentos que se incluyen(4).
- Resolución de la AEMPS sobre la clasificación del estudio
- Protocolo completo, incluidos los anexos, y donde conste el número de pacientes que se pretenden incluir en España, desglosado por Comunidad Autónoma.
- Dictamen favorable del estudio por un CEIC acreditado en España.
- Listado de Centros Sanitarios donde se pretende realizar el estudio, desglosado por Comunidad Autónoma
- Listado de investigadores participantes en la Comunidad Autónoma.
- Si el estudio se pretende realizar en otros países, situación del mismo en éstos
- Documento acreditativo de haber satisfecho las tasas correspondientes, en aquellas CC.AA. donde se exijan.

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8

Aim of this thesis

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8 AIM OF THIS THESIS

8.1 STATEMENT OF THE PROBLEM

IFIs are a high morbidity and mortality infection diseases with a great socioeconomic impact. The incidence of IFIs has increased worryingly in the past few years even in patients without any of the classic risk factors for fungal infections. Simultaneously, with the introduction of new targeted, biological, and cellular therapies in patients with onco-hematologic malignancies a new population at risk of invasive fungal infection has appeared.

Candida spp. infections continue to be the most frequent type of fungal infection. Candidemia has an important health impact, especially in critical patients admitted to the Intensive Care Units where invasive candidiasis has high incidence and mortality. In the last few years, however, there has been a notable shift in the etiology of candidiasis with *non-albicans Candida* species gaining prominence. Filamentous fungal infections, much less frequent, are however extremely serious infections and a really important problem in some patients, such as haematological patients undergoing a hematopoietic stem cell transplant. In these cases, the elevated mortality of these infections implies that antifungal prophylaxis and treatment should be performed as optimally as possible to avoid therapeutic failure. In this context, voriconazole continues to be the drug of choice for the treatment of aspergillosis invasive.

Voriconazole is an antifungal drug belonging to the triazole antifungals. Available in oral and intravenous formulation, it has an acceptable safety profile and it is effective in the treatment of invasive aspergillosis and infections caused by *Candida*. However, this drug has a high inter and intra individual variability and narrow therapeutic index, making difficult its management. The pharmacokinetic variability of voriconazole is mainly due to its hepatic metabolism. Voriconazole is metabolized in the liver by the cytochrome CYP3A4 isoenzyme, mainly through the CYP2C19. The gene encoding the CYP2C19 isoenzyme is highly polymorphic, which explains much of the interindividual variability of voriconazole pharmacokinetics.

TDM of voriconazole has proven to be a useful tool in clinical practice providing therapeutic success. However, TDM implementation in routine clinical practice is not yet fully established. The analytical method used for the determination of voriconazole plasma voriconazole has also not been standardized. Finally, although several studies have demonstrated the relationship between plasma voriconazole concentrations and clinical outcomes, the optimal target therapeutic index remains to be clarified.

Pharmacogenetics is a branch of personalized medicine that has gained importance in recent years. Due to the genetic polymorphisms in *CYP2C19*, the main enzyme responsible for voriconazole metabolism, the use of pharmacogenetics has been considered as a complementary tool to pharmacokinetics, allowing different doses to be selected based on the genetic condition of individuals. However, the clinical impact of pharmacogenetics on antifungal treatment with voriconazole has not been demonstrated sufficiently to implement the pharmacogenetics of voriconazole in routine clinical practice.

Finally, other causes of pharmacokinetic variability of voriconazole are drug interactions. Voriconazole data sheet contains some drugs whose concomitant administration with voriconazole is contraindicated due to its strong inducing or inhibiting effect of CYP450. However, the effect of other commonly used drugs like corticosteroids or proton pump inhibitors on plasma concentrations of voriconazole is not clear. Knowing the impact of voriconazole drug interactions on voriconazole plasma concentrations remains essential to make recommendations in selecting the best concomitant treatment of patients treated with voriconazole.

In summary, despite having different classes of antifungals in the market and new molecules in clinical research, improving the treatment of existing antifungals is essential. Voriconazole continues to be the drug of choice in the treatment of the serious infection caused by *Aspergillus* spp.. Therefore, having tools that allow optimizing the antifungal therapy with voriconazole can ben our best ally in the treatment of invasive fungal infections.

8.2 GENERAL AIM

The general aim of this thesis was to evaluate the use of voriconazole in daily clinical practice and to investigate the impact of *CYP2C19* genotype and drug-drug interactions on voriconazole plasma concentrations.

8.3 SPECIFIC AIMS

i. To evaluate the use of voriconazole in daily clinical practice in adult patients not undergoing TDM.

   a. To investigate the treatment characteristics or voriconazole treatment: indication, route of administration, dose and duration.
   b. To investigate the patient characteristics: main underlying disease and risk factors of IFI.
   c. To investigate the potential pharmacokinetics drug interactions with concomitant medications.

ii. To develop and validate an HPLC-UV method for measuring voriconazole in human plasma samples.
   a. To perform a comparison of the HPLC-UV method with and ARK^{TM} Immunoassay in order to evaluate the agreement between both methods.

iii. To analyse plasma concentrations of voriconazole in acute patients treated with voriconazole and the percentage of patients with concentrations outside the optimal therapeutic range.

iv. To investigate the impact of genotype on voriconazole pharmacokinetics.
   a. To evaluate the frequency of the different polymorphism of *CYP2C19*, *CYP3A4*, *CYP3A5*, and *CYP2C9* in patients treated with voriconazole.
   b. To study the influence of polymorphism of *CYP2C19* on voriconazole plasma concentrations.

v. To evaluate the impact of drug interactions on voriconazole pharmacokinetics.
   a. To analyse the relationship of different PPIs and voriconazole plasma concentrations.
   b. To analyse the influence of glucocorticoids on voriconazole plasma concentrations.

9

Cohesion of this thesis

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9 COHESION OF THIS THESIS

The cohesion of this thesis comes from the development of a method for the pharmacokinetic determinations of voriconazole and its practical implementation in patients with invasive fungal infections treated with this antifungal.

Until the development of this work, patients with invasive fungal infections treated with voriconazol were no subjected to TDM of voriconazol in our hospital. In these patients, the dose of voriconazole was usually the standard dose included in the drug's data sheet. The same dosage recommendation was used for all patients, without any type of individualization. In the face of evidence that patients treated with voriconazole did not obtain the expected therapeutic results, with high rates of therapeutic failure, we propose a first retrospective study to know the efficacy and safety of voriconazole in the real-life clinical setting. In this study we evaluate outcomes, safety, drug interactions and characteristics of voriconazole treatment demonstrating the wide variety strategies in the voriconazole using and the large number of drugs susceptible to pharmacokinetic interactions. This initial study, which served as the starting point for this thesis, is included in the ***chapter 9***.

This retrospective study reinforces the need to implement TDM of voriconazole in order to optimize the antifungal therapy. Because of that, we developed a chromatographic analytical method for the voriconazole pharmacokinetic measurement in human plasma samples. This method was validated and compared with an

immunoassay method used in other pharmacokinetic laboratories. The detailed methodology and the results of this work are collected in ***chapter 10*** of this thesis.

Once we had an analytical method for the pharmacokinetic determination of voriconazole, we set out to evaluate the factors that explain the pharmacokinetic variability. In this sense, we developed a work that focused on how genetics affects voriconazole treatment. For that purpose, a prospective and multicentre study was carried out to evaluate the impact of polymorphisms in the genes involved in the metabolism of voriconazole on the pharmacokinetics of voriconazole. The methodology and results of this study are detailed in ***chapter 11*** of this thesis.

Finally, another aspect of special interest in the pharmacokinetic variability of voriconazole is the presence of drug-drug interactions. Therefore, we developed two works focused on this aspect. In the first, we focused on the effect of proton pump inhibitors on voriconazole plasma concentrations, analysing the differences between pantoprazole and omeprazole, the two most commonly used IBPs in our setting. The details of this work are included in the ***chapter 12*** of this work**.** The second one, a case report included in the ***chapter 13***, show the effect of glucocorticoids on the pharmacokinetics of voriconazole.

10
An observational study of the efficacy and safety of voriconazole in a real-life clinical setting

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Capitulo 10

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11
Therapeutic drug monitoring of voriconazole: validation of a high performance liquid chromatography method and comparison with an ARK™ inmmunoassay

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Therapeutic drug monitoring of voriconazole: validation of an ultra-high performance liquid chromatography method and comparison with an ARK immunoassay

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ABSTRACT

Objective Voriconazole is an antifungal agent used in the treatment of aspergillosis and fluconazole-resistant *Candida* infections. Therapeutic drug monitoring (TDM) of voriconazole is recommended to optimise clinical results. The aim of this study was the development and validation of a high performance liquid chromatography (HPLC) method for measuring voriconazole in human serum, and comparison with an ARK immunoassay method.

Methods Linearity, precision, accuracy and stability of the HPLC method were validated according to the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines. The method was applied to the analysis of 58 trough serum samples from patients treated with voriconazole, and the HPLC-UV (ultraviolet) method was compared with an ARK immunoassay. The correlation of both methods was studied by the Pearson regression coefficient and the concordance of the values was evaluated by the Bland-Altman and Passing-Bablok methods.

Results All validation parameters met the criteria set out in the FDA and EMA guidelines. The standard curve was linear over a concentration range of 0.25–16 µg/mL with a limit of quantification of 0.125 µg/mL. No interactions between voriconazole and other drugs was observed and voriconazole was stable after 1 month at −80°C. Comparison of the HPLC method and the enzyme immunoassay method showed a linear correlation with a systematic error of −0.61 µg/mL between both methods.

Conclusion The method developed is simple and fast and can be easily applied for routine therapeutic drug monitoring of voriconazole. The HPLC-UV method was more sensitive than the immunoassay method and there was concordance with the immunoassay. Consequently both methods could be used, considering the correlation between them.

INTRODUCTION

Voriconazole is a triazole antifungal agent with potent broad-spectrum activity. It is currently recommended as a first-line agent for the treatment of invasive aspergillosis, fluconazole-resistant serious invasive *Candida* species infections, and infections caused by emerging pathogens, such as *Fusarium* and *Scedosporium*.[1–4] Voriconazole has a narrow therapeutic index, large interpatient variability and non-linear pharmacokinetics. Several studies have found a relationship between trough plasma concentrations (Cmin) and clinical response in terms of efficacy as well as toxicity. Poor treatment outcomes have been reported in patients with Cmin <1 mg/L,[5 6] concentrations >2 mg/L have been related to optimal responses,[7–9] and Cmin >4.5–5.5 mg/L have been associated with adverse events such as vision disturbances, rash and hepatotoxicity.[5 10 11] In this context, therapeutic drug monitoring (TDM) represents a useful tool to optimise dosing regimens and clinical outcome.[12 13]

Several factors contribute to the wide intervariability of voriconazole. These include genetic polymorphism in the *CYPC2C19* gene, drug–drug interactions and patient characteristics such as age, weight and liver function.[14 15] The large variability in voriconazole plasma concentrations together with the narrow therapeutic window make individualised dosing adjustments based on TDM necessary to optimise therapeutic response and to minimise the probability of toxicity.[16]

Different methods, including agar well diffusion bioassays, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS) and enzyme immunoassays (EIA) have been described for the determination of voriconazole in biological fluids. Table 1 shows the advantages and disadvantages of most widely used methods in clinical pharmacokinetic laboratories.

Currently, in clinical pharmacokinetic laboratories there is no standardisation regarding the method used for plasma voriconazole determination. The choice depends on the availability of equipment, reagents and staff experience. HPLC and EIA are the methods most commonly used. Given the recommendation to perform voriconazole TDM in clinical practice, it is essential to have chromatographic and immunoassay methods suitable for pharmacokinetic determination in clinical practice as well as for evaluating the relationship between both methods.

We therefore conducted a study to develop and validate an HPLC-UV (ultraviolet) method for measuring voriconazole in human plasma samples and to perform a comparison with an ARK

Table 1 Methods for measuring voriconazole in human plasma

	HPLC	LC-MS	EIA
Advantages	► Sensitive and specific ► Possibility of simultaneous analysis of multiple drugs ► Less expensive equipment than LC-MS	► Highly sensitive and specific ► Possibility of simultaneous analysis of multiple drugs ► Small sample volume	► Fast results ► No need for specific equipment ► No need for a sample preparation step
Disadvantages	► Large sample volume to ensure sensitivity ► Subject to interference from multiple substances ► Requires a sample preparation step	► Expensive equipment ► Requires a high degree of specialty and experience ► Not widely available ► Requires a sample preparation step	► Low specificity ► High cost per determination
References	25–30	31–36	23 24

EIA, enzyme immunoassays; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry.

immunoassay in order to evaluate the agreement between both methods.

MATERIALS AND METHODS

Development and validation of an HPLC-UV method for measuring voriconazole in human plasma

Voriconazole pure drug substance was kindly supplied by Pfizer (Pfizer SA, Madrid, Spain). HPLC grade acetonitrile was purchased from Panreac Química SAV (Castellar del Vallés, Spain). HPLC water from Millipore's Milli-Q System was used throughout the analysis. Stock solutions of voriconazole were prepared in dimethyl sulfoxide (DMSO) (Sigma–Aldrich Química). Drug-free human serum from healthy donors was supplied by the blood bank department.

The HPLC system consisted of an Agilent 1260 series HPLC system (Agilent Technologies, USA) equipped with Diode Array Detector HS, a solvent delivery quaternary pump system, maximum pressure 400 bar and an autosampler with thermostat. The software model OpenLAB CDS 3D UV (PDA) was used for the data processing. The mobile phase consisted of a filtered and degassed mix of acetonitrile: ultrapure water (60:40, v/v). Chromatographic and detection conditions are described in table 2.

Stock solutions of voriconazole were prepared for the calibration standards (CS) and quality control (QC) samples, respectively, by dissolving 40 mg of voriconazole in 25 mL of DMSO for each solution. Using this solution we prepared eight CS at final concentrations of 0.125, 0.25, 0.50, 1, 2, 4, 8 and 16 µg/mL by spiking the appropriate amounts of the voriconazole stock solution into drug-free human serum.

Both the CS and the samples of our patients were processed in the same way. Protein precipitation was performed by the addition of acetonitrile in a 1 to 1 ratio. The final mixture was then shaken for 30 s on a Vortex Shaker at maximum speed followed by centrifugation at 13 800 *g* at 25°C for 15 min.

Table 2 Chromatographic and instrumental conditions

Instrumental parameters	Conditions
Elution mode	Isocratic
Flow rate	0.8 mL/min
Volume of injection	50 µL
Wave length detection	255 nm
Column	SunFire C18 5 µm 4.6×150 mm
Guard column	SunFire C18 5 µm 4.6×20 mm
Temperature of the column	25°C
Temperature of the autosampler	25°C
Pressure of the system	900–1200 psi
Retention time voriconazole	3.20 min

For method comparison, the results were evaluated with the ARK Voriconazole Assay (ARK Diagnostics, Inc, Fremont, CA, USA) that is based on competition between the drug in the specimen and voriconazole labelled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. On binding to the antibody, G6PDH enzyme activity decreases. However, in the presence of the drug from the specimen, enzyme activity increases in a manner that is directly proportional to the drug concentration. Active enzyme converts the cofactor nicotinamide adenine dinucleotide (NAD) to NAD + hydrogen (NADH) that is measured spectrophotometrically at 340 nm. For this study, the ARK Voriconazole Assay was applied to an Architect c4000 clinical chemistry analyzer (Abbot Diagnostics, Illinois, USA), fully automated platform with random access (800 test/hour). CS and QC provided with the kit by ARK comprised a synthetic protein matrix with the following concentrations: 0.0, 0.5, 1.5, 4.0, 8.00 and 16.0 µg/mL (CS) and 1.0, 5.0 and 10.0 µg/mL (QS). CS and patient samples were centrifuged (1500 *g* for 10 min at 4°C) before analysis.

Validation procedure of the HPLC-UV method

The linearity, precision and accuracy of the assay were validated according to US Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines.[17 18]

Linearity was evaluated by analysing standard voriconazole solutions in the range of 0.125–16.0 µg/mL. Experiments were performed in triplicate and on two non-consecutive days. Linearity was assessed by linear regression.

Intra-/inter-day precision and accuracy of the analytical method were evaluated by triplicate processing and analysis of seven CS samples (0.25, 0.5, 1, 2, 4, 8 and 16 µg/mL). Precision was expressed as coefficient of variation, calculated as CV%=(-standard deviation/mean of measured values)×100; whereas accuracy was expressed as a percentage of the relative error, determined with the formula RE%=([mean measured concentration−nominal concentration]/nominal concentration)×100. Criteria for acceptability of data included accuracy within ±15% deviation from the nominal values and precision within ±15% of CV%, except for the lower limit of quantification (LLOQ), for which values should not exceed 20% of CV%.

Limit of detection (LOD) is defined as the concentration at which the analyte can be distinguished from background signal. This was determined by measuring the peak area that was greater than or equal to the average of the blanks +3 SD. The limit of quantification (LOQ) was defined as the analyte response that was at least five times the response of a blank sample and whose precision and accuracy were within 20%.

Stability of voriconazole was tested in low (0.5 µg/mL), medium (2 µg/mL) and high (4.0 µg/mL) concentrations, after

long-term storage (freeze for 1 month at −80°C). Percentage deviations of measured peak areas were compared with those obtained at the beginning of the study.

Clinical application and comparison with an ARK immunoassay method

The previously described method was applied to the analysis of 58 trough serum samples from patients treated with voriconazole. Using these 58 human samples, the HPLC method developed was compared with the routinely used ARK immunoassay carried out at the General University Hospital Castellón.

Blood samples of voriconazole were collected at steady state just before the next dose of voriconazole (trough level). After centrifugation (1500 *g* for 10 min at 4°C), the serum was further aliquoted into two polypropylene Eppendorf tubes and then stored at −80°C until analysis.

Correlation between HPLC and immunoassay methods

The correlation or linearity of both methods was studied by the Pearson regression coefficient. Subsequently, concordance of the values obtained with the two methods was evaluated by the Bland-Altman method, which graphically represents the differences of the analysed concentrations with respect to their mean value. Finally, the concordance was also evaluated by the Passing-Bablok method, the coefficient of concordance of Lin (rc) was estimated and deviations from linearity were determined by the CUSUM test. Results were expressed as the median and IQR; a statistical study was performed using the Mann-Whitney U test for quantitative tests using the STATA/IC-14.1 program.

Patient care relied solely on the ARK method. The study was approved by the local ethics committee and written informed consent was obtained from each participant.

RESULTS

Development and validation of an HPLC-UV method for measuring voriconazole in human plasma

The standard curve was linear over a concentration range of 0.25–16.0 µg/mL for voriconazole, with a correlation coefficient of 0.9999 (figure 1).

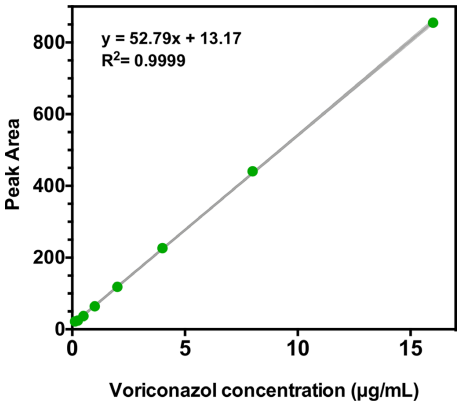

Figure 1

Table 3 Intra-day and inter-day precision (expressed as coefficient of variation, CV%) and accuracy (expressed as mean percentage relative error, RE%) of determined voriconazole concentrations in serum quality controls

Cnom (µg/ml)	C (µg/ml) (mean±SD)	Precision (CV%)	Accuracy (RE%)
Intra-assay (within run) (n=3)			
0.25	0.22±0.04	8.96	−12.12
0.5	0.46±0.04	6.09	−8.04
1	0.97±0.09	6.47	−2.54
2	2.01±0.17	6.51	0.15
4	4.04±0.25	5.19	1.09
8	8.10±0.71	7.39	1.27
16	15.94±0.99	5.31	−0.38
Inter-assay (between run) (n=6)			
0.25	0.28±0.12	10.24	−0.58
0.5	0.56±0.19	6.92	1.90
1	1.05±0.16	12.53	2.86
2	2.08±0.21	9.01	3.96
4	4.11±0.26	5.91	4.68
8	8.15±0.58	7.02	11.03
16	15.90±0.80	5.04	11.13

The LLOQ and LOD of voriconazole was determined to be 0.25 µg/mL and 0.125 µg/mL, respectively. The precision and accuracy ranged from 5.19% to 8.96% and from −13.12% to 8.04%, respectively. The intra-day precision and inter-day precision for QC samples were <15%. The method also showed accuracy within 15%. The CV% was within the acceptable limits stated for bioanalytical method validation (table 3).

Voriconazole-free serum samples did not show any interference with the signal. No interactions between voriconazole and matrix components were detected. In addition, when analysing patient samples, we did not observe any chromatographic interference with other drugs used in our patients.

Post-preparation stability testing showed a slight loss of stability after long-term storage at −80°C for 1 month in the lowest voriconazole concentrations, although in no case did the difference exceed 20% as proposed by the international guidelines (table 4).

Clinical application and comparison with an ARK immunoassay method

The suitability of this analytical method to determine voriconazole concentration was investigated using a total of 58 trough serum samples. No concentrations below LLOQ (<0.25 µg/mL) were observed in our patients. Concentrations above 5.5 µg/mL (associated with potential toxic effects) were observed in 8.62% (5 out of 58) of samples. A high percentage of samples analysed (25.86%, 15 out of 58) showed concentrations <1 µg/mL, which corresponded to the minimal therapeutic targets for voriconazole proposed by Pascual *et al*.[5]

Table 4 Stability of voriconazole after 1 month long-term storage at −80°C

Cnom (µg/ml)	Peak area 1	Peak area 2	Peak area difference (%)
0.5	37	43	83
2	118	124	94
4	226	245	91

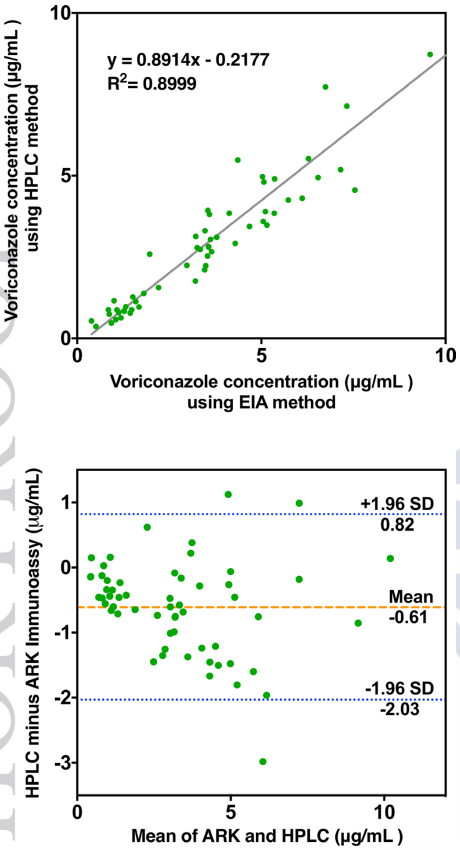

Figure 2

Correlation between the HPLC and ARK immunoassay method

These 58 serum samples were determined with both methods, obtaining a median concentration of 2.80 µg/mL (IQR 0.97–4.01) and of 3.52 µg/mL (95% CI 1.47–5.08) (p=0.082) in the determinations with HPLC and ARK immunoassay, respectively.

Ccomparison of the HPLC and ARK immunoassay methods showed a significant linear correlation (Pearson's r: 0.90, p<0.0001). Passing-Bablok regression showed an intercept of −0.22 (95% CI −0.45 to −0.03) and a slope of 0.89 (95% CI 0.77–0.97), with a substantial agreement between both methods, as indicated by a concordance correlation coefficient of 0.91. The Bland-Altman plot revealed a small systematic error of −0.61 µg/mL (95% CI −2.03 to −0.82) between both methods. The CUSUM test was indicative of non-significant linearity deviation (p>0.20) (figure 2A,B).

DISCUSSION

Therapeutic failure of invasive fungal infections is potentially life threatening. Achieving optimal voriconazole concentrations is essential to achieve clinical success. In this study we found a high percentage of patients with infra-therapeutic concentrations, similar to previous studies.[6] We also found a high interpatient variability similar to other publications.[19 20] The importance of TDM of antifungal agents is recognised by clinical guidelines, and accumulating evidence supports TDM for voriconazole, making it necessary to have adequate analytical methods for voriconazole measurement.[2 21 22]

A simple and fast HPLC-UV method was developed and validated to quantify voriconazole in patient samples using a small volume of human serum and providing information for clinical decisions in less than 1 hour. The mobile phase is a mixture of water and acetonitrile without buffers or gradient separation. Therefore, the method is considered easy and simple. The method has met the validation criteria of regulatory agencies (EMA and FDA) but has also been validated by a comparison with an ARK immunoassay method used for the routine determination of voriconazole in a pharmacokinetic laboratory.

The ARK immunoassay consists of convenient, liquid-stable, ready-to-use reagents for homogeneous enzyme immunoassays, which makes it very attractive for pharmacokinetic determinations. Two previous studies have validated this method for voriconazole determination in human samples. These methods showed good performance in the concentration range between 1 and 5.5 µg/mL. However, our HPLC method guarantees higher sensitivity (LLOQ=0.25 µg/mL), so it can be applied for the determination of both therapeutic and prophylactic low concentrations. For this reason, we consider HPLC as the method of choice for the pharmacokinetic determination of voriconazole whenever it is available.

Despite the advantages of chromatographic methods, many clinical pharmacokinetic laboratories employ immunoassay techniques to determine plasma concentrations of voriconazole. Being able to correlate the results obtained between both techniques is very useful due to the variability in the methods of determination according to the laboratory, as mentioned above. We made a method comparison between the previously developed and validated HPLC method and an ARK immunoassay method used routinely. This comparison showed a significant linear correlation and a slight overestimation in the determination of voriconazole by the ARK immunoassay method compared with HPLC, but with a relationship between both methods that was linear and constant.

To our knowledge, there are only two previous studies that have made a comparison between chromatographic and EIA methods used for voriconazole determination. The first one is a validation of an ARK immunoassay method and comparison with an ultra-HPLC with photodiode array detention (UPLC).[23] In this study a linear correlation was observed between both methods, with a concordance correlation coefficient of 0.96. Similar to our study, the Bland-Altman plot also revealed a slight overestimation of the EIA over the UPLC with a systematic error of −0.29 mg/L. The second study is a validation of an ARK immunoassay method and comparison with liquid chromatography-tandem mass spectrometry (LC-MS/MS).[24] In this second study a good correlation was also observed, with a correlation coefficient of 0.98 between both methods.

There is no study that compares ARK immunoassay and conventional HPLC. Having this comparison is very useful when considering the availability of UPLC or mass spectrometry is unusual in clinical pharmacokinetic laboratories.

This study shows the existence of concordance between simple HPLC-UV and ARK immunoassay in the determination of voriconazole in patient serum samples. Like the results of these

previous studies, voriconazole concentrations determined by HPLC or immunoassay could be used indistinctly, considering the correlation between the two methods. Clinical laboratories and hospitals that use one of these two techniques for the pharmacokinetic monitoring of voriconazole can use this information in their clinical practice. However, it should be taken into account that the analytical method must be correctly validated according to international guidelines, and we must also consider the limit of quantification of the method when making analytical interpretations.

In conclusion, having a sensitive and specific method for the determination of voriconazole is essential, since TDM is recommended to guarantee optimal antifungal therapy. This chromatographic method can be applied easily for routine TDM in pharmacokinetic laboratories. This simple and fast method can be offered to physicians to optimise drug dosage and improve clinical results.

What this paper adds

What is already known on this subject?

- EIA (enzyme immunoassays) or HPLC-UV (high-performance liquid chromatography-ultraviolet) are two analytical methods used by laboratories for the pharmacokinetic determination of voriconazole. The use of one method or the other depends on the availability of the equipment and the experience of the personnel.

What this study adds?

- In this study, we developed and validated a simple HPLC-UV method for measuring voriconazole in human serum samples and propose a correlation with an ARK immunoassay.

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12
Impact of *CYP2C19* genotype and drug interactions on voriconazole plasma concentrations: a Spain pharmacogenetic-pharmacokinetic prospective multicenter study

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Capitulo 12

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13
A multicenter prospective study evaluation the impact of Proton Pump Inhibitors omeprazole and pantoprazole on voriconazole plasma concentrations

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Capitulo 13

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14
Voriconazole hepatotoxicity as a result of steroid withdrawal in a patient with allergic bronchopulmonar aspergillosis

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15

Discussion

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15 DISCUSSION

Voriconazole is an antifungal drug with crucial role in the management of invasive fungal infections. This drug has a broad-spectrum antifungal activity and is effective in the treatment of different fungal infections including aspergillosis, candidiasis and infections caused by emerging pathogens such as *Fusarium* and *Scedosporoum* (38). Voriconazole has non-linear pharmacokinetic, narrow therapeutic index and high variability in plasma concentrations both within and between individuals. Several factors such as its saturable hepatic clearance, age, *CYP2C19* genetic polymorphism, drug-drug interactions, inflammation or hepatic dysfunction can influence voriconazole plasma concentrations (119,123). In view of this unpredictability of plasma concentrations, TDM of voriconazole has been proposed as a tool to guide antifungal therapy. In addition, *CYP2C19* genotype has been demonstrated to influence voriconazole trough concentrations and appears to be an excellent complement to pharmacokinetics (17).

As a first part of this thesis, we carried out an observational retrospective study to evaluate clinical outcomes in adult patients hospitalized who received systemic treatment with voriconazole without TDM. The main contribution of this study was the assessment of the voriconazole use in real clinical practice. In fact, only a few studies assessed real-life data of voriconazole therapy after the commercialization of this drug in 2002 (94,199,203). In this first investigation we could observed that voriconazole was used for a wide variety of clinical indications, not only for IA. Apart from this

indication, other reasons for starting treatment with voriconazole were observed, such as fluconazole-resistant *Candida* infections, non-invasive *Aspergillus* spp. infections (like CPA or ABPA), as well as the prophylactic use of voriconazole.

Another interesting result observed in this first study was the low percentage of patients with proven or probable IA. This seems mainly due the complexity on the diagnosis of IA. According to the revised definitions for invasive fungal disease of the EORTC/MSG (59). A proven IA requires histopathology evidence of fungal invasion. A diagnosis of probable IA is based on the presence of a combination of host factors, clinical features, and positive mycology. And, finally, a diagnosis of possible IA is made in the presence of host factors and clinical features but in the absence of or with negative mycological criteria. These diagnostic criteria have been validated in immunocompromised patients and have demonstrated to be very helpful in the diagnosis of IFIs in this group of patients (204,205). However, in immunocompetent patients the diagnosis of IFI is much more complex. According to these criteria, since no host risk factor is met, the patient would be considered unclassifiable and therefore we could not categorize aspergillosis as probable or possible. Host risk factors that are encompassed in the EORTC-MSG criteria include immunodeficiency states like neutropenia, HSCT, prolonged use of corticosteroids or treatment with other immunosuppressant (59). However, in recent years, the risk of developing IA has been associated with new underlying diseases or comorbidities that are not included in this classification. This is the case of cirrhosis, COPD or previous influenza infection. All these factors have currently been associated with an increased risk of IFI (206,207). This phenomenon could also be corroborated in our study, since 30% of the patients included presented some respiratory underlying condition, being the COPD the most common comorbidity. Therefore, EORTC-MSG

criteria are not appropriate in non-haematological patients and, in addition, the risk factors for IFI must also be reviewed and updated.

Regarding to voriconazole treatment, in real-life setting, we found a surprisingly large number of patients (almost a quarter) that did not receive the loading dose or voriconazol on the first day of treatment. These results are worrying, since the absence of the loading dose delays the achievement of optimal therapeutic plasma concentrations (112). This fact is probably due to prescribing errors that can be avoided with the implementation of an AFS program. Another interesting aspect analysed in this part of the thesis was the concomitant prescription of drugs susceptible to interact with voriconazole. We reported a high rate of patients treated concomitantly with voriconazole and omeprazole; witch is expected to inhibit the CYP2C19 enzyme. We found also a high number of patient treated with voriconazole and glucocorticoids that are know inductors of CYP enzymes. Efficacy was similar to previous studies (208) with successful outcomes in only less than a half of patients. Both crude mortality and related-mortality were high (56% and 36% respectively) and adverse events were observed in 37.5% of the patients. Abnormal liver function was the most frequent adverse event, with three cases of toxic hepatitis related to voriconazole.

In summary, in this first part of the thesis we could verify the following aspects: first, voriconazole was used to treat different fungal pathologies, including IA and other types of fungal infections. In addition, patients treated with voriconazole were not all immunosuppressed, but many of them were immunocompetent patients with COPD as risk factor for IFI. Secondly, the doses used were standard doses recommended without TDM of voriconazole and in a large number of patients the loading dose was not administered. Finally, the therapeutic results were not good, since we observed a

high rate of therapeutic failures and a significant incidence of adverse side effects.

This initial work reinforces the need to improve the antifungal therapy with voriconazole since despite having other antifungal drugs, voriconazole remains the drug of choice in the treatment of IA according to the international guidelines of the management of fungal infections (89,90,92,206). TDM is recommended by the these guidelines (206,92,90,89) as it showed to improve voriconazole outcomes with higher efficacy and lower drug toxicity (124,125,132,134,210–212). Therefore, implementing the TDM of voriconazole as well as analyzing factors effecting the kinetic variability of this drug will allow achieving better patient clinical outcomes and will also improve the management of antifungal drugs. This will be beneficial for the global population since it will avoid the generation of resistances.

For that purpose, in the second part of this thesis, an analytical method was developed for measuring voriconazole in human plasma samples was developed. The method consists of a HPLC witch uses a mixture of water and acetonitrile as the mobile phase. No buffers or gradient separation are needed, so the method is easy and simple to apply. The method has also met the validation criteria of regulatory agencies EMA and FDA (159,160). The main advantage of our method is its high sensitivity. The lower limit of quantification (LOQ) allows us to quantify voriconazole prophylactic plasma concentrations (C_{min}<1 µg/mL). However, the voriconazole determination assay is not standardized and some pharmacokinetic laboratories use the ARK™ immunoassay method. The immunoassay use ready-to-use reagents and avoid the specialized chromatography equipment what makes it very attractive in pharmacokinetic determinations. Therefore, despite being less sensitive than HPCL, ARK™

Immunoassays are frequently used. In this thesis, we also made a correlation between both methods, HPLC and ARK^{TM}. The results obtained showed a significant linear correlation and a slight overestimation in the determination of voriconazole by the ARK^{TM} compared to the HPLC but with a relationship between both methods linear and constant. These results were concordant with a previous comparison between an ultra-high performance liquid chromatography with photodiode array detention (UPLC) and an ARK^{TM} immunoassay (157) and also with a another study that compares a liquid chromatography-tandem mass spectrometry (LC-MS/MS) with an ARK^{TM} immunoassay (156).

With an analytical method available for the pharmacokinetic determination, the next step of this thesis was to analyse factors affecting interindividual variability of voriconazole pharmakokinetics. For this reason, we carry out a prospective, multicenter and observational study including 78 Caucasian adult patients treated with systemic voriconazole. This study examined the impact of different factors affecting voriconazole concentrations, especially the *CYP2C19* polymorphisms and drug-drug interactions.

Fist of all, we found high inter-individual variability in voriconazole exposure, with voriconazole trough plasma concentration ranged from 0.06 to 14.47 µg/mL. In addition, nearly half of the patients had subtherapeutic voriconazole trough concentrations, which was slightly higher than what was observed in other studies (120,129,195,213). This result is really alarming; since it has been shown that subtherapeutic concentrations are associated with lack of antifungal response (87,128,132).

Regarding to the genotype, a high percentage of patients were carriers of the *CYP2C19*17* allele, likewise previous reports in the Caucasian population (120,195,210). This allele is a gain of function

variant witch codifies an increased enzyme activity and it has been targeted as a potential cause of subtherapeutic voriconazole concentrations (176). The present study showed that a high percentage of patients with the *CYP2C19*17/*17* and **1/*17* genotypes failed to achieve therapeutic trough voriconazole concentrations. These results were concordant with previous reports (120,195,213). However, we couldn't find statistically significant differences in plasma concentrations of voriconazole between the different phenotypic groups. This is probably because despite being one of the largest studies evaluating the impact of *CYP2C19* genotype on voriconazole plasma concentrations, it was still insufficient number of patients to reach statistical power. Larger multicenter studies are needed to confirm these results.

In addition to the relationship between voriconazole phenotype and voriconazole plasma concentrations, this work also investigates the impact of *CYP2C19* genetic polymorphisms and outcomes in terms of efficacy and toxicity. In this sense, we found a lower percentage of RM and UM patients among those patients who developed voriconazole toxicity suggesting a lower risk of toxicity in patients carrying the *CYP2C19*17* allele. The relationship between genotype and toxicity has been studied in several studies with controversial results. A previous meta-analysis has found that patients with the PM phenotype demonstrated a trend towards an increased incidence of voriconazole-associated adverse events when compared with other patients, although statistical significance was not reached (207). Later, another study also showed that patients with at least one loss of function allele were more likely to experience adverse events from voriconazole therapy, but these results could not be proven with a statistical significance (215). In our study only one patient was a PM, therefore we could not obtain significant results related to PM phenotype and risk of toxicity. Studies with a larger number of

patients and a greater proportion of PMs are needed to evaluate the use of *CYP2C19* genetic polymorphism as a predictive biomarker for voriconazole toxicity.

Apart from safety, another significant aspect is the clinical response. Given the high mortality rate associated with IFIs (40,51), efficacy is essential in treatment with voriconazole. However, we were unable to study the relationship between polymorphisms and clinical outcomes in this study because out of the 78 patients included only 16 were categorized as proven or probable aspergillosis according to the EORTC-MSG criteria. In the rest of the patients (those with possible or empirical IFIs), we had no objective way of assessing clinical efficacy. Given that, lack of clinical outcome data was an additional limitation of our study.

In addition to *CYP2C19* polymorphisms, voriconazole concentrations may be influenced by other variables, such as co-medications. Although the effects of some drugs on voriconazole are well known and their administration is contraindicated (rifampicin, phenytoin or St. John's worth), the effects on plasma concentrations of voriconazole (and the subsequent clinical impact), of other drugs of very common use have not been completely explored. This is the case of PPIs and glucocorticoids.

Seventy-two percent of the patients included in our work received a PPI. PPI are competitive inhibitors of voriconazole (177). However, despite there are pharmacokinetic studies evaluating the effect of PPIs on voriconazole plasma concentrations, the net effect of PPIs on voriconazole pharmacokinetics as well as the differences according to the type of PPI has not been delineated. For this reason, a specific part of the thesis focused on investigating this aspect. Unlike other studies (119,120,130,170,173–176) no significant differences were found in our study between patients without PPIs and patients

treated with some kind of PPI in our study. However, the results showed that omeprazole produced a significative greater increase on voriconazole levels compared with pantoprazole regardless of *CYP2C19* polymorphism. This observation is consistent with the results of a retrospective study in 33 patients that revealed a statistically significant association between increased voriconazole concentrations and esomeprazole use but not with rabeprazole or pantoprazole use (215). However, these authors did not include omeprazole, one of the most widely used PPI. In contrast, all PPIs were included in the *in vitro* and *in vivo* study of Yan *et al.* (176). These authors revealed an increase on voriconazole concentrations in patients treated with lansoprazole, omprazole or esomeprazole whereas there was no significant association with pantoprazole use. Another multicenter study of voriconazole pharmacokinetics in 201 patients suggests a weaker interaction between voriconazole and pantoprazole compared with other PPIs, including omeprazole, although the effect was not statistically significant and, besides, this study did not include *CYP2C19* genetic information (119).

Another drug-drug interaction, between voriconazole and glucocorticoids, has been suggested previously related to the CYP induction by glucocorticoids. In this sense, An *in vitro* study has identified glucocorticoid receptor binding sites in the promoter region of the *CYP2C19* gene and demonstrated up-regulation of *CYP2C19* in response to dexamethasone, supporting an inductive effect of glucocorticoids on *CYP2C19* (162,163). There are limited data regarding the interaction between voriconazole and glucocorticoids in clinical practice. Dolton *et al.* found that concomitant dexamethasone was related to a 3.75 fold decrease in voriconazole concentrations (119). This authors also demonstrated substantial differences in voriconazole serum level changes depending on the specific steroid: dexamethasone > methylprednisolone > prednisone/prednisolone).

Cojutti *et al.* also found in a recent study that coadministration of methylprednisolone and dexamethasone was associated with a decrease in voriconazole concentrations (170). Similary, Wallace *et al.* observed a 2-fold increase in the plasma concentration of voriconazole when the dexamethasone dose was reduced in one patient with a fungal brain abscess (171). However, our study and similar to other authors (120) did not found any significant interaction between voriconazole and concomitant steroid use. These conflicting results could be explained by the heterogeneity of the studied populations and the type and dose of the glucocorticoid employed.

However, despite these global results, a clinically relevant interaction between voriconazole and glucocorticoids was observed in one patient included in our study. This is the case of an 82 years-old woman diagnosed of ABPA that under treatment with glucocorticoids and voriconazole developed symptoms of hepatotoxicity after glucocorticoids withdrawal. A possible mechanism responsible for decreased voriconazole elimination could be a pharmacokinetic interaction between glucocorticoids and voriconazole. The patient presented plasma concentrations of voriconazole in a therapeutic range while under treatment with glucocorticoids. Upon discontinuation of prednisone, an increase in voriconazole plasma concentration was observed along with toxicity, suggesting that prednisone had been inducing metabolism of voriconazole. The Drug Interaction Probability Scale (DIPS Algorithm) (215) was used to assess the causation of a potential drug interaction between prednisone and voriconazole. The score obtained was 7 points, suggesting this drug interaction as the probable cause of the patient's hepatotoxicity.

In summary, voriconazole remains the drug of choice in the management of invasive aspergillosis. In addition, early optimal

antifungal therapy improves clinical outcomes in patients with IFIs and reaching voriconazole concentrations in the therapeutic range early in the course of therapy is related to lower mortality (129). In this sense, TDM of voriconazole remains the best way to improve antifungal treatment outcomes, but pharmacogenetics can complement TDM in the optimal management of antifungal treatment. *CYP2C19* polymorphisms have been shown to be an important source of variability in plasma voriconazole concentrations. Indeed, in the Caucasian population being a carrier of allele **17* may lead to an increased risk of subtherapeutic concentrations.

The combination of pharmacogenetics and pharmacokinetics could be of great interest in in patients who are expected to receive the drug, such as haematology adults or paediatric patients or patients who are going to receive a solid organ transplant. This strategy could be also useful in the management of infections in witch the drug must reach difficult-to-reach tissues, such as the central nervous system. In these patients, having genetic information could facilitate a more appropriate initial dose selection of voriconazole, which would later be guided by TDM. Although there is a lack of further data, especially cost/effectiveness studies, supporting the implementation of voriconazole pharmacogenetics on clinical practice. Preliminary data suggest certain groups of patients may already benefit from *CYP2C19* genetic determination. In this sense, TDM of voriconazole remains the best way to improve antifungal treatment outcomes, but pharmacogenetics can complement TDM in the optimal management of antifungal treatment.

Finally, in the management of antifungal therapy we cannot forget the importance of AFS programs. The AFS multidisciplinary team must ensure the proper use of antifungal therapy in hospitals, with the aim not only of improving patient clinical outcomes, but also to avoid antifungal resistance, and ultimately to benefit society.

Clinical pharmacists, with knowledge of antifungal PK/PD and potential drug–drug interactions, are key members of the AFS team. Moreover, clinical pharmacists are uniquely suited to interpret and apply genetic information to the therapeutic decision-making process and should lead the effort to incorporate pharmacogenetic information into patient care. In short, clinical pharmacists should be instrumental in leadership, development, and implementation of clinical pharmacogenomics initiatives in the field of fungal diseases.

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Conclusions

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16 CONCLUSIONS

a) Las infecciones fúngicas invasivas (IFIs) presentan una elevada morbilidad y mortalidad, especialmente en los pacientes críticos y onco-hematológicos. Dado que el voriconazol continúa siendo el fármaco de primera elección en el tratamiento de la aspergilosis invasiva, diseñar estrategias que garanticen el adecuado manejo de estas infecciones resulta crucial.

b) En este trabajo hemos evaluado el uso de voriconazol en la práctica clínica real a través de un estudio retrospectivo observacional inicial.

    1) Se observó que los pacientes tratados con este fármaco presentaban diferentes comorbilidades y edad avanzada. Muchos de ellos recibiendo tratamiento simultáneo con múltiples fármacos, incluyendo glucocorticoides e inhibidores de la bomba de protones (IBPs) sugiriendo la existencia de múltiples factores de variabilidad farmacocinética.

    2) Se observó una alta incidencia de IFI en los pacientes que presentaban como comorbilidad principal EPOC, indicando la necesidad de actualizar los factores de riesgo de IFI así como los criterios empleados para categorizar estas infecciones en probadas, probable o posibles.

3) El voriconazol se empleó para tratar diferentes patologías, incluyendo tratamientos dirigidos, empíricos así como profilaxis antifúngica indicando la necesidad de instaurar equipos multidisciplinares que aseguren un adecuado uso de los fármacos antifúngicos y que minimicen la aparición de resistencias.

4) La eficacia del tratamiento con voriconazol en práctica clínica real fue baja con una alta incidencia de efectos adversos. Esto hace necesario la implementación de estrategias como la monitorización farmacocinética de voriconazol.

c) En este trabajo hemos desarrollado un método analítico basado en HPLC para la determinación farmacocinética de voriconazol en muestras plasmáticas.

1) El método desarrollado fue rápido, simple, sensible y específico, por lo que podría ser empleado en la práctica clínica asistencial como método para la monitorización farmacocinética de voriconazol.

2) Este método se comparó con el ARK™ Inmunoensayo obteniéndose una correlación linear entre ambos métodos, sugiriendo el empleo de uno u otro siempre que estén adecuadamente validados y considerando la correlación existente.

d) Los factores que afectan a la farmacocinética de voriconazol fueron analizados en un estudio multicéntrico y prospectivo.

1) Se observó una alta proporción de pacientes con concentraciones subterapéuticas sugiriendo la necesidad

inmediata de diseñar estrategias que garanticen alcanzar concentraciones plasmáticas dentro del intervalo terapéutico de forma precoz.

2) Se confirmó la existencia de una alta tasa de pacientes portadores del alelo **17* corroborando la alta incidencia del mismo en población caucásica.

3) Los portadores del alelo **17* presentaron concentraciones plasmáticas de voriconazol más bajas y una mayor proporción de pacientes con concentraciones subterapéuticas comparados con el resto de genotipos. Por este motivo, se propone el genotipado de *CYP2C19* como herramienta para la selección de la dosis inicial de los pacientes tratados con voriconazol.

4) Los glucocorticoides no tuvieron un impacto significativo en las concentraciones plasmáticas de voriconazol en nuestro trabajo. Sin embargo, la variedad en las dosis y tipo de corticoide empleado indican la necesidad de confirmar estos resultados en futuros estudios.

5) Se observó un mayor efecto inhibidor enzimático de omeprazol comparado con pantoprazol. Por ello, la selección del IBP es un punto clave para minimizar el riesgo de variabilidad farmacocinética en los pacientes tratados con voriconazol.

e) En base a los resultados obtenidos podemos concluir que la farmacogenética de voriconazol es una herramienta útil para la selección inicial de la dosis de voriconazol y posteriormente realizar modificaciones en la dosis guiadas por farmacocinética. Sugerimos por tanto implementar el

genotipado de *CYP2C19* no como un sustituto sino como un complemento de la farmacocinética en el manejo de los pacientes a tratamiento con voriconazol.

f) El farmacéutico clínico tiene un papel clave en la aplicación de la medicina personalizada en el tratamiento antifúngico. Como experto en medicamentos, debe formar parte de los equipos de uso adecuado de antifúngicos en los hospitales y liderar estrategias que combinen farmacogenética , farmacodinámica y farmacocinética para optimizar la terapia antifúngica.

17 List of tables and figures

Tables

Figures

18 References

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