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CAMBIOS EN LAS CARACTERÍSTICAS DE LA ENFERMEDAD FÚNGICA INVASORA EN LOS PACIENTES INMUNODEPRIMIDOS

Tesis presentada por

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Para optar al grado de Doctor

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Certifican que la tesis titulada

"Cambios en las características de la enfermedad fúngica invasora en los pacientes inmunodeprimidos"

Que presenta la licenciada **Maddalena Peghin**, ha sido realizada bajo su dirección en el Servicio de Enfermedades Infecciosas y Neumología del Hospital Universitari Vall d'Hebron, la consideran finalizada y autorizan su presentación para que sea defendida ante el tribunal que corresponda.

Dr. M. Isabel Ruiz Camps **Dr. Víctor Monforte Torres**

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“Nella vita non bisogna mai rassegnarsi, arrendersi alla mediocrità, bensì uscire da quella zona grigia in cui tutto è abitudine e rassegnazione passiva, bisogna coltivare il coraggio di ribellarsi”

RITA LEVI-MONTALCINI

“Amare il proprio lavoro è la cosa che si avvicina più concretamente alla felicità sulla terra”

RITA LEVI-MONTALCINI

Producción científica

Los resultados obtenidos durante el proceso de investigación que ha llevado a la elaboración de esta tesis doctoral han sido publicados previamente en revistas científicas indexadas:

Artículos originales

"Unusual forms of subacute invasive pulmonary aspergillosis in patients with solid tumors". **Peghin M**, Ruiz-Camps I, Garcia-Vidal C, Cervera C, Andreu J, Martin M, Gavaldá J, Gudiol C, Moreno A, Felip E, Pahissa A. J Infect. 2014 Oct (Impact factor 2014: 4.441)

"10 years of prophylaxis with nebulized liposomal amphotericin B and the changing epidemiology of *Aspergillus* spp. infection in lung transplantation"

Peghin M, Monforte V, Martin-Gomez MT, Ruiz-Camps I, Berastegui C, Saez B, Riera J, Ussetti, P, Solé J, Gavaldá J, Roman A. Tansplant International 2015 Sept. (Impact factor 2014: 2.599)

"Epidemiology of Invasive Respiratory Disease Caused by Emerging Non-*Aspergillus* molds in Lung Transplant Recipients".

Peghin M, Monforte V, Martin-Gomez MT, Ruiz-Camps I, Berastegui C, Saez B, Riera J, Solé J, Gavaldá J, Roman A. Transplant Infectious Disease. 2015 Oct. (Impact factor 2014: 2.064)

Artículos originales relacionados (Anexo)

"Environmental variables associated with an increased risk of invasive aspergillosis" Garcia-Vidal C, Royo-Cebrecos C, **Peghin M**, Moreno A, Ruiz-Camps I, Cervera C, Belmonte J, Gudiol C, Labori M, Roselló E, de la Bellacasa JP, Ayats J, Carratalà J. Clin Microbiol Infect. 2014 Nov. (Impact factor 2014: 5.768)

"Causes of death in a contemporary cohort of patients with invasive aspergillosis"

Garcia-Vidal C, **Peghin M**, Cervera C, Gudiol C, Ruiz-Camps I, Moreno A, Royo-Cebrecos C, Roselló E, de la Bellacasa JP, Ayats J, Carratalà J. PLoS One. 2015 Mar. (Impact factor 2014: 3.234)

Artículos de revisión (Anexo)

"Infecciones por hongos filamentosos en el paciente inmunosuprimido: profilaxis y tratamiento" Ruiz-Camps I, **Peghin M**. Rev Esp Quimioter. 2015 Sept. (Impact factor 2014: 0.797)

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ABREVIATURAS

ABPA: aspergilosis broncopulmonar alérgica

APC: aspergilosis pulmonar crónica

API: aspergilosis pulmonar invasiva

AB: anfotericina B

CI: intervalo de confianza

CMV: citomegalovirus

DCI: disfunción crónica del injerto

EGC: enfermedad granulomatosa crónica

EICH: enfermedad del injerto contra el huésped

EPOC: enfermedad pulmonar obstructiva crónica

HIV: virus de inmunodeficiencia humana

IFI: infección fúngica invasora

LBA: lavado broncoalveolar

n-AB: anfotericina B nebulizada

n-ABD: anfotericinaB nebulizada desoxicolato

n-ABL: anfotericina B nebulizada liposomal

OR: razón de probabilidades

VIH: virus de la inmunodeficiencia humana

SIDA: síndrome de inmunodeficiencia adquirida

TBC: tuberculosis

TOS: trasplantados de órgano sólido

TP: trasplantados de pulmón

TPH: trasplante de progenitores hematopoyéticos

I. INTRODUCCIÓN

I. INTRODUCCIÓN

I.1. Cambios en las características de la infección fúngica invasora en los pacientes inmunodeprimidos

En la última década, las infecciones fúngicas invasoras (IFI) siguen aumentando su incidencia en pacientes inmunodeprimidos u hospitalizados con graves enfermedades de base (1) (2) (3) (4). Estas infecciones presentan elevadas tasas tanto de morbilidad como de mortalidad y originan un consumo elevado de recursos para su prevención, diagnóstico y tratamiento con una importante carga de trabajo asistencial (5) (6).

En los últimos años, la población de pacientes susceptibles de desarrollar IFI se ha ampliado de forma importante y han surgido nuevos grupos de riesgo que están adquiriendo un notable protagonismo (7) (8) (9) (10). Además, se ha modificado la visión clásica de la IFI y ahora sabemos que los diversos síndromes clínicos pueden ser vistos como un espectro continuo de la enfermedad, cuyas manifestaciones se definen por la interacción entre patógeno y huésped. (11). Finalmente, se han identificado cambios en la cronología de estas infecciones, debido a los avances médicos contra las enfermedades subyacentes clásicas, la presencia de nuevas estrategias profilácticas y terapéuticas, la aparición de resistencias antifúngicas y de nuevas herramientas diagnósticas (12) (13).

Conocer los cambios asociados a las características de la IFI resulta de especial importancia para optimizar el manejo clínico de estas infecciones, su prevención y su tratamiento.

I.2. Patogénesis de la infección fúngica invasora

Los hongos son un grupo heterogéneo de microorganismos eucariotas que interaccionan de manera constante con el ser humano. Como resultado de esta interacción, se puede producir un amplio espectro de situaciones que oscilan desde cuadros de hiperreactividad, a la eliminación del hongo sin causar patología o a diferentes infecciones graves. Solo una mínima proporción de hongos tiene la capacidad de ser patógena para el ser humano. En la actualidad,

las IFI más frecuentes son aquellas causadas por las especies de *Candida*, *Aspergillus*, *Cryptococcus*, *Pneumocystis* y hongos filamentosos distintos de *Aspergillus*, que pueden presentar diferentes factores de virulencia que potencian su capacidad invasora. La distribución de los agentes causales varía en función de la geografía, de las condiciones de los pacientes y de las unidades de hospitalización (14). Frente a la infección fúngica, el ser humano se defiende utilizando diferentes estrategias, que van desde los mecanismos de protección de la inmunidad innata (macrófagos, neutrófilos, monocitos, células natural killer) a los mecanismos de la inmunidad adaptativa, inducidos específicamente durante la infección y la enfermedad (15). El mecanismo innato de primera línea es la presencia de barreras físicas tales como la piel y las membranas mucosas, que se complementa con las membranas celulares, los receptores celulares y los factores humorales. Existe controversia sobre la contribución relativa de la inmunidad humoral y celular en la defensa del huésped contra las infecciones fúngicas. Durante mucho tiempo se consideró que la inmunidad mediada por células era importante, pero la inmunidad humoral tenía poco o ningún papel. Sin embargo, se acepta ahora que la inmunidad celular es el principal mecanismo de defensa, pero que ciertos tipos de respuesta de anticuerpos son protectores. En general, los anticuerpos de tipo Th1 favorecen la respuesta a la infección por hongos, mientras que los Th2 condicionan susceptibilidad a la infección. En diferentes situaciones en la que estos mecanismos de defensa se ven comprometidos, el huésped es más susceptible a padecer una IFI (14).

I.3. Enfermedad fúngica invasora por hongos filamentosos

Dentro de los hongos causantes de IFI, los hongos filamentosos, que incluyen *Aspergillus* spp. y otras especies, tienen gran interés y están emergiendo como problema clínicos mayores de la micología moderna (16). Los hongos filamentosos son ubicuos en el ambiente y la inhalación de esporas en un fenómeno continuo. El género *Aspergillus* spp. incluye unas 200 especies, de las que más de 20 pueden causar infecciones en humanos (15). Aunque su principal nicho

ecológico es la tierra, el agua o la vegetación, las esporas de *Aspergillus* spp. se pueden dispersar fácilmente en el aire y sobrevivir en diferentes condiciones ambientales. Este hongo, que crece preferiblemente a 37°, tiene un ciclo biológico muy simple reproduciéndose por esporas, las cuales germinan y posteriormente las hifas constituirán las formas invasivas del hongo. La simplicidad del ciclo biológico favorece una alta capacidad del hongo para la esporulación y, como consecuencia, la presencia de concentraciones altas de esporas en el aire. Entre las especies patógenas, la más frecuente es *Aspergillus fumigatus* seguida de *Aspergillus flavus*, *Aspergillus niger* y *Aspergillus terreus* (15). La inhalación de las pequeñas esporas de *Aspergillus* spp. (2-3 µm) es la etapa inicial de la patogenia de este hongo (17). Aunque la inhalación de estas esporas por el ser humano es muy frecuente, habitualmente no produce ninguna enfermedad al ser eliminada por el sistema inmunitario. Sin embargo, en algunos huéspedes, la espora tiene mayor facilidad para alcanzar el tracto respiratorio inferior. Una vez depositado y, de nuevo, dependiendo de la respuesta del huésped y su interacción con el patógeno, se puede producir un amplio espectro de enfermedades, que manifiestan una desregulación del sistema inmunitario: desde manifestaciones de hiperreactividad (aspergilosis broncopulmonar alérgica, neumonía por hipersensibilidad, neumonitis eosinofílica, granulomatosis broncocéntrica), hasta la enfermedad invasora característica del paciente inmunodeprimido (Figura 1, Figura 2) (11).

Figura 1. Interacción de *Aspergillus* spp. con el huésped de Hope et al. modificada (18).

Abreviaturas: ABPA, aspergilosis broncopulmonar alérgica; API, aspergilosis pulmonar invasiva

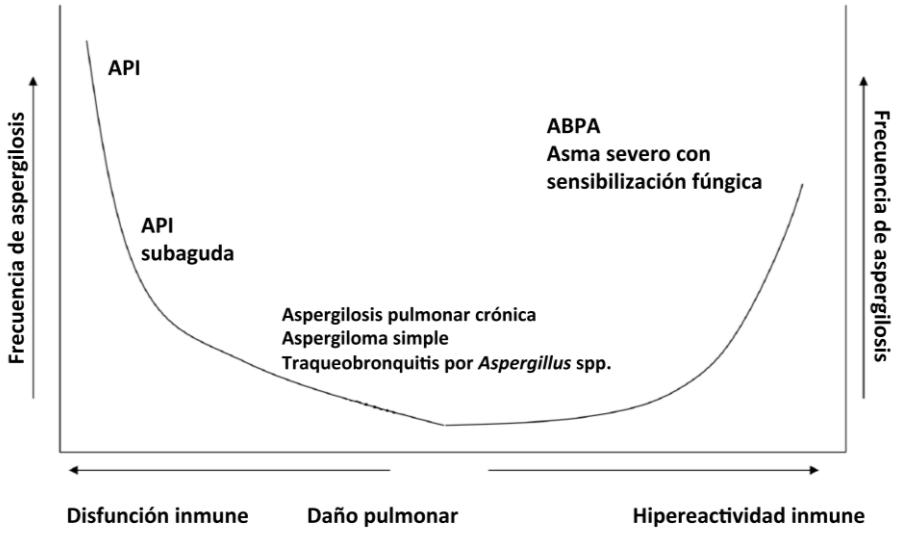
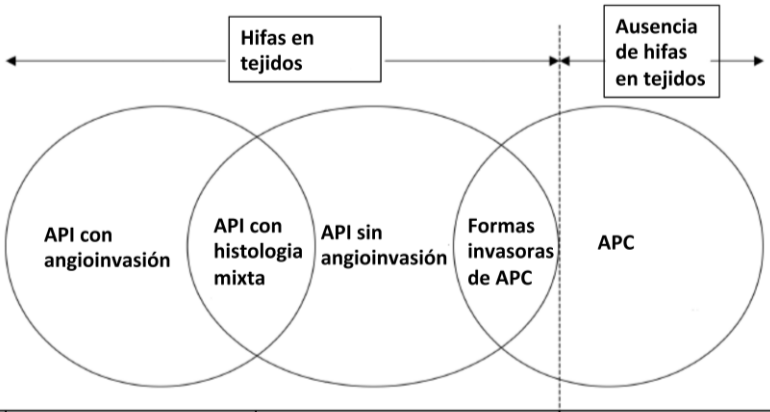


Figura 2. Características patológicas de diferentes formas de aspergilosis de Hope et al. modificada (18). Abreviaturas: APC, aspergilosis pulmonar crónica; API, aspergilosis pulmonar invasiva; EGC, enfermedad granulomatosa crónica; EICH, enfermedad del injerto contra el huésped; SIDA, síndrome de inmunodeficiencia adquirida; TPH, trasplante de progenitores hematopoyéticas; VIH, virus de inmunodeficiencia humana.



Patología	Angioinvasión, necrosis coagulativa, infartos hemorrágicos	Ausencia de angioinvasión, piogranulomatosis inflamatoria, necrosis inflamatoria	Hifas no invasoras confinadas en cavidad pulmonar previa
Húesped	Neutropenia profunda y prolongada	Pacientes con EGC y TPH no neutropenicos, terapia corticoidea, VIH/SIDA , EICH	Enfermedad pulmonar estructural previa, deficiencias inmunologicas menores
Tiempo-curso	Días- Semanas	Semanas- Meses	Meses - Años

I.4. Nuevas poblaciones de riesgo y formas clínicas de infección fúngica invasora por *Aspergillus* spp.

La aspergilosis pulmonar invasiva (API) incluye un espectro de enfermedades relacionadas con diferentes condiciones basales que deterioran la respuesta inmune a la inhalación pulmonar y sistémica de *Aspergillus* spp. (18). Clásicamente, la API se ha descrito como una enfermedad aguda en pacientes severamente inmunodeprimidos, como los pacientes con neutropenia prolongada (19), los que reciben un trasplante de progenitores hematopoyéticos (TPH) , los tratados con dosis altas de corticosteroides (20), los pacientes con síndrome de inmunodeficiencia adquirida avanzada (21) o los afectos de enfermedad granulomatosa crónica (22) .

De los pacientes sometidos a trasplante de órgano sólido (TOS), tanto adultos como niños, los que presentan una mayor incidencia de IFI son los trasplantados de pulmón (TP) (23). Además en este subgrupo de pacientes, la infección aspergilar se puede manifestar como enfermedad aislada de la vía respiratoria traqueobronquial, en forma de traqueobronquitis simple (secundaria a inflamación y producción excesiva de moco) o de traqueobronquitis ulcerativa o traqueobronquitis pseudomembranosa (a menudo alrededor de la línea de sutura)(24). La traqueobronquitis ulcerativa es una forma broncoinvasora localizada de API, sin evidencia de afectación del parénquima pulmonar y la broncoscopia muestra típicamente la presencia de ulceraciones o pseudomembranas (25).

Hoy en día, el perfil de los pacientes considerados de riesgo de aspergilosis invasiva está en expansión, habiéndose descrito la presencia de API en los sujetos con formas menos graves de inmunosupresión, como los pacientes con enfermedad pulmonar obstructiva crónica (EPOC) (26), enfermedad hepática crónica (27), ingresados en unidades de cuidados intensivos (28) o en tratamiento con fármacos biológicos (9).

Además, en los pacientes con formas de inmunosupresión menor, la aspergilosis pulmonar puede manifestarse con diferentes formas clínicas y patológicas, como la API subaguda o la aspergilosis pulmonar crónica (APC), una forma localmente invasiva de esta infección (29) (30) (Figura 2). En la actualidad podríamos considerar que la APC está infradiagnosticada, ya que incluye un grupo de enfermedades en la frontera entre la enfermedad saprofítica e invasiva, cuya nomenclatura y criterios diagnósticos (microbiológicos, radiológicos, histológicos) (29) (30) han sido definidos y reconocidos sólo recientemente como un importante problema de salud mundial (31, 32). La compleja relación entre las condiciones subyacentes y *Aspergillus* spp. abarca un espectro de diferentes formas de APC (Tabla 1) incluyendo aspergiloma, aspergilosis pulmonar cavitaria crónica, aspergilosis pulmonar fibrosante crónica y aspergilosis necrotizante crónica o subaguda invasiva. La duración de la enfermedad superior a tres meses puede ser uno de los criterios que ayude a diferenciar entre las formas crónicas y agudas de esta entidad (Tabla 1).

Los pacientes con APC presentan, generalmente, una enfermedad pulmonar estructural subyacente, como tuberculosis (TBC), EPOC, infecciones por micobacterias atípicas, aspergilosis broncopulmonar alérgica, sarcoidosis fibrocavitaria, tumores sólidos y cirugía torácica (30). También la APC suele ocurrir en pacientes con formas leves de inmunosupresión, como los pacientes sometidos a uso crónico de corticosteroides inhalados, tratamientos inmunosupresores nuevos y diabéticos (33). La presentación clínica de esta forma de APC está enmascarada a menudo por la patología crónica de base y se desarrolla de forma más prolongada en el tiempo. Los síntomas más comunes son la pérdida de peso, la tos productiva crónica, la hemoptisis de gravedad variable, la fatiga, la disnea y ocasionalmente fiebre y sudores. La prueba cardinal para el diagnóstico de la aspergilosis pulmonar crónica es la positividad de los anticuerpos frente a *Aspergillus* (precipitinas) (29) (30). Sin embargo, esta prueba no está bien estandarizada para esta indicación. Pocos pacientes (10-40%) con APC tienen cultivos positivos para *A. fumigatus* (o raramente otras especies de *Aspergillus*) en

esputo, pero la PCR de *Aspergillus* spp. suele ser positiva. La APC se asocia con una significativa morbilidad y mortalidad y el manejo terapéutico óptimo no está bien definido, siendo los fármacos de elección los triazoles (voriconazol o itraconazol) (29) (30). Debido a la frecuencia de recaídas, el tratamiento es a menudo a largo plazo o continúo y, por tanto, puede ser limitado por la presencia de efectos secundarios o el desarrollo de resistencias (33).

Tabla 1. Glosario de definiciones según Denning y colaboradores modificado (29) (30).

ASPERGILOSIS PULMONAR INVASIVA (API) AGUDA	La aspergilosis pulmonar invasiva aguda se desarrolla por lo general en pacientes marcadamente inmunocomprometidos en menos de 1 mes. Puede ser angioinvasiva o no. Las características radiológicas incluyen nódulos o consolidaciones progresivas a menudo con el signo del '' halo '', la formación de cavidades (en aire o en media luna), de derrame pleural y la forma miliar (si se asocia con la exposición masiva a esporas). Si se realiza una biopsia, las hifas son visibles en el tejido pulmonar destruido. La mayoría de los diagnósticos se realizan por las lesiones radiológicas típicas en la tomografía computarizada, los resultados microbiológicos (cultivos, antígenos positivo para *Aspergillus* spp.), estado de inmunosupresión del paciente y rápida evolución de la enfermedad.
ASPERGILOSIS PULMONAR CRONICA (APC)	
Aspergilosis pulmonar invasiva subaguda o crónica necrotizante	Aspergilosis invasiva en pacientes ligeramente inmunocomprometidos, que se produce entre 1 y 3 meses, con rasgos radiológicos marcados (cavitaciones, nódulos y consolidaciones progresivas con ''formación de abscesos''), con hifas visibles en el tejido pulmonar destruido o que se diagnostica por los resultados microbiológicos (ej. antígeno de *Aspergillus* spp. positivo) y por la evolución de la enfermedad.
Aspergilosis pulmonar crónica fibrosante	Destrucción fibrótica severa de al menos dos lóbulos de pulmón que complican la aspergilosis pulmonar crónica cavitaria con una importante pérdida de la función pulmonar. Por lo general, la fibrosis es en forma de consolidación, pero puede presentarse con grandes cavidades con fibrosis alrededor. La destrucción fibrótica grave de un lóbulo con una cavidad se clasifica como aspergilosis pulmonar cavitaria crónica que afecta a ese lóbulo.
Aspergilosis pulmonar crónica cavitaria	Una o más cavidades pulmonares que pueden o no contener una bola fúngica, con evidencia serológica o microbiológica que indica la presencia de *Aspergillus* spp. Se describe en pacientes no inmunocomprometidos (o uno cuya condición de inmunosupresión ha remitido o es mínima) con síntomas pulmonares o sistémicos significativos y progresión radiológica evidente (nuevas cavidades, aumento de infiltrado pericavitario o aumento de la fibrosis) durante al menos 3 meses de observación. Si se realiza una biopsia de la zona afectada, se demuestra la presencia de hifas y zonas de inflamación crónica y fibrosis, pero sin invasión de tejidos.
Aspergiloma simple	Cavidad pulmonar individual que contiene una bola de hongos, con evidencia serológica o microbiológica que indica la presencia de *Aspergillus* spp. en un paciente no inmunodeprimido con síntomas menores o ausencia de síntomas y ausencia de progresión radiológica durante al menos 3 meses de observación.
Aspergiloma	Una sombra aproximadamente esférica con aire alrededor (también llamado bola fúngica) en una cavidad pulmonar, con evidencia serológica o microbiológica de la presencia de *Aspergillus* spp. en el material. Esta es una descripción radiológica o morfológica, no es una descripción de la enfermedad.

I.5. Cambios en los factores predisponentes para el desarrollo de aspergilosis invasora

El conocimiento más detallado de los factores que contribuyen a las patogénesis de la aspergilosis invasora es necesario para optimizar la gestión de esta infección y mejorar las medidas profilácticas. Los factores que aumentan el riesgo de infección por *Aspergillus* spp. han sido bien definidos e incluyen:

(a) factores de inmunidad individuales del huésped, como las citopenias, la enfermedad por citomegalovirus (CMV), el trasplante, la enfermedad de injerto contra el huésped, el rechazo agudo, el uso de dosis altas de corticosteroides, el VIH, el uso de agentes anti-células T, la edad avanzada, las transfusiones de sangre, el uso de fármacos biológicos, la diabetes mellitus, la insuficiencia renal crónica y los factores genéticos (34) (35) (36);

(b) presencia de enfermedad pulmonar estructural (fibrosis quística, sarcoidosis, EPOC, TBC);

(c) factores que pueden promover la colonización por hongos filamentosos (uso más amplio de antimicrobianos, factores ambientales, virus respiratorios) (37).

En los pacientes TOS, históricamente, la API se ha considerado una complicación del periodo inmediato en el post-trasplante. Sin embargo, estudios más recientes han puesto de manifiesto que la incidencia de la API persiste elevada una vez superado ese periodo (38) (36). Asimismo, diferentes series han demostrado que la API se concentra en subpoblaciones específicas dentro de los receptores de TP (Tabla 2) (36).

Sin embargo, la información es más escasa sobre otras variables que pueden promover la colonización por hongos, tales como son las variables ambientales y los virus respiratorios. Con la finalidad de estudiar estos factores ambientales, hemos realizado un estudio (Anexo 1) retrospectivo multicéntrico (2008-2011), incluyendo un número elevado de pacientes hospitalizados con API (165 pacientes) en el área de Barcelona. Específicamente, en nuestra población de pacientes (con unos estados de inmunidad muy variada) hemos encontrado que la presencia de un recuento elevado de esporas en el aire entre 28 y 42 días antes de la

infección se asociaba con un aumento de tasas de ingresos por API. Estos resultados de nuestro estudio coinciden con publicaciones anteriores (39) (40) y apoyan la teoría de que la relación entre *Aspergillus* spp. y el huésped se inicia varios días antes del diagnóstico de la API y que la mayoría de los pacientes con API adquieren la infección fuera del hospital. Sin embargo, no hemos encontrado ninguna relación entre el recuento de esporas y las variables climáticas en nuestra área o entre las condiciones climáticas y los ingresos por API, como se había comunicado previamente (39). Este hallazgo sugiere que la patogénesis de la API es compleja y que sin duda se deben de tener en cuenta las diferencias geográficas. Por otra parte, la relación entre las condiciones climáticas y el recuento de esporas puede estar condicionada por otros factores ambientales como el aire, la polución, el uso de la tierra, etc. Finalmente, hemos encontrado una relación significativamente estrecha entre los virus respiratorios circulantes (virus respiratorio sincitial, influenza H1N1, adenovirus) y el riesgo de API. Se ha descrito previamente que la infección por diferentes tipos de virus respiratorios aumenta el riesgo de desarrollar API (34) (41) (42) (43). En primer lugar los virus respiratorios producen una ruptura de la mucosa respiratoria que favorece la invasión por *Aspergillus* spp. y en segundo lugar pueden alterar las defensas locales y sistémicas del huésped, produciendo un estado de adaptación inmunitaria que facilita la patogenia de *Aspergillus* spp.

Tabla 2. Factores de riesgo de aspergilosis pulmonar invasiva en receptores de trasplante de pulmón, según Fortun y colaboradores modificado (44).

	API precoz	**API tardía (>90 días postrasplante)**
Trasplante pulmonar	Isquemia de la anastomosis bronquial o colocación stent bronquial Infección por CMV Rechazo agudo Trasplante unipulmonar Colonización por *Aspergillus* spp. pretrasplante o en primer año Hipogammaglobulinemia (IgG <400mg/dl)	Rechazo crónico

I.6. Novedades en el diagnóstico de la aspergilosis invasora

La aproximación diagnóstica a las micosis invasoras y a la aspergilosis ha cambiado en los últimos años, pero sigue teniendo un bajo rendimiento. Dos avances clave en el diagnóstico de la enfermedad micótica invasiva han sido las pruebas de detección antigénica y las técnicas moleculares. Asimismo, otras herramientas más antiguas (precipitinas) han sido revaloradas en los actuales paradigmas diagnósticos de diferentes formas de aspergilosis (45).

La detección de galactomanano (antígeno de *Aspergillus*) en fluidos es más sensible que el cultivo para el diagnóstico de aspergilosis invasiva. En el suero, la sensibilidad es variable (17-100%), siendo el corte de sensibilidad más alto en pacientes con enfermedad hematológica neutropénica que no reciben profilaxis (45) (46). La evidencia de publicaciones recientes apoya que el cribado de serie de muestras de sangre en pacientes de alto riesgo es apropiado cuando la prevalencia de la aspergilosis invasiva supera el 7% y no se da ninguna profilaxis antifúngica (45). En otras condiciones no se considera coste–efectivo (47). Más recientemente, se ha ido utilizando la detección de galactomanano en muestras de lavado broncoalveolar (LBA), con buenos valores predictivos con resultados superiores a 1, especialmente en los pacientes trasplantados de pulmón, ingresados en cuidados intensivos o con APC (48).

El 1,3-β-D-glucano (BDG) es un resto de carbohidrato presente en las paredes de muchos hongos y se produce in vivo durante infección por varios organismos fúngicos (incluyendo dentro de los hongos filamentosos *Aspergillus* spp, pero no especies del orden *Mucorales*). El test del BDG está indicado para el diagnóstico presuntivo de IFI y parece ser sensible con un buen valor predictivo negativo. Esta prueba podría tener un papel en algunos algoritmos diagnósticos, sobre todo a expensas de su alto valor predictivo negativo, pero en caso de resultados positivos siempre se necesitaría la realización de otras investigaciones (49).

La detección de anticuerpos para *Aspergillus* spp. (principalmente *A.fumigatus*) es útil para el diagnóstico de varias formas de aspergilosis en pacientes inmunocompetentes. A pesar de la

ausencia de estudios comparativos sobre este método diagnóstico, el aumento de las concentraciones de IgG frente a *Aspergillus* spp. (precipitinas) son útiles para confirmar el diagnóstico de aspergilosis pulmonar crónica y aspergiloma (29).

Las técnicas moleculares de amplificación de los ácidos nucleicos potencialmente pueden mejorar el diagnóstico de la IFI. A pesar de la ausencia de estandarización de esta técnica, la utilidad de la PCR ha sido revisada para el diagnóstico de la aspergilosis invasiva en un metaánalisis, apoyando con moderada evidencia el uso de técnicas moleculares en sangre en subgrupos de pacientes inmunocomprometidos (50). En el momento actual, los datos para el uso de la PCR de *Aspergillus* spp en LBA y muestras de esputo son insuficientes para recomendar esta técnica como método diagnóstico (51).

Como resumen, galactomanano, BDG, y PCR para *Aspergillus* spp. tienen un alto valor predictivo negativo y son ideales para excluir el diagnóstico de aspergilosis invasiva. Los valores predictivos positivos son subóptimos si la prevalencia de la enfermedad es baja. Sin embargo, la combinación de biomarcadores parece aumentar la sensibilidad para el diagnóstico de API, precediendo al desarrollo de enfermedad manifiesta y permitiendo un uso racional y temprano de los agentes antifúngicos (52).

En otras micosis los recursos diagnósticos son más reducidos, pero la radiología, los estudios anatomopatológicos y el diagnóstico microbiológico pueden ser útiles.

I.7. Avances en las estrategias de profilaxis antifúngica

Los estudios realizados en la década de 1990 y principios de 2000 aportaban resultados muy pobres sobre la evolución de la aspergilosis invasiva. Las tasas de mortalidad variaban entre el 60 y el 90 por ciento y dependían en gran medida de la enfermedad de base (53). La supervivencia global ha mejorado en parte por el gran esfuerzo que se ha puesto en la prevención de las infecciones fúngicas mediante la utilización de estrategias profilácticas y en el tratamiento precoz de la aspergilosis invasiva (54).

La utilidad de medidas en la prevención de la IFI depende de las características basales del paciente y la epidemiología de las IFIs en las diferentes instituciones (54). Sin embargo, es necesario considerar que la eficacia en la reducción de este tipo de infecciones no debe ser atribuida únicamente a la utilización de profilaxis primaria con antifúngicos. En determinadas situaciones es necesario asociar otro tipo de medidas, posiblemente más importantes, como son la optimización de los procedimientos quirúrgicos, el manejo adecuado de la inmunosupresión, el control ambiental de determinados hongos, el correcto manejo de la infección hospitalaria y la adecuada cumplimentación y seguimiento de las medidas por parte de los pacientes (44).

Actualmente, el uso de la profilaxis antifúngica ha demostrado ser eficaz en los pacientes con neoplasia hematológica de elevado riesgo (leucemia mieloide aguda) y trasplantados de progenitores hematopoyéticos (ECIL 5-2013). Sin embargo, otras situaciones que también presentan riesgo de aspergilosis, como son por ejemplo la leucemia linfoide aguda, no tienen recomendaciones claramente establecidas. En la actualidad, el disponer de diferentes antifúngicos permite realizar una profilaxis individualizada valorando eficacia, tolerabilidad e interacciones.

Sin embargo, en el paciente sometido a TOS la profilaxis antifúngica no está tan estandarizada como en el paciente hematológico. Como se ha comentado previamente, los pacientes TP presentan un mayor riesgo de desarrollo de IFI (1, 55), debido posiblemente a la presencia de factores de riesgo característicos de los TP como son un elevado nivel de inmunosupresión, la presencia de alteración de las defensas pulmonares locales secundarias al trasplante (pérdida de vasos linfáticos, motilidad mucociliar reducida, disminución del reflejo de la tos) y el contacto constante del aloinjerto con los hongos filamentosos que son ubicuos en al ambiente. A pesar de los avances en medicamentos antimicóticos, la infección por *Aspergillus* spp. se asocia con una elevada mortalidad (52-54%) en este grupo de población (56). Este hecho ha condicionado que la mayoría de centros que realizan trasplante de pulmón hayan adoptado

estrategias profilácticas antifúngicas (57-59), aprobadas en la mayoría por las Sociedades Internacionales, aunque con un bajo nivel de evidencia (60). Sin embargo, queda todavía por determinar el fármaco antifúngico óptimo, si la profilaxis debe ser universal o dirigida y la duración de la estrategia profiláctica (61).

En nuestro centro, en abril de 1993 empezamos a utilizar una pauta de profilaxis para la infección por *Aspergillius* spp. con anfotericina B desoxicolato nebulizada (n-ABD) en todos los TP y de por vida. En julio de 2003, n-ABD se cambió a anfotericina liposomal nebulizada (n-LAB) por una rotura de stock de n-ABD en el mercado español. Los primeros resultados sobre el uso de n-LAB fueron reportados en un estudio previo, que incluía los primeros 104 TP, que habían recibido n-LAB profiláctica, seguidos durante al menos 12 meses. La estrategia con n-LAB resultó eficaz, con una incidencia de 1.2% de API (62). Además, a lo largo de estos años, hemos observado en estudios de farmacocinética que tras la administración de n-LAB (25 mg), las concentraciones se mantenían elevadas a nivel pulmonar durante 14 días y eran las adecuadas para la prevención de la IFI, por lo que decidimos usar este intervalo de administración (63). Otras publicaciones han informado de la buena tolerabilidad y de un perfil óptimo de seguridad de la n-LAB (63) (64) (65), sin evidencia de absorción sistémica significativa, efectos sobre la función respiratoria, o cambios en el contenido de lípidos del surfactante pulmonar (66), resultados que permiten la administración a largo plazo.

I.8. Aparición de resistencias a los fármacos antifúngicos

Aunque la resistencia a los antifúngicos no se consideraba un problema significativo en los hongos filamentosos, en los últimos años están emergiendo problemas de resistencia con un profundo impacto en la salud humana. Los mecanismos de resistencia antifúngica pueden ser innatos, primarios y secundarios y dependen de las características intrínsecas o adquiridas de los patógenos fúngicos (67) (68).

Voriconazol es el antifúngico de elección para la API, siendo considerada la anfotericina B como una alternativa y las equinocandinas como fármacos de segunda línea (69) (70). Se han aislado *Aspergillus* spp. resistentes a voriconazol tanto en pacientes naïve, no tratados previamente, como en pacientes expuestos a voriconazol por resistencia adquirida o por selección de especies intrínsecamente resistentes (71) (72) (73). Además, se ha comprobado que algunas de esta resistencia son cruzadas entre azoles (voriconazol, itraconazol, ravuconazol o posaconazol). La resistencia de *Aspergillus* spp. a los azoles podría estar relacionada con el uso masivo de antifúngicos en tratamientos empíricos y en profilaxis. Además, al ser los únicos agentes disponibles en la formulación oral, estos se utilizan en las infecciones crónicas y, a menudo durante largos períodos de tiempo, pudiendo aumentar las resistencias (74). Por otra parte, los triazoles son utilizados tanto en la medicina clínica como en la agricultura, Recientemente ha sido reportada en la literatura la aparición de cepas resistentes en el "medio ambiente" y la presencia de genes con mecanismo de resistencia (mutación TR-L98H del gen que codifica cyp51A) con carácter de clonalidad, siendo este fenómeno particularmente extendido en algunos países, como en los Países Bajos y en el Norte Europa (75) (76, 77) (78) (79). El peligro real del uso masivo de azoles en agricultura supone la ocupación del nicho ecológico cercano al ser humano por especies resistentes, que podrían desplazar a las especies sensibles y cambiar la población de hongos que infectan al ser humano.

En el momento actual los mecanismos de resistencia de *Aspergillus* spp. a otros fármacos antifúngicos son poco conocidos. Se ha observado la presencia de resistencia intrínseca a la anfotericina B (AB) en cepas de *A. terreus*, A. *flavus* (resistencia ente 10-15%) y de otras especies menos comunes (80) (81). Además, a pesar que el desarrollo de resistencia durante el tratamiento con AB es raro, en estudios anteriores, los aislamientos de *Aspergillus* spp. en pacientes en tratamiento previo han mostrado concentraciones mínimas inhibitorias más altas

que los de los pacientes sin exposición a AB (82) (83). Finalmente, ha sido también descrita la aparición de resistencia *in vitro* e *in vivo* para equinocandinas durante el tratamiento (84).

Estos datos evidencian, por una parte, la necesidad de un uso correcto de los antifúngicos a las dosis indicadas y durante el tiempo adecuado. En segundo lugar, sugieren la importancia de realizar estudios de vigilancia (tanto local como regional) y finalmente, de optimizar los métodos para la detección de las resistencias y la caracterización de los mecanismos asociados para mejorar la eficacia de la terapia antifúngica.

I.9. Cambios en la mortalidad por aspergilosis

Pese a que datos recientes sugieran una mayor supervivencia de los pacientes al compararla con la década del los 90, la mortalidad relacionada con la API sigue siendo alta (mayor del 50%) en la mayoría de los casos (85)(86)(1).

El conocimiento sobre la real causa de muerte en pacientes con API sigue siendo escaso. Se carece de estudios enfocados concretamente a los procesos que conducen a la muerte en estos enfermos y no hay un consenso claro acerca de cómo definir la causa de la muerte de un paciente, el papel que la API puede haber jugado en este evento y el tiempo trascurrido desde el inicio de la infección hasta el exitus (en la mayoría de los estudios se habla de mortalidad global a los 90 días) (85) (86) (1). Esta falta de conocimiento limita la comprensión de las estrategias óptimas de tratamiento, que podrían ayudar a mejorar el pronóstico.

Con la finalidad de describir las causas actuales de mortalidad en los pacientes con API, hemos realizado un estudio (Anexo 2) retrospectivo multicéntrico (2008-2011) incluyendo un número elevado de pacientes con API (152 pacientes). En este estudio la mortalidad global a los 90 días era del 60.5%. La mortalidad fue clasificada como relacionada a la API en el 67.4% de los exitus, siendo la principal causa, la presencia de insuficiencia respiratoria. La mayoría de las muertes relacionadas con la API (83.9%) ocurrieron dentro de los primeros 21 días después del diagnóstico. De hecho, la mortalidad relacionada con API representó el 97.6% de las muertes

que ocurrieron dentro de los primeros 14 días. Los restantes casos de muerte fueron clasificados como no-relacionados (32.6%), siendo la principal causa la presencia de bacteriemia y representando el 68.6% de las muertes entre los días 21 y 90 después del diagnóstico. Estos resultados sugieren que la evaluación de la supervivencia a los 90 días después del diagnóstico (un criterio utilizado por muchos investigadores) (85) (86) es un indicador impreciso de la eficacia del tratamiento, porque incluye un gran número de muertes por causas que no están relacionadas con la IFI.

Otro hallazgo del estudio es que la presencia de la enfermedad hepática se asociaba de forma independiente con un mayor riesgo de mortalidad relacionada con la API, tal como ha sido previamente documentado en estudios realizados en receptores de TPH (85). Hemos constatado que el tratamiento con voriconazol se asociaba de forma independiente con un menor riesgo de muerte relacionada a la API, tal como se había descrito recientemente en estudios observacionales (85) (86) y en un ensayo aleatorizado (69).

I.10. Enfermedad fúngica causada por hongos filamentosos emergentes

Aunque *Aspergillus* spp. siguen siendo el hongo filamentoso más comúnmente asociado a IFI, durante la pasada década, se han ido comunicando infecciones debidas a hongos considerados poco frecuentes, pero que han adquirido creciente protagonismo como causantes de infección invasora (87). Estudios recientes sobre hongos filamentosos distintos de *Aspergillus* spp. han reportado cuadros de infecciones posteriores a desastres naturales (88) y brotes de casos después de inoculaciones yatrógenas (89) (90), evidenciando el potencial de estos patógenos ambientales para causar enfermedad grave tanto en pacientes inmunodeprimidos como en inmunocompetentes.

Dependiendo del estado inmunológico del huésped y la vía de infección (inoculación cutánea o inhalación) los hongos filamentosos distintos de *Aspergillus* spp. (fundamentalmente las especies de los géneros *Mucorales*, *Fusarium* spp., *Scedosporium* spp. y otros dematiáceos)

pueden causar un amplio espectro de enfermedades (87)(91). La incidencia de estos hongos varía según la zona geográfica. En la última década supone alrededor del 10% de las micosis por hongos filamentosos en los TPH y hasta un 19-30% en los TOS (23, 56, 92) (93). La aparición de estos patógenos raros parece estar en relación con el uso de profilaxis antifúngica. El reciente incremento de los casos mucormicosis ha sido temporalmente vinculado en muchos centros de trasplante a la introducción y el uso generalizado de voriconazol y equinocandinas, fármacos que carecen de actividad contra los *Mucorales* (94) (95). No es sorprendente que el aumento de los casos de mucormicosis se asocie a menudo con una disminución en el incidencia de casos documentados de aspergilosis invasiva (95). Sin embargo, no se sabe si esta asociación refleja una verdadera relación epidemiológica o representa un marcador de cambio en la inmunosupresión que ocurre en paralelo con la evolución de prácticas de trasplante y las estrategias terapéuticas (95).

Este cambio epidemiológico es preocupante, ya que los hongos filamentosos distintos de *Aspergillus* spp. son a menudo resistentes a los agentes antifúngicos convencionales. Anfotericina B liposomal presenta un espectro antifúngico amplio; sin embargo, ciertos hongos (por ejemplo, *Scedosporium apiospermum*, *Scedosporium prolificans*, *Paecilomices* spp.) pueden presentar resistencia innata o susceptibilidad variable a la AB. Otros hongos tales como *S. prolificans* son resistentes a todos los antifúngicos actualmente disponibles, condicionando un manejo clínico complejo y una elevada mortalidad (96) (97) (98) (99).

En los TP hay pocos datos sobre la epidemiologia de la IFI causada por estos hongos (100) (56) (23) (87) y se desconoce la influencia que pueda tener el uso de profilaxis antifúngica previa sobre la selección de estas especies en este grupo de población.

II. JUSTIFICACIÓN DEL ESTUDIO E HIPÓTESIS DE TRABAJO

II. JUSTIFICACIÓN DEL ESTUDIO E HIPÓTESIS DE TRABAJO

La enfermedad fúngica invasora causada por hongos filamentosos representa posiblemente el paradigma más representativo de los cambios epidemiológicos y clínicos, que se han observado en los últimos años. Esta entidad incluye diversos síndromes clínicos que podrían considerarse como un espectro continuo de una misma enfermedad y que son el resultado de la interacción estrecha entre el hongo y el huésped, con un estado de inmunosupresión variada, que puede ser moderado como en el paciente con neoplasia sólida o más severa como en los TP. La IFI causada por hongos filamentosos en conjunto, desde el aspecto puramente epidemiológico hasta el clínico, ha sido el hilo conductor de los diferentes estudios.

II.1. Estudio y trabajo 1

II.1.1. Justificación del estudio 1

La APC incluye un grupo de enfermedades en la frontera entre la enfermedad saprofítica e invasiva. Actualmente esta forma de aspergilosis puede ser infradiagnosticada porque la nomenclatura y los criterios diagnósticos se han definido sólo en los últimos años y porque la sintomatología puede ser a menudo enmascarada por la patología crónica de base.

A lo largo de la práctica clínica, hemos observado la aparición de formas compatibles con APC en los pacientes con neoplasia sólida con afectación pulmonar primaria o secundaria, decidiendo enfocar nuestro interés en este grupo de pacientes de riesgo. La falta de estudios en este campo justifica el interés de desarrollar el presente estudio.

II.1.2. Hipótesis de trabajo 1

Entre los pacientes con neoplasia sólida, aquellos que presentan una afectación pulmonar primaria o metastásica son los que desarrollan con mayor frecuencia una APC debido a la enfermedad pulmonar subyacente y a los diferentes grados de inmunosupresión que presentan.

II.2. Estudio y trabajo 2

II.2.1. Justificación del estudio 2

Aspergillus spp. es la causa más común de infección fúngica invasiva en los TP, asociándose persistentemente con una elevada mortalidad en este subgrupo de población, y parece ser un factor de riesgo para el desarrollo de rechazo crónico. Entre las diferentes estrategias profilácticas con fármacos antifúngicos, la profilaxis usada en nuestro centro con n-LAB ha demostrado tener un elevado perfil de seguridad. La pauta inhalada tiene la ventaja de llegar a las zonas más distales del árbol bronquial evitando, al mismo tiempo, las interacciones medicamentosas y efectos secundarios sistémicos. Sin embargo, se carece de información amplia sobre la eficacia de n-LAB para prevenir la infección por *Aspergillus* spp. y el impacto que la exposición prolongada a n-LAB podria tener sobre las especies de *Aspergillus*.

II.2.2. Hipótesis de trabajo 2

Después de 10 años de experiencia con el uso de este tratamiento preventivo en nuestro centro, consideramos que esta profilaxis podría ser es eficaz, bien tolerada y segura. Sin embargo el uso de n-LAB profiláctica a largo plazo podría favorecer la aparición especies resistentes ya sea como colonizadores o como causa de infección. Asimismo la colonización o infección por *Aspergillus* spp. podrían estar asociadas con la aparición de disfunción crónica del injerto en los TP.

II.3. Estudio y trabajo 3

II.3.1. Justificación del estudio 3

Entre los receptores TOS, las infecciones por hongos filamentosos distintos de *Aspergillus* spp. están emergiendo en los últimos años y se ha descrito un aumento en de estas infecciones en los pacientes que reciben agentes antifúngicos activos frente a *Aspergillus* spp. El espectro y el

impacto global de estos hongos patógenos antes y después del trasplante no han sido definidos plenamente en los receptores de trasplante de pulmón y no hay series publicadas en Europa. Asimismo, se desconoce si este fenómeno de presión fúngica con la aparición de hongos emergentes se asocia también al uso de AB.

II.3.2. Hipótesis de trabajo 3

El aislamiento de hongos filamentosos diferentes de *Aspergillus* spp. antes y después del trasplante podría influir en el posterior riesgo de progresión a IFI. La profilaxis con AB nebulizada podría condicionar la epidemiología de los hongos filamentosos emergentes como causa de infección fúngica invasora en los TP en nuestro medio.

III. OBJETIVOS

III. OBJETIVOS

III.1. Objetivos del primer trabajo

1. Describir los factores predisponentes, la presentación clínica y la evolución de los pacientes con cáncer de pulmón primario o con metástasis pulmonares secundarias que presentan una APC.
2. Definir en qué pacientes el aislamiento de *Aspergillus* spp. en muestras respiratorias debe inducir un elevado nivel de sospecha de infección para un diagnóstico precoz.

III.2. Objetivos del segundo trabajo

1. Evaluar la eficacia, tolerabilidad y seguridad de la profilaxis con n-LAB tras 10 años de experiencia en los pacientes TP.
2. Investigar el impacto que puede tener la profilaxis con n-LAB a largo plazo sobre la evolución de la infección y/o colonización por *Aspergillus* spp. en los TP.
3. Definir si existe asociación entre infección por *Aspergillus* spp. y disfunción crónica del injerto.

III.3. Objetivos del tercer trabajo

1. Describir el impacto clínico que puede tener el aislamiento de hongos filamentosos no-*Aspergillus* spp. pre y post-trasplante en el riesgo de progresión a IFI en los pacientes TP.
2. Investigar la relación existente entre el uso de profilaxis con n-LAB y la infección por hongos filamentosos no-*Aspergillus.*

IV. MÉTODOS

IV. MÉTODOS

IV.1. TRABAJO 1

IV.1.1. Población y diseño del estudio

En el trabajo 1 se realizó un estudio observacional, retrospectivo incluyendo todos los casos de pacientes adultos con neoplasia sólida diagnosticados de APC en el Hospital Vall d'Hebron, hospital universitario terciario de Barcelona, entre los años 2008 y 2011. Para ampliar el número de pacientes se solicitó la colaboración de otros hospitales que estaban realizando estudios de seguimiento epidemiológico similares. En concreto, se colaboró con el Hospital de Bellvitge y el Hospital Clínic (Barcelona).

IV.1.2. Criterios de inclusión

Se estudiaron los pacientes diagnosticados de APC, que cumplían los siguientes criterios:

- Pacientes mayores de 18 años
- Pacientes con neoplasia sólida primaria pulmonar
- Pacientes con neoplasia sólida con metástasis secundarias pulmonares
- Diagnóstico de APC (subtipo IPA subaguda), establecido siguiendo los criterios de Denning y colaboradores (29) (30) (Tabla 1)

IV.1.3. Recogida de información

La recogida de información de los pacientes se llevó a cabo a través de las bases de datos hospitalarios del Hospital General, Servicio de Microbiología y Servicio de Anatomía Patológica (Hospital Vall d'Hebron, Hospital Clínic, Hospital de Bellvitge), con un protocolo de recogida de datos previamente definido. De forma esquemática las variables que se incluyeron son:

- Datos demográficos: datos de filiación
- Enfermedad actual: enfermedad neoplásica de base y estadio de clasificación

- Antecedentes patológicos: historia de enfermedades previas o concomitantes pulmonares (TBC antigua, EPOC, neumotórax etc.) y tratamientos que condicionen mayor susceptibilidad a tener una APC (corticoides, quimioterapia, radioterapia, anticuerpos monoclonales)

- Datos de presentación clínica de la enfermedad:

- Síntomas clínicos (fiebre, tos, hemoptisis, disnea, dolor pleurítico etc.…).
- Patrón radiológico valorado por tomografía computarizada (TC) torácica a elevada resolución previa al diagnóstico de APC y al diagnóstico de APC (masa, cavidad, masa cavitada, consolidación, nódulos, cavitación, absceso, derrame pleural)

- Datos microbiológicos de la enfermedad: muestras respiratorias (esputo, LBA, broncoaspirado, punción aspiración con aguja fina pulmonar, biopsia pulmonar), con aislamiento, sensibilidad antifúngica e identificación de especie de *Aspergillus* spp.

- Datos respecto a tratamientos: tratamiento antifúngico, cambio de tratamiento antifúngico, duración del tratamiento, necesidad de intervención quirúrgica

- Datos evolutivos de la enfermedad:

- respuesta al tratamiento
- evolución del patrón radiológico por TC torácico (masa, cavidad, masa cavitada, consolidación, nódulos, cavitación, absceso, derrame pleural)
- mortalidad relacionada o no relacionada

IV.1.4. Definiciones

Las definiciones se establecieron siguiendo los criterios existentes en la literatura.

Para definir los casos de aspergilosis pulmonar invasora probada o probable se usaron los criterios de la European Organization for Research and Treatment of Cancer /Mycoses Study (70). El diagnóstico de APC se hizo según la clasificación de Denning y colaboradores como se describe en la Tabla 1. La APC incluye el aspergiloma, la aspergilosis pulmonar crónica cavitaria, aspergilosis pulmonar fibrosante crónica y la API subaguda (29) (30).

Se consideró neutropenia un recuento absoluto de neutrófilos <500/ mm3 en el momento de inicio de la infección.

Se consideró el tratamiento esteroideo cuando los pacientes recibían una dosis diaria de prednisona de 10 mg o equivalente durante el último mes, dado como tratamiento habitual o en bolus.

El antígeno de galactomanano se detectó en el lavado broncoalveolar utilizando el kit de inmunoensayo enzimático comercial Platelia ™ Aspergillus (Bio-Rad), de acuerdo con las instrucciones del fabricante. Un valor > 0.5 se consideró positivo.

La respuesta al tratamiento antifúngico se definió como resolución, mejoría o estabilidad de la clínica, de las muestras microbiológicas y de los hallazgos radiológicos. El fracaso del tratamiento antifúngico se definió como la progresión de los síntomas clínicos y/o persistencia de cultivo positivo para las mismas especies de *Aspergillus* spp y/o empeoramiento de los hallazgos radiológicos a pesar del tratamiento.

IV.2. TRABAJO 2 Y TRABAJO 3

IV.2.1. Población y diseño del estudio

En el trabajo 2 y 3 se estudiaron todos los pacientes adultos trasplantados de pulmón desde Julio 2003 hasta Julio 2013 en el Hospital Vall d'Hebron. La recogida de datos se realizó de forma retrospectiva hasta Julio 2014.

IV.2.2. Criterios de inclusión

Se estudiaron todos los pacientes trasplantados de pulmón, que cumplían los siguientes criterios:

- Pacientes mayores de 18 años.
- Pacientes que habían sobrevivido más de 24 horas después del trasplante.
- Paciente con seguimiento mínimo durante al menos 1 año o hasta la muerte.

- Pacientes en tratamiento profiláctico con n-LAB.

IV.2.3. Recogida de información

La recogida de información de los pacientes se llevó a cabo a través de las base de datos hospitalarios del Hospital General, Servicio de Microbiología y Servicio de Anatomía Patológica, a través de un protocolo de recogida de datos previamente definido. De forma esquemática las variables que se incluyeron fueron:

- Datos demográficos: datos de filiación.

- Enfermedad previa a trasplante (EPOC, fibrosis, fibrosis quística etc.).

- Presencia de colonización previa (hasta 1 año antes) y posterior al trasplante por hongos filamentosos.

- Factores de riesgo para IFI: colonización crónica por bacterias gram negativas, estenosis bronquial, disfunción crónica del injerto (DCI), rechazo agudo, enfermedad por CMV, stent bronquial, colonización pre-trasplante por hongos filamentosos, inhalación masiva, aumento de inmunosupresión, abandono de profilaxis, inmunosupresión de inducción, trasplante unipulmonar.

- Datos de presentación clínica de la enfermedad:

  - Patrón broncoscópico (mucosidad, placas, pseudomembranas, úlceras necrosantes, mucosa eritematosa, normal).
  - Patrón radiológico valorado por TC de alta resolución al diagnóstico de IFI (nódulos, cavitaciones, infiltrados, consolidaciones, normal).

- Datos microbiológicos de la enfermedad: muestras respiratorias (esputo, LBA, broncoaspirado, aspirado traqueal, biopsia pulmonar) con aislamiento, sensibilidad antifúngica e identificación de la especie de los hongos filamentosos.

- Datos respecto a tratamientos: tratamiento antifúngico, cambio de tratamiento antifúngico, duración del tratamiento, necesidad de intervención quirúrgica.

- Datos respecto a la profilaxis: efectos adversos (broncoespasmo, nauseas, mareos), abandono de la profilaxis.

- Datos evolutivos de la enfermedad:

- respuesta al tratamiento.
- mortalidad relacionada o no relacionada.

IV.2.4. Definiciones

Las definiciones se establecieron siguiendo los criterios presentes en la literatura.

Para definir los casos de aspergilosis pulmonar invasora probada o probable se usaron los criterios de la European Organization for Research and Treatment of Cancer /Mycoses Study. (70). La clasificación de aislamiento único, colonización e infección por *Aspergillus* spp. y otros hongos filamentosos esta descrita en la Tabla 3 (62) (70) (101).

Para definir la colonización pre-trasplante por *Aspergillus* spp. y hongos filamentosos no-*Aspergillus* spp. se incluyeron las colonizaciones desde como mínimo 1 año antes del trasplante en adelante y las muestras respiratorias intraoperatorias positivas del órgano explantado. La traqueobronquitis ulcerativa y la aspergilosis pulmonar invasiva se consideraron como infecciones fúngicas invasoras (IFI), mientras que traqueobronquitis simple, la infección del stent bronquial y el aspergiloma del pulmón nativo fueron clasificados como infecciones fúngicas no invasoras (no-IFI).

La infección y colonización por hongos filamentosos se clasificaron en inicio temprano, si ocurrían <90 días después del trasplante y de inicio tardío, si ocurrían > 90 días después. (36)

La respuesta al tratamiento se clasificó como éxito o fracaso, tal como se ha descrito previamente en la literatura (69).

La mortalidad fue considerada relacionada con la enfermedad fúngica si era la causa o jugaba un papel importante en la muerte del paciente, y sin relación si jugaba un papel menor o ningún papel.

Tabla 3. Clasificación de aislamiento, colonización e infección por hongos filamentosos modificada (62) (70) (102).

Aislamiento único	Un cultivo positivo de esputo o aspirado traqueal para hongos filamentosos en pacientes asintomáticos con mucosa respiratoria de apariencia normal y ausencia de lesiones endobronquiales.
Colonización	Un cultivo único positivo de muestras de broncoscopio (broncoaspirado, lavado broncoalveolar o biopsia bronquial) o al menos dos cultivos positivos de esputo o aspirado traqueal positivos para hongos filamentosos en pacientes asintomáticos con aspecto normal de la mucosa respiratoria y ausencia de lesiones endobronquiales.
INFECCION POR HONGOS FILAMENTOSOS	
Traqueobronquitis simple	Aislamiento de hongo filamentosos, asociado a síntomas clínicos (por ejemplo, producción de esputo purulento), además de los resultados de la broncoscopia con la presencia de mucosidad o mucosa roja edematosa, habiéndose descartado la presencia de infección bacteriana.
Infección de stent bronquial	Aislamiento de hongo filamentosos, asociado a síntomas clínicos (por ejemplo, producción de esputo purulento), además de los resultados de la broncoscopia con presencia de mucosidad o mucosa roja edematosa (habiéndose descartado la presencia de infección bacteriana) en paciente portador de stent bronquial.
Traquobronquitis ulcerativa o pseudomembranosa	Aislamiento de hongos filamentosos en la biopsia bronquial y/o hallazgos en la broncoscopia de úlceras necróticas o pseudomembrana en la anastomósis o en el árbol traqueobronquial que desaparecen después del tratamiento.
Infección fúngica pulmonar invasiva (incluye API)	Aislamiento de hongos filamentosos con evidencia de daño tisular en la histopatología pulmonar o signos radiológicos de infección fúngica invasiva.

IV.2.5. Procedimientos de Laboratorio de Microbiología

En nuestro hospital las muestras broncoscopicas (LBA, broncoaspirado, biopsia bronquial) se someten de rutina a un examen microscópico antes del cultivo (tinción de Gram) y se inoculan en medios sólidos y líquidos (agar Sabouraud suplementado con gentamicina y cloranfenicol, y la infusión de cerebro y corazón suplementado con gentamicina y cloranfenicol). Tanto el esputo como el aspirado traqueal se inoculan solamente en el agar Sabouraud suplementado con antibióticos. Las muestras se incuban a 25ºC en atmósfera ambiente durante al menos 15 días, prolongando el período de incubación, cuando hay una elevada sospecha de infección por hongos.

IV.2.6. Análisis estadístico

Para el análisis estadístico se utilizó el programa estadístico STATA (versión 11.0). Las variables categóricas se definieron como número de casos y proporción, y se compararon mediante la prueba de Chi-cuadrado o, en los casos que lo requerían, el Test Exacto de Fisher. Las variables cuantitativas se definieron como media y desviación estándar, y se compararon con la prueba T de Student o, en los casos necesarios con la prueba no paramétrica de U de Mann-Whitney. Las diferencias de medias de incidencia entre los periodos se evaluaron utilizando el test de Mantel-Haenszel. El porcentaje de cambio (razón de odds) en la incidencia se presentó asociado a su intervalo de confianza del 95%. La influencia de colonización o infección por hongos filamentosos en el tiempo para el desarrollo posterior de la DCI y la relación inversa se estudiaron con el análisis de regresión de Cox de riesgos proporcionales: el inicio del DCI y la colonización o infección por hongos filamentosos eran covariables dependientes del tiempo. Todos los análisis estadísticos se llevaron a cabo considerando un nivel de significación o valor de p menor de 0.05.

IV.2.7. Confidencialidad de los datos y consentimiento informado

La recogida de datos se hizo siguiendo los protocolos previamente establecidos y los datos se introdujeron en una base de datos con un sistema de codificación con el fin de proteger la información personal de cada paciente. En la hoja de recogida de datos, el código se relacionó con el número de historia clínica. Estas hojas son custodiadas por el investigador responsable. El estudio fue aprobado por el Comité Ético del hospital coordinador (Hospital Vall d'Hebron). El consentimiento informado no fue necesario dado el carácter retrospectivo del estudio.

V. RESULTADOS

V. RESULTADOS

Partiendo de la hipótesis de trabajo, el estudio se llevó a cabo mediante la elaboración de tres trabajos diferentes, que tratan de dar respuesta a cada uno de los objetivos planteados. La exposición de los resultados se estructurará en tres partes, correspondientes a cada uno de los trabajos.

Además, considerando que este trabajo constituye un estudio relativo a IFI en pacientes inmunodeprimidos, a continuación, como parte final de la memoria, se añaden en forma de apartado Anexo varias publicaciones relacionadas que se realizaron durante la elaboración del presente trabajo, consistentes en dos artículos originales y una revisión.

El primer artículo (Anexo 1), titulado "Environmental variables associated with an increased risk of invasive aspergilosis", trata de identificar la relación entre el recuento de esporas en el ambiente y los ingresos hospitalarios por API. Además en este estudio se evalúan los efectos de las variables climáticas y de los virus respiratorios sobre el riesgo de desarrollo de la API.

En la segunda publicación (Anexo 2), titulada "Causes of death in a contemporary cohort of patients with invasive aspergilosis" se trata de estudiar las causas inmediatas de mortalidad en los pacientes con API y el papel de la API como causa de exitus. Asimismo se evalúa el "timing" del evento exitus en estos pacientes y los factores de riesgo asociados a la mortalidad relacionada con la API.

El tercer articulo (Anexo 3) titulado "Infecciones por hongos filamentosos en el paciente inmunosuprimido: profilaxis y tratamiento" revisa las novedades y cambios más importantes que se han producido en la profilaxis y el tratamiento de la IFI en los últimos años.

V.1. Trabajo 1

Estudio que describe las características de la APC en los pacientes con cáncer de pulmón primario o metástasis pulmonares secundarias.

V.1.1. Artículo

Peghin M, Ruiz-Camps I, Garcia-Vidal C, Cervera C, Andreu J, Martin M, Gavaldá J, Gudiol C, Moreno A, Felip E, Pahissa A. "Unusual forms of subacute invasive pulmonary aspergilosis in patients with solid tumors". J Infect. 2014 Oct.

V.2. Trabajo 2

Estudio que evalúa la eficacia, tolerabilidad y seguridad la profilaxis con n-LAB para la prevención de la infección por Aspergillus spp. en los pacientes trasplantados de pulmón. Además investiga el impacto a largo plazo del uso de n-LAB profiláctica sobre la evolución de la infección y colonización por *Aspergillus* spp.

V.2.1. Artículo

M. Peghin, V. Monforte, M. Martin-Gomez, I. Ruiz-Camps, C.Berastegui, B. Saez, J. Riera, P. Usetti, J. Solé, J. Gavaldá, A. Roman. "10 years of prophylaxis with nebulized liposomal amphotericin B and the changing epidemiology of *Aspergillus* spp. infection in lung transplantation" **.** Transpl Int. 2015 Sep.

V.3. Trabajo 3

Estudio que describe la epidemiología de las infecciones por hongos filamentosos no-*Aspergillus* spp. en los pacientes trasplantados de pulmón bajo profilaxis con n-LAB y el valor de los cultivos positivos pre y post-trasplante.

V.3.1. Artículo

M. Peghin, V. Monforte, M. Martin-Gómez, I. Ruiz-Camps, C.Berastegui, B. Saez, J. Riera, J. Solé, J. Gavaldá, A. Roman. "Epidemiology of Invasive Respiratory Disease Caused by Emerging Non-*Aspergillus* molds in Lung Transplant Recipients"**.** Transpl Infect Dis. 2015 Oct.

Anexo 1:

Estudio que evalúa la relación entre el recuento de esporas en el ambiente, los efectos de las variables climáticas y los virus respiratorios sobre el riesgo de desarrollo de API.

Artículo

Garcia-Vidal C, Royo-Cebrecos C, Peghin M, Moreno A, Ruiz-Camps I, Cervera C, Belmonte J, Gudiol C, Labori M, Roselló E, de la Bellacasa JP, Ayats J, Carratalà J. "Environmental variables associated with an increased risk of invasive aspergillosis". Clin Microbiol Infect. 2014 Nov

Anexo 2:

Revaloración del concepto de mortalidad en los pacientes con AI. Estudio del papel de la API como causa de exitus y de los factores de riesgo asociados a la mortalidad relacionada con la API.

Artículo:

Garcia-Vidal C, Peghin M, Cervera C, Gudiol C, Ruiz-Camps I, Moreno A, Royo-Cebrecos C, Roselló E, de la Bellacasa JP, Ayats J, Carratalà J. "Causes of death in a contemporary cohort of patients with invasive aspergilosis". PLoS One. 2015 Mar.

Anexo 3

Revisión de las novedades y de los cambios más importantes que se han producido e la profilaxis y en el tratamiento de la enfermedad fúngica invasiva en la última década. En este trabajo se repasa desde la aparición de nuevos factores de riesgo de presentar IFI, hasta las nuevas estrategias profilácticas y terapéuticas.

Revisión:

Ruiz-Camps I, Peghin M. "Infecciones por hongos filamentosos en el paciente inmunosuprimido: profilaxis y tratamiento". Rev Esp Quimioter. 2015 Sep.

V.4. Artículos publicados

V.4.1. ARTÍCULO 1

Journal of Infection (2014) 69, 387–395

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Unusual forms of subacute invasive pulmonary aspergillosis in patients with solid tumors

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KEYWORDS
Aspergillus;
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Chronic pulmonary aspergillosis;
Solid tumors;
Solid cancer;
Lung cancer;
Lung metastasis

Summary *Objectives: Aspergillus* spp. can cause acute invasive disease in severely immunocompromised patients. Nonetheless, there are few reports of solid tumors complicated with subacute invasive pulmonary aspergillosis (subacute IPA).
Methods: Retrospective observational cohort study, performed in patients with primary lung cancer or secondary lung metastasis complicated with subacute IPA in three referral hospitals.
Results: From 2008 to 2011, 14 episodes of subacute IPA were diagnosed, including 11 (78.6%) probable and 3 proven (21.4%). Nine patients (64.3%) had primary lung cancer. Thirteen patients (92.9%) had more than one local or systemic predisposing factor for subacute IPA. No patient had previous fungal colonization. *Aspergillus* spp. was isolated in 6 specimens of bronchoalveolar lavage, 6 sputum, 2 biopsies, and 1 percutaneous lung puncture. At the time *Aspergillus* spp. was isolated, the most common radiologic findings on chest computed tomography (CT) were cavitary masses, and development or expansion of cavitation in existing masses or nodules (10/14, 71.4%). On CT follow-up, most patients (8/12, 66.7%) had new cavity

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; CPA, chronic pulmonary aspergillosis; CNPA, chronic pulmonary aspergillosis; CT, computed tomography; HIV, human immunodeficiency virus; IPA, invasive pulmonary aspergillosis.

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formation or expansion of one or more existing cavities. All patients were treated with azoles and two underwent surgery. Ten (71.4%) patients died after *Aspergillus* spp. was detected (median time 73 days, IQR 33–243): 2 (20%) deaths were subacute IPA-attributable and 6 (60%) were related.

Conclusions: Primary lung cancer and secondary lung metastasis seem to be triggering factors for *Aspergillus* spp. implantation, and predispose to subacute IPA. Once localized in the damaged lung, the mold can grow and cause or expand cavities. In lung cancer patients, *Aspergillus* spp. detection is associated with a very poor prognosis.

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Introduction

Invasive pulmonary aspergillosis (IPA) encompasses a spectrum of disease related to underlying conditions that compromise the pulmonary and systemic immune response to inhaled *Aspergillus* spp.[1] Classically, IPA has been described as a serious acute disease in severely immunocompromised hosts, such as those with prolonged neutropenia[2] and recipients of high doses of glucocorticoids.[3] Nowadays, the profile of patients considered at risk of invasive aspergillosis is expanding, as IPA is seen to occur in hosts that are less immunosuppressed, such as patients with chronic obstructive lung disease (COPD)[4] or chronic liver disease[5] and those hospitalized in the intensive care unit.[6] Furthermore, in less severely immunosuppressed patients, IPA can manifest with unusual clinical and pathologic features, such as those of subacute invasive pulmonary aspergillosis (subacute IPA) or chronic necrotizing pulmonary aspergillosis (CNPA), a locally invasive form of this infection.[7,8]

Growing evidence suggests that patients with solid malignancies are an emerging group among the new hosts at risk.[9] However, the current knowledge of solid lung tumors complicated with IPA has come from single case reports or single-center series.[9–14] There are no reports specifically focused on subacute IPA in patients with primary lung cancer and secondary lung metastasis. The diagnosis is difficult in this population, because the clinical and radiologic signs are nonspecific and could be attributed to cancer, and the sensitivity of fungal culture is low. Hence, this entity may be underdiagnosed.

The aim of this study is to describe the predisposing factors, clinical presentation, and outcome of patients with primary lung cancer or secondary lung metastasis complicated with subacute IPA.

Patients and methods

Study setting and patient population

A retrospective, observational, cohort study of all consecutive cases of invasive aspergillosis from January 2008 to December 2011 was performed in three tertiary care university hospitals in Barcelona, Spain. We identified all cases of proven or probable subacute IPA in patients with primary lung cancer or secondary lung metastasis. Cases were detected through the General Hospital, Microbiology, and Histopathology databases, using a standardized protocol. All case report forms and available radiologic results were carefully reviewed by a single investigator.

Definitions

The criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG)[15] were used to define cases of proven or probable IPA.

Chronic pulmonary aspergillosis (CPA) includes aspergilloma, chronic cavitary pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA), and CNPA or subacute IPA. Classification of subacute IPA was made following Denning's et al. definitions,[1,7,8] as described in Table 1 and patients with subacute IPA were included.

Neutropenia was established on an absolute neutrophil count of $<500/mm^3$ at the onset of infection.

Corticosteroid therapy was defined as an amount of corticosteroid (the equivalent of 10 mg daily doses of prednisone during the last month) given as therapy or pulses.

Galactomannan was detected in bronchoalveolar lavage (BAL) using the Platelia™ *Aspergillus* commercial enzyme immunoassay kit (Bio-Rad), according to the manufacturer's instructions. A value >0.5 was considered positive.

Response to antifungal treatment was defined as resolution, improvement, or stability of clinical, microbiological, and radiologic manifestations of subacute IPA. Failure of antifungal treatment was established on progression of the clinical symptoms and/or positive culture for the same *Aspergillus* species and/or worsening of radiologic manifestations of subacute IPA, despite therapy.

Mortality was classified as follows: *subacute IPA-attributable* if subacute IPA was the cause of death or played a major role in the patient's death; *subacute IPA-related* if subacute IPA played a minor role in the patient's death; and *subacute IPA-unrelated* if subacute IPA had no role in the cause of death.

Statistical analysis

A descriptive analysis was performed. Continuous variables were expressed as the median and range. All proportions were calculated as percentages of patients with available data. Data analysis was performed with SPSS Statistics 16 (IBM SPSS, Chicago, IL).

Results

Epidemiology of invasive aspergillosis and solid cancer

During the four-year study period, we identified 180 patients with invasive aspergillosis, and 90% of them had lung involvement (IPA). Overall, a solid tumor was a host factor for IPA in 18 patients (10%). Fourteen cases (77.7%) were ultimately classified as subacute IPA.

Table 1 Glossary of terms by Denning's et al. modified.

Acute invasive aspergillosis	Invasive aspergillosis, usually in markedly immunocompromised patients, occurring over,1 month. May be angioinvasive or not. Radiological features include nodules or progressive consolidation often with a ''halo'' sign inferring angioinvasive disease, cavity (or air crescent) formation, pleural effusion and miliary appearance (if associated with massive spore exposure). If biopsy is performed, hyphae are visible in destroyed lung tissue. Most diagnoses inferred from typical radiological appearances on computed tomography scanning, microbiological investigations (i.e. positive Aspergillus antigen), immunocompromised status and rapid pace of disease
Chronic pulmonary aspergillosis (CPA)	
Subacute invasive aspergillosis or chronic necrotizing pulmonary aspergillosis (CNPA)	Invasive aspergillosis, usually in mildly immunocompromised patients, occurring over 1–3 months, with marked pleitrophic radiological features (cavitation, nodules and progressive consolidation with ''abscess formation''), with hyphae visible in destroyed lung tissue or inferred from microbiological investigations (i.e. positive Aspergillus antigen) and pace of disease
Chronic fibrosing pulmonary Aspergillosis (CFPA)	Severe fibrotic destruction of at least two lobes of lung complicating chronic cavitary pulmonary aspergillosis leading to a major loss of lung function. Usually the fibrosis is in the form of consolidation, but it may be large cavities with surrounding fibrosis. Severe fibrotic destruction of one lobe with a cavity is simply referred to as chronic cavitary pulmonary aspergillosis affecting that lobe
Chronic cavitary pulmonary Aspergillosis (CCPA)	One or more pulmonary cavities which may or may not contain a fungal ball, with serological or microbiological evidence implicating Aspergillus spp. in a non-immunocompromised patient (or one whose immunocompromising condition has remitted or is trivial) with significant pulmonary or systemic symptoms and overt radiological progression (new cavities, increasing pericavity infiltrates or increasing fibrosis) over at least 3 months of observation. If biopsy of the affected area is performed, it demonstrates hyphae with surrounding chronic inflammation and fibrosis but not tissue invasion
Simple aspergilloma	Single pulmonary cavity containing a fungal ball, with serological or microbiological evidence implicating Aspergillus spp. in a non-immunocompromised patient with minor or no symptoms and no radiological progression over at least 3 months of observation
Aspergilloma	An approximately spherical shadow with surrounding air, also called a fungal ball, in a pulmonary cavity, with serological or microbiological evidence that Aspergillus spp. is present in the material. This is a radiological or morphological description, not a disease descriptor

Patient background

The clinical characteristics, microbiological and radiologic findings, treatment, and outcome of 14 patients with primary lung cancer or secondary lung metastasis complicated with subacute IPA are reported in Table 2.

Eleven (78.6%) patients met the criteria of probable subacute IPA and 3 (21.4%) of proven subacute IPA. The patients' characteristics are listed in Table 3. Nine patients (64.3%) had primary lung cancer and 5 (35.7%) secondary lung metastasis. The diagnosis of cancer was histologically proven in all episodes. The most common underlying primary oncological disease of the lung was non-small-cell cancer in 8 patients (57.1%), with a histological diagnosis of adenocarcinoma in 4 cases (28.6%) and squamous cell carcinoma in 4 others (28.6%). Of the 5 patients with secondary lung metastasis, the primary tumor affected the thymus in 2, and the cervix, sigma, and tongue in 1 patient each.

According to the response to oncological treatment, 8 patients presented progressive disease (57.1%), 5 a partial response (35.7%), and 1 stable disease (7.1%).

Local and systemic risk factors

Apart from cancer, 13 cases (92.9%) had more than one local or systemic factor compromising the immune response

Table 2 Clinical characteristics, microbiological and radiologic findings, treatment, and outcome of 18 patients with primary lung cancer or secondary lung metastasis complicated with subacute IPA CTx, chemotherapy during the last month; DM, diabetes; F, female; HIV; Y, years; M, male; NSCLC, no small cell lung cancer; PD, progressive disease; PR, partial response; PTX, pneumothorax; RT, previous radiotherapy; SCLC, small cell lung cancer; SD, stable disease; ST, steroids; TBC, previous tuberculosis.

	Sex/Age	Neoplasia	Comorbidities	Results of cultures for *Aspergillus* spp.	Type of IPA	Chest CT before *Aspergillus* spp. diagnosis	Chest CT at *Aspergillus* spp. diagnosis	Chest CT after Aspergillus spp. diagnosis	Treatment	Death
1	M, 58y	Primary NSCLC, stage III, PR	COPD, CTx , HIV, RT	BAL: *A. fumigatus*	Probable	Mass	Cavitary mass and nodules	Improvement	Voriconazole + amphotericin B.	No
2	M, 73y	Metastatic sigma neoplasia, stage IV, PR	Alcoholism, COPD, PTX, RT, surgery	BAL: *A. fumigatus*	Probable	Cavitary mass and consolidation	Expansion of existing and new cavities formation	Expansion of existing cavity	Voriconazole	No
3	M, 53y	Metastatic tongue neoplasia, stage IV, PD	Cirrhosis, CTx	BAL: *A. fumigatus*	Probable	Cancer and IPA diagnosis overlapped	Cavitary mass and nodules	No CT	Voriconazole	Attributable
4	M, 65y	Primary NSCLC stage III, PD	Alcoholism, COPD, PTX	BAL: *A. flavus*	Probable	Nodule	Mass	Improvement	Voriconazole	Unrelated
5	M, 56y	Metastatic thymoma, stage IV, PD	CTx, myasthenia gravis, RT, ST, tacrolimus	Sputum: *A. fumigatus*	Probable	Nodule	Cavitary Nodule	Expansion of existing cavity with abscess formation	Itraconazole	Related
6	M, 65y	Primary SCLC, stage IV, PD	COPD, DM, PTX, TBC	BAL: *A. fumigatus*	Probable	Cancer and IPA diagnosis overlapped	Mass	Stable consolidation, pleural effusion	Voriconazole	Related
7	F, 55y	Primary NSCLC, stage IV, PD	erlotinib, ST	Lung puncture: *A. fumigatus*	Proven	Mass	Cavitary mass	Expansion of existing cavity	Voriconazole	Unrelated
8	M, 39y	Metastatic thymoma, stage IV, PD	m-Tor inhibitors, RT, surgery	Sputum: *A. fumigatus*	Probable	Multiples masses and nodules	New cavities and abscess formation	No CT	Voriconazole	Related
9	F, 68y	Metastatic cervical neoplasia, stage IV, PR	Alcoholism, CTx	Cutaneous puncture: *A. flavus*	Proven	Cancer and IPA diagnosis overlapped	Cavitary nodule with abscess	No CT	Voriconazole	No
10	M, 62y	Primary NSCLC, stage IV, PD	COPD, ST	Sputum: *A. fumigatus*	Probable	Mass	Mass	New cavity and abscess formation	Itraconazole	Related
11	M, 66y	Primary NSCLC, stage IV, SD	COPD, CTx	Sputum: *A. terreus*	Proven	Cancer and IPA diagnosis overlapped	Mass	Lobectomy	Voriconazole	No

12	M, 64y	Primary NSCLC, stage III, PD	CTx	Sputum: *A. terreus*	Probable	Cancer and IPA diagnosis overlapped	Cavitary mass	Expansion of existing cavities and new cavity formation with abscess	Voriconazole	Related
13	F, 46y	Primary NSCLC, stage III, PR	COPD, alcoholism, neutropenia, CTx, surgery	Sputum: *A. fumigatus*	Probable	Cavitary mass	Expansion of existing and new cavities formation	Improvement	Voriconazole	Related
14	M, 76y	Primary NSCLC, stage III, PR	CTx, RT	BAL: *A. terreus*	Probable	Cavitary mass and nodules	Expansion of existing cavity with abscess formation	Expansion of existing cavity with abscess formation	Voriconazole	Attributable

and predisposing for subacute IPA (Table 3). Only 1 patient in the series had neutropenia.

Clinical features

The median time between the cancer diagnosis and *Aspergillus* spp. detection was 252 days (IQR 42—740). *Aspergillus* spp. colonization had not been detected in any of the patients' previous microbiological studies.

Symptoms at the time of the diagnosis varied and included fever in 5 patients (35.7%), cough in 4 (28.6%), hemoptysis in 4 (28.6%), dyspnea in 4 (28.6%), and chest pain in 4 (28.6%).

Microbiological and histological results

Three (21.4%) patients had a proven diagnosis of aspergillosis by percutaneous lung puncture, surgical lung biopsy and skin biopsy (disseminated disease), respectively. The other patients (78.6%) were diagnosed through a combination of clinical features, radiologic abnormalities, and mycologic evidence (at least one positive culture). Microbiological diagnosis of aspergillosis was performed with 6 (42.9%) BAL specimens, 6 (42.9%) sputum samples, 1 percutaneous lung puncture, 1 surgical lung biopsy, and 1 skin biopsy. *A. fumigatus* was the most common species isolated in 9 samples (64.3%), followed by *A. terreus* in 3 (21.4%), and *A. flavus* in 2 (14.3%). In 8 (57.1%) patients, respiratory samples yielded concomitant bacterial isolates. Only 1 of 3 patients in whom galactomannan was measured in BAL had a positive assay. Galactomannan antigen in serum and *Aspergillus* IgG antibodies (precipitins) were not determined in any case.

Radiological findings

The most common radiologic findings on chest computed tomography (CT), observed in 9 of 14 patients before *Aspergillus* spp. detection, were masses or nodules (6 patients, 66.7%) and cavitary masses (3 patients, 33.3%). In 5 patients, the cancer and subacute IPA diagnosis overlapped. At the time *Aspergillus* was first detected, chest CT showed cavitary masses or expansion of cavitation in existing masses or nodules (10 patients, 71.4%), or the presence of masses (4 patients, 28.6%). CT controls were performed over time in 12 of the 14 patients. Eight (66.7%) patients had a new cavitation or expansion of one or more existing cavities (associated with progressive abscess formation in 4 cases), 3 patients improved, and 1 cured (after surgery) (Figs. 1 and 2). Five of the 8 patients who presented radiologic changes over time had progressive disease (62.5%) and 3 (37.5%) had a partial response.

Treatment and outcome

Once the diagnosis of subacute IPA was suspected or established, all patients received oral or intravenous systemic antifungal therapy. Voriconazole was given as the primary therapy in 11 patients (78.6%), itraconazole in 2 (14.3%) and combined voriconazole plus liposomal amphotericin in 1 (7.1%).

Table 3 Characteristics of 14 patients with primary lung cancer or secondary lung metastasis complicated with subacute IPA. (1) 1 HIV infection, 1 myasthenia gravis, 1 cirrhosis, 1 diabetes (2) 1 erlotinib, 1 M-Tor inhibitor, 1 tacrolimus.

Characteristics	Number of patients (%)
Mean age (SD), years	60.1 (SD 10.1)
Sex	
Male	11 (78.6%)
Female	3 (21.4%)
Type of lung cancer	
Primary lung cancer	9 (64.3%).
Secondary lung metastasis	5 (35.7%)
Cancer stages	
Stage IV	9 (64.3%)
Stage III	5 (35.7%)
Local comorbid lung conditions	
COPD	7 (50%)
Radiotherapy	5 (35.7%)
Previous surgery	4 (28.6%)
Pneumothorax	2(14.3%)
Tuberculosis	1 (7.1%)
Systemic comorbid conditions	
Alcoholism	3 (21.4%)
Others (1)	4 (28.6%)
Previous treatment	
Chemotherapy (last month)	9 (64.3%)
Radiotherapy	3 (21.4%)
Steroids	3 (21.4%)
Others (2)	3(21.4%)
Neutropenia	1 (7.1%)

Two patients underwent surgical resection due to persistent hemoptysis, and one of them died during the intervention. The duration of therapy ranged from 18 days to 20 months. The response to subacute IPA therapy (antifungals, surgery, or both) after 6 weeks of follow-up was 35.7% (5 patients). Overall, 10 patients (71.4%) died after *Aspergillus* spp. were detected (median time 73 days, IQR 33–243), with 2 subacute IPA-attributable (20%) and 6 subacute IPA -related (60%) deaths.

Discussion

Patients with solid malignancies are an emerging group at risk of IPA infection. Previous articles[9–14] have provided some data on invasive aspergillosis in lung cancer patients, but to our knowledge, there are no reports specifically focused on characterizing subacute IPA in a population with primary lung cancer and secondary lung metastasis.

The invasive aspergillosis infection rate in patients with solid tumors varies from 0.70% to 2.63%.[10,13] Some studies[9,11] have reported an association between bronchogenic carcinoma and secondary aspergillosis, with an incidence of IPA in the range of 14.2%–40.6% in this population. This high incidence[9] may be partly explained by systematic performance of follow-up bronchoscopy and various non-standardized tests (*Aspergillus* spp. PCR and serologic tests) in all patients with bronchogenic carcinoma, which could result in detection of *Aspergillus* spp. colonization rather than infection in some cases.

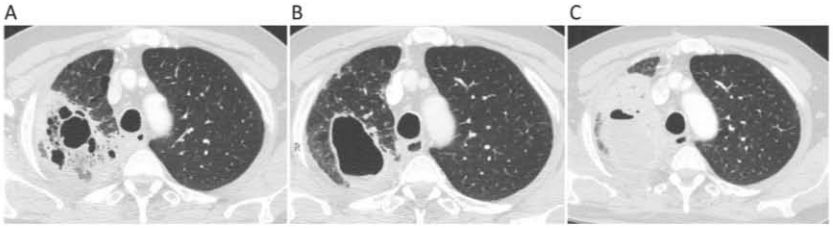

Figure 1 A–C. Patient 14. A) Chest CT scan shows a mass with multiple cavities at the cancer diagnosis. (August 2010). B) Progressive cavitary infiltrate at *Aspergillus* spp. detection (September 2010). C) Progressive consolidation with abscess formation after *Aspergillus* spp. detection (October 2010).

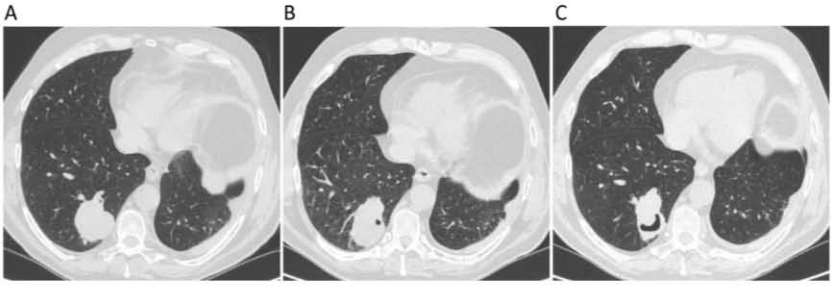

Figure 2 A–C. Patient 7. A) Chest CT scan shows a mass at the cancer diagnosis (May 2010). B) At *Aspergillus* spp. detection (October 2010), a cavitation is seen in the mass. C) Progression of the cavitation after *Aspergillus* spp. isolation (March 2011).

In our cohort, 10% of patients with IPA had a solid tumor as a risk factor for invasive aspergillosis, as has been described in other series (range 2.4%–10.3%).[16,17] The most important study focused on CPA identified the presence of previous treated lung cancer in 13 (10.3%) of 126 patients as a risk factor for CPA.[8]

The determinant factors related to subacute IPA are the presence of anatomical lung changes and local and systemic factors that compromise the immune response.[8] The complex relationship between underlying conditions and *Aspergillus* spp. encompasses a spectrum of different forms of IPA.[18] Chronic disease is a form of IPA on the borderline between colonization and invasive disease, developing over weeks or months.[7] In our cohort, the first line of pulmonary host defense against *Aspergillus* spp. was altered in all patients because of local pathological lung changes due to primary and metastatic lung cancer. Other local factors seen in our patients were COPD comorbidity (50%), radiation therapy (35.7%), previous resectional surgery (28.6%), previous pneumothorax (14.2%), and previous pulmonary tuberculosis (7.1%). Our patients also had systemic factors that compromise the immune response, such as chemotherapy (64.3%), prolonged corticosteroid therapy (21.4.%), other immunosuppressive treatments (21.4%), alcoholism (21.4%), diabetes (7.1%), cirrhosis (7.1%), and HIV infection (7.1%).

Of note, all patients presented advanced neoplastic disease (stage III or IV) with a limited response to oncological treatment (57.1% progressive disease, 35.7% partial response). As has been reported,[13] these data suggest that advanced oncological disease is associated with severely decreased immunity. Although neutropenia is a well-recognized risk factor for IPA in hematological patients,[2] only one patient in our cohort (7.1%) had neutropenia. In other solid cancer series with invasive aspergillosis,[10,13] neutropenia rates varied from 7.7% to 31.1%. This higher incidence may be explained by the choice of a different threshold to define neutropenia (absolute neutrophil count $<2000/mm^3$) and inclusion of both acute and chronic pulmonary aspergillosis cases.[13] The presence of a normal leukocyte count could explain the histological and radiologic pattern of inflammatory necrosis and the development of subacute forms of IPA, rather than the angioinvasive pattern commonly seen in neutropenic hematological patients.[19]

Subacute IPA may be difficult to diagnose in patients with primary lung cancer or pulmonary metastatic disease for several reasons. First, because the clinical presentation of subacute IPA is variable and nonspecific, and symptoms such as fever (35.7%), cough, hemoptysis, dyspnea, and chest pain (each observed in 28.5% of our patients, respectively) might be attributed to cancer rather than infection. Second, *Aspergillus* spp. infection can coexist in time with bacterial infections, a finding reported in other series.[14] In our study, 10 (71.4%) patients presented a concomitant bacterial respiratory infection.

Third, oncological patients can have radiologic abnormalities (lung cavitations or nodules) caused by their malignant disease that may be difficult to interpret.[10] In our series, the most common radiologic findings on chest CT performed before *Aspergillus* spp. detection were masses or nodules (66.7%), and cavitary masses (33.3%). Following the subacute IPA diagnosis, 8 of 12 patients (66.7%) showed new cavity formation or expansion of one or more existing cavities on CT controls. This radiologic pattern is in keeping with the findings of previous studies.[7,20] Cavity formation could be related to proteolytic destruction of the lung parenchyma by enzymes released from neutrophils and elastase produced by *Aspergillus*.[21]

Regarding the microbiological isolates, *A. fumigatus* was the most common species (64.3%), in keeping with a previous report on CPA[7] and a recent study on the epidemiology of the predominant *Aspergillus* species isolated in Spain.[22] Of particular note, the percentages of *A. flavus* and *A. terreus* in our patients were higher than the reported rates in a previous study.[22] This data has therapeutic implications because of the low sensitivity of these species to amphotericin.

Voriconazole is the reference standard treatment for invasive aspergillosis.[23] Current recommendations for treating CPA include oral therapy using one of the triazoles, but voriconazole is now recommended as the drug of choice by some authors.[24,25] In the present study, all patients received triazoles.

Even with proper treatment, the outcome of this infection may be unsatisfactory. The response to subacute IPA therapy at 6 weeks' follow-up was found to be quite poor (35.7%) in our population. In contrast, other studies on CPA have reported a more favorable outcome of antifungal treatment (stability or improvement) in the range of 43.5%–83.1%.[24,25] This favorable outcome may be explained by less severe baseline diseases and a longer follow-up, which was limited in our cohort by oncological disease.

Overall, 10 of the 14 patients (71.4%) died after *Aspergillus* spp. detection at a mean of 73 days; 2 deaths were subacute IPA -attributable (20%) and 6 subacute IPA-related (60%). Previous studies have reported mortality rates of 51%–60% in patients with solid cancer and invasive aspergillosis.[13,26] These data suggest that detection of *Aspergillus* infection in oncological patients heralds a very poor prognosis. Mortality is likely related to the underlying comorbid conditions, delayed introduction of antifungal treatment and poor drug penetration to lung areas devitalized by surgery, radiation, and tumor necrosis. It is likely that early diagnosis and prompt treatment would have a beneficial impact on the management of this condition.[27]

The primary limitation of our cohort is the low rate of proven subacute IPA diagnoses (21.4%), which was due to the high risk of performing invasive diagnostic interventions in a population with a poor baseline status and advanced-stage cancer. Transbronchial biopsy, percutaneous aspirates and thoracoscopic or open-lung biopsy are rarely performed in these patients. In our study, bronchoalveolar lavage was carried out in only 6 (42.9%) cases. The higher use of this invasive diagnostic tool in the study by Shaid et al.[9] was related to exclusive patient selection among those undergoing diagnostic bronchoscopy and bronchoalveolar lavage. The diagnosis of subacute IPA can, however, be made based on clinical and radiologic features consistent with the diagnosis, isolation of *Aspergillus* spp. on culture of sputum, bronchoscopy, or percutaneous specimens, and exclusion of other conditions with similar presentations.[1]

It is also difficult to establish the real value of *Aspergillus* spp. isolation from respiratory specimens. Some authors believe that because of underlying structural lung disease and chronic airway colonization, *Aspergillus* spp. isolation from respiratory secretions has little significance.[10] In keeping with the opinion of other authors,[13] we believe that although *Aspergillus* can colonize the airways without causing infection, isolation of *Aspergillus* spp. from respiratory secretions is of value in this at-risk population and that it is crucial to make a diagnostic effort to recognize the possibility of subacute IPA. We stress that even though our patients might have had chronic colonization, *Aspergillus* was not detected in any case before the diagnosis of subacute IPA. Another limitation of our study is the fact that *Aspergillus* IgG antibody (precipitins) detection in blood was not performed. Denning has proposed this test as a diagnostic tool, associated with clinical, microbiological, and radiologic features consistent with the diagnosis of CPA.[7] Detection of precipitin antibodies may be useful to achieve an earlier diagnosis of CPA, but the test is not well-standardized for this indication[7] and because of the retrospective nature of our study, this information was not available. Besides, we think that the microbiological diagnosis of *Aspergillus* spp. in all patients offsets this limitation.

Aspergillus galactomannan antigen in BAL (cutoff >0.5) can also be used in the diagnosis of CPA and is reported to be an accurate method.[28] In our cohort, it was performed in four patients with only one positive result, probably because in two cases testing was done after the start of mold-active drugs. Serum galactomannan antigen was not measured in any case because the use of this test has not been clearly addressed in non-hematological neutropenic patients.[29]

In conclusion, in patients with primary or secondary metastatic lung cancer, the presence of anatomical neoplastic lung changes and local and systemic factors compromising the immune response seem to be factors predisposing to subacute IPA. Currently, this entity may be under-diagnosed because the baseline disease may mask the signs and symptoms of subacute IPA. Isolation of *Aspergillus* spp. from respiratory secretions should elicit a high level of suspicion of infection in these vulnerable patients, in whom subacute IPA is associated with a poor prognosis.

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V.4.2. ARTÍCULO 2

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ORIGINAL ARTICLE

10 years of prophylaxis with nebulized liposomal amphotericin B and the changing epidemiology of *Aspergillus* spp. infection in lung transplantation

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antifungal prophylaxis, fungal infections, invasive aspergillosis, lung transplant, nebulized liposomal amphotericin B.

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Conflicts of interest
None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

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Summary

The aim of this study was to assess the outcome and tolerability of prophylactic nebulized liposomal amphotericin B (n-LAB) in lung transplant recipients (LTR) and the changing epidemiology of *Aspergillus* spp. infection and colonization. We performed an observational study including consecutive LTR recipients (2003–2013) undergoing n-LAB prophylaxis lifetime. A total of 412 patients were included (mean postoperative follow-up 2.56 years; IQR 1.01–4.65). Fifty-three (12.8%) patients developed 59 *Aspergillus* spp. infections, and 22 invasive aspergillosis (overall incidence 5.3%). Since 2009, person-time incidence rates of *Aspergillus* spp. colonization and infection decreased (2003–2008, 0.19; 2009–2014, 0.09; $P = 0.0007$), but species with reduced susceptibility or resistance to amphotericin significantly increased (2003–2008, 38.1% vs 2009–2014, 58.1%; $P = 0.039$). Chronic lung allograft dysfunction (CLAD) was associated with *Aspergillus* spp. colonization and infection (HR 24.4, 95% CI 14.28–41.97; $P = 0.00$). Only 2.9% of patients presented adverse effects, and 1.7% required discontinuation. Long-term administration of prophylaxis with n-LAB has proved to be tolerable and can be used for preventing *Aspergillus* spp. infection in LTR. Over the last years, the incidence of *Aspergillus* spp. colonization and infection has decreased, but species with reduced amphotericin susceptibility or resistance are emerging. CLAD is associated with *Aspergillus* spp. colonization and infection.

Introduction

Aspergillus spp. is the most common cause of invasive fungal infection in lung transplant recipients (LTR) [1]. Despite the advances in antifungal drugs, infection by *Aspergillus* spp. is associated with persistently high mortality in this population [2]. Therefore, preventive measures are preferred over treatment, but the optimal antifungal drug, use of universal or targeted prophylaxis, and duration of the prophylactic strategy remain to be determined [3].

Among available antifungal agents, amphotericin B by nebulized administration (n-AB) reaches the most distal areas of the bronchial tree while avoiding drug interactions and systemic side effects [4]. However, information is

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lacking on the efficacy of n-AB, the incidence of breakthrough aspergillosis, and the impact of n-AB exposure on *Aspergillus* speciation or susceptibility to this drug [5].

Several types of n-AB preparations are available. In April 1993, we began using prophylactic nebulized B deoxycholate (n-ABD) for *Aspergillus* spp. infection in all LTR. In July 2003, n-ABD was switched to nebulized liposomal amphotericin (n-LAB) because n-ABD supply was lacking on the Spanish market. We then attempted to work out a lifetime n-LAB prophylactic strategy. The initial results were reported in a previous study [6], which included the first 104 LTR receiving prophylactic n-LAB in two centers and followed up for 12 months. The n-LAB strategy proved effective, with a 1.2% incidence of invasive aspergillosis (IA). We also observed [7] that drug concentrations after n-LAB remained high and adequate for *Aspergillus* spp. prophylaxis during 14 days, a convenient administration interval. Other publications have reported good tolerance to n-LAB [6] and an optimal safety profile [8], with no evidence of significant systemic absorption [7,9], effects on respiratory function [7], or changes in lipid content of pulmonary surfactant[10], permitting long-term administration.

After 10 years of experience with this therapy in our center, we have now set out to reassess the outcome and tolerability of prophylactic nebulized liposomal amphotericin B (n-LAB) in a large number of LTR over lengthy follow-up. In addition, we have investigated the long-term impact of prophylactic n-LAB use on the evolution of *Aspergillus* spp. infection and colonization in LTR, and associations between these infections and chronic lung allograft dysfunction (CLAD).

Materials and methods

Study setting and patient population

A retrospective, observational study was performed on all consecutive adult patients undergoing lung transplantation in Hospital Univeristari Vall d'Hebron (Barcelona, Spain) from July 2003 to July 2013, receiving lifetime n-LAB prophylaxis.

We included all patients older than 18 years who had survived more than 24 h after transplantation. All patients had been followed up for at least 1 year or until death. Cases of *Aspergillus* spp. infection were identified through the General Hospital, Microbiology, and Histopathology databases, using a standardized protocol. The study protocol was approved by the Vall d'Hebron Ethics Committee for Clinical Research.

Prophylaxis for *Aspergillus* spp. infection

Since July 2003, all patients undergoing lung transplantation in our center receive 25 mg (6 ml) of n-LAB thrice weekly for the first 60 days, 25 mg once weekly between 60 and 180 days, and 25 mg once every 2 weeks thereafter, for life. Routinely, all patients with episodes of *Aspergillus* spp. colonization and all high-risk patients (suture abnormalities, post-transplantation culture isolation of *Aspergillus* spp., CMV disease, or increased immunosuppression) are treated by maintaining or increasing the n-LAB dose to thrice weekly.

In our center, prophylaxis with azoles or echinocandins is not routinely performed, except in patients with pretransplant colonization with AB-resistant fungi (*A. terreus*, *Scedosporium* spp.) or with severe intolerance to inhaled n-LAB.

Disease definitions

Classification of *Aspergillus* spp. colonization and infection is described in Table 1. Pretransplant *Aspergillus* spp. colonization included colonization from minimum 1 year before the transplant onwards and positive intra-operative respiratory samples from the explanted organ. Ulcerative tracheobronchitis and invasive pulmonary aspergillosis (IPA) were regarded as invasive aspergillosis (IA), whereas simple tracheobronchitis, bronchial stent infection, and native-lung aspergilloma were classified as noninvasive aspergillosis (NIA). The EORTC/MSG [11] and International Society of Heart and Lung Transplantation (ISHLT) [12] criteria were used to define IA cases, and only proven and probable cases were included.

We examined factors that were associated with *Aspergillus* spp. infection in previous studies: single lung transplant, chronic gram-negative bacteria colonization, bronchial stenosis, acute rejection, CMV disease, bronchial stent, pretransplant and post-transplant *Aspergillus* spp. colonization, massive inhalation, excessive immunosuppression, abandonment of prophylaxis, and induction immunosuppression therapy [13,14]. Excessive immunosuppression was defined as an amount of corticosteroid (the equivalent of 1 mg/kg daily doses of prednisone during the last month) given as therapy or pulses, and/or treatment with thymoglobulin, basiliximab, OKT3, and total lymphoid irradiation (TLI).

Aspergillus spp. infection and colonization were categorized into early-onset, occurring <90 days after transplantation and late-onset, occurring >90 days after. [15] Therapy response was categorized as success or failure, as has been described elsewhere. [16] Mortality was considered IA-related if IA was the cause or played a major role in the patient's death, and IA-unrelated if IA played a minor or no role.

Statistics

A descriptive analysis was performed. Continuous variables are expressed as the median and range. All proportions were calculated as percentages of patients with available data. Categorical variables were analyzed using the

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Table 1. Classification of *Aspergillus* spp. colonization and infection modified [6,11,12].

Colonization	Single positive bronchoalveolar lavage or bronchial aspirate; or positive bronchoalveolar lavage galactomannan test; or at least two positive sputum cultures or tracheal aspirate for *Aspergillus* spp. in asymptomatic patients with normal-appearing respiratory mucosa or absence of endobronchial lesions
Aspergillus infection	
Simple tracheobronchitis	Detection of *Aspergillus* spp. and clinical symptoms (e.g, purulent sputum production) plus bronchoscopy findings of mucus and edematous red mucosa, with bacterial infection ruled out.
Bronchial stent infection	Detection of *Aspergillus* spp. and clinical symptoms (e.g, purulent sputum production) plus bronchoscopy findings of mucus and edematous red mucosa, with bacterial infection ruled out in patients with bronchial stent
Native lung aspergilloma	An approximately spherical shadow with surrounding air, also called a fungal ball, in a pulmonary cavity, with serological or microbiological evidence that *Aspergillus* spp. is present in the material
Ulcerative/pseudomembranous tracheobronchitis	Detection of *Aspergillus* spp. with bronchial biopsy and/or bronchoscopy findings of necrotic ulcers or pseudomembrane in the anastomosis or in the tracheobronchial tree that disappeared after treatment
Invasive pulmonary aspergillosis	Detection of *Aspergillus* spp. with evidence of tissue damage on lung histopathology or radiological signs of invasive aspergillosis

chi-square test. *Aspergillus* spp. colonization and/or infection incidence rates were calculated as number of cases at risk during follow-up time. We assessed the influence of *Aspergillus* spp. colonization/infection on the time to subsequent development of CLAD and the reverse relationship with Cox proportional hazards regression: onset of CLAD and *Aspergillus* spp. colonization/infection were time-dependent covariates. Differences were considered significant at a value of $P < 0.05$.

Results

Patients and baseline characteristics

A total of 412 patients were included, and mean postoperative follow-up was 2.56 years (IQR 1.01–4.65). Clinical characteristics of the study population are reported in Table 2.

Table 2. Demographic data and patient characteristics.

Variable	Number of patients (%)
Patients	412
Age, mean (SD), years	49.9 (±11.4)
Sex, *n* (%)	
Male	257 (62.4)
Female	155 (37.6)
Pretransplant diagnosis, *n* (%)	
Idiopathic pulmonary fibrosis	159 (38.6)
Chronic obstructive pulmonary disease	152 (36.9)
Cystic fibrosis	26 (6.3)
Primary pulmonary hypertension	18 (4.4)
Bronchiectasis	17 (4.1)
Lymphangioleiomyomatosis	15 (3.6)
Others	25 (6.1)
Pretransplant *Aspergillus* colonization, *n* (%)	74 (18)
Transplant type, *n* (%)	
Double	264 (64.1)
Single	148 (35.9)

Aspergillus spp. infections

Overall, 53 (12.9%) patients developed 59 *Aspergillus* spp. infections: most patients manifested only noninvasive forms (31/412, 7.5%) and the remaining patients, invasive disease (22/412, 5.3%), which yielded a 3.6% 1-year cumulative incidence of IA. Of the 22 IA patients, 15 (3.6%) had IPA and 7 (1.7%) ulcerative tracheobronchitis. Of the 31 NIA patients, 23 (5.6%) had simple tracheobronchitis, 6 (1.5%) bronchial stent infections, and 2 (0.7%) native-lung aspergillomas (Fig. S1). Six patients (1.5%) presented 2 episodes of infection. None of the patients had disseminated infection and two patients had only extrapulmonary involvement (1 sternal osteomyelitis, 1 wound infection).

Median time from transplantation to the first *Aspergillus* spp. infection was 266 days (107–884 IQR). Based on the time, infection was diagnosed after transplantation; 50 (84.7%) were classified as late-onset (Fig. 1). Overall about half of *Aspergillus* spp. infections (30/59; 50.8%) occurred < 270 days (9 months) after transplant. Fifteen of 22 IA (68.1%) occurred <12 months after transplant (Fig. 1).

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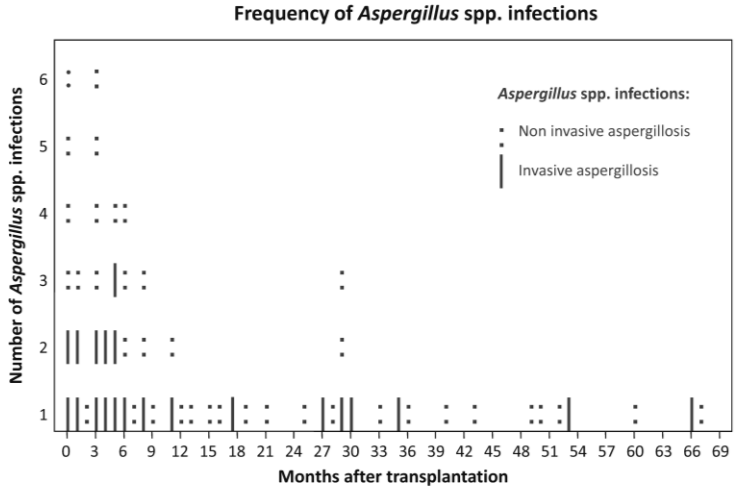

Figure 1 Onset of *Aspergillus* spp. infection after transplantation.

Findings on bronchoscopy, performed in 58 of 59 patients, are reported in Tables 3 and 4. The clinical characteristics, microbiological, bronchoscopy and radiologic findings, treatment, and outcome of IA patients are reported in Table 3. Thirteen IA patients (59.1%) met criteria for proven IA and 9 (40.9%) for probable IA.

In addition, 68 post-transplant *Aspergillus* spp. colonizations were noted in 61 LTR (14.8%). Six LTR (9.8%) developed *Aspergillus* spp. infection at any time after post-transplant colonization (only 1 IA, 1.6%). Person-time incidence rates of *Aspergillus* spp. colonization and infection were lower in the last 5 years of the study (2003–2008, 0.19; 2009–2014, 0.09; $P = 0.0007$; Fig. 2).

Tolerability

Over the 10-year study period, only 12 (2.9%) of 412 patients with lifetime prophylaxis and prolonged follow-up (2.56 years, IQR 1.01–4.65) experienced mild adverse effects associated with n-LAB. Mild, transitory breathing difficulty occurred in 8 patients (1.9%), nausea in 3 (0.7%), and dizziness in 1 (0.2%). Prophylaxis had to be stopped in 7 (1.7%) because of secondary effects, and 7 (1.7%) other patients abandoned n-LAB prophylaxis spontaneously.

Etiology

Infections due to nonfumigatus *Aspergillus* species (*A. flavus, A. niger, A. nidulans,* and *A. terreus*) were more common (35/59, 59.3%) than those caused by *A. fumigatus* (11/59, 18.6%). Mixed infections by 2 or 3 *Aspergillus* species occurred in 22% of episodes. Of interest, 1 cryptic *Aspergillus flavus* complex (*A. alliaceus*) was isolated in 1 patient with IA.

Although *Aspergillus* colonization and infection rates decreased over the last 5 years (Fig. 2), cases involving *Aspergillus* species with reduced susceptibility or resistance to amphotericin (*A. flavus*, *A. terreus,* and *A. alliaceus*) significantly increased (2003–2008, 38.1% vs 2009–2014, 58.8%; $P = 0.04$; Fig. 2).

Factors predisposing to *Aspergillus* spp. infection

Factors predisposing to *Aspergillus* spp. infection are listed in Table 5. Of note, more than half the *Aspergillus* spp. infection episodes were associated with chronic gram-negative colonization (57%), mainly *P. aeruginosa*.

No significant differences were observed between early and late IA episodes, although there was a trend toward greater frequency of association with bronchial stenosis (55.1% vs 10%, $P = 0.13$) and CLAD (42.9% vs 0%, $P = 0.01$) in late episodes. The rate of *Aspergillus* spp. infections in double LTR was similar to that of single LTR (13.6% vs 11.5%, $P = 0.6$).

Aspergillus spp. and chronic lung allograft dysfunction

Time-dependent Cox regression analysis showed that CLAD was associated with the development of *Aspergillus* spp. colonization and infection in all patients (HR 24.4, 95% CI 14.28–41.97; $P = 0.00$; Fig. 3a).

We found no time-dependent relationship between colonization and infection by *Aspergillus* spp. in general and development of CLAD (HR 0.77, 95% CI 0.44–1.34, $P = 0.3$; Fig. 3b).

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Table 3. Clinical characteristics, microbiological, bronchoscopic, and radiologic findings, treatment, and outcome of patients with invasive aspergillosis (IA).

	Sex, Age, y	Transplant	Time elapsed, days	Risk factors	Type of infection	Type of IA	*Aspergillus* spp.	Bronchoscopy	Chest CT	Treatment	Death
1	M, 63	Single lung	162	8,10	IPA	Probable	*A. flavus* + *A. niger*	mucus or mucus plaques	nodules	voriconazole + anidulafungin – n-LAB	Unrelated
2	F, 64	Single lung	98	1,10	IPA	Probable	*A. alliaceus*	plaque	nodules	voriconazole + anidulafungin – n-LAB	No
3	M, 34	Double lung	2000	6,7,8,10	IPA	Proven	*A. flavus*	plaque	nodules	voriconazole + n-LAB	Related
4	M, 43	Double lung	521	1,4,8,11	IPA	Proven	*A. flavus* + *A.terreus*	erythema	cavitation	voriconazole + n-LAB	Related
5	M, 52	Double lung	831	4,11	IPA	Probable	*A. fumigatus* + *A. terreus*	erythema	nodules	voriconazole + n-LAB	No
6	M, 29	Double lung	1611	8,1	IPA	Proven	*A.fumigatus* – *A. flavus*	ulcerations	nodules	voriconazole + caspofungin + n-LAB	Related
7	M, 57	Double lung	900	8,1	IPA	Proven	*A. fumigatus*	no performed	cavitation	voriconazole + caspofungin + n-LAB	Related
8	F, 18	Double lung	883	6,7,8,10	IPA	Probable	*A. fumigatus*	mucus or mucus plaques	cavitation	voriconazole + AMB + n-LAB	Related
9	M, 55	Double lung	31	1,10	IPA	Proven	*A. fumigatus*	mucus or mucus plaques	cavitation	voriconazole + micafungin + n-LAB	Related
10	M, 57	Single lung	252	4,6,10	IPA	Proven	*A. flavus*	mucus or mucus plaques	cavitation	voriconazole + n-LAB	Related
11	M, 38	Double lung	285	8,10	IPA	Proven	*A. flavus* + *A.terreus*	plaque	cavitation	voriconazole + n-LAB	No
12	M, 60	Double lung	1050	5,8,9	IPA	Proven	*A. flavus*	plaque	new or progressive and persistent inflitrate	voriconazole + n-LAB	Related
13	M, 44	Single lung	20	1,5	IPA	Proven	*A. fumigatus* + *A. nidulans*	plaque	consolidation	AB – n-LAB	Unrelated
14	F, 33	Double lung	161	8	IPA	Probable	*A. flavus*	normal	nodules	voriconazole + n-LAB	No

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Table 3. continued

	Sex, Age, y	Transplant	Time elapsed, days	Risk factors	Type of infection	Type of IA	*Aspergillus* spp.	Bronchoscopy	Chest CT	Treatment	Death
15	F, 58	Double lung	177	1,9	IPA	Probable	*A. fumigatus*	normal	nodules	voriconazole – caspofungin – n-LAB	No
16	M, 59	Double lung	203	1,2,6,10	Ulcerative TB	Proven	*A. flavus*	plaque	negative	voriconazole – n-LAB	No
17	M, 49	Double lung	17	3	Ulcerative TB	Proven	*A. flavus*	pseudomembrane formation	consolidation	voriconazole – n-LAB	Related
18	M, 51	Double lung	330	4,6,9	Ulcerative TB	Proven	*A. terreus* + *A. niger*	plaque	new or progressive and persistent infiltrate	AB + n-LAB	Unrelated
19	M, 43	Double lung	107	1,4,9	Ulcerative TB	Probable	*A. fumigatus* + *A. flavus*	plaque	new or progressive and persistent infiltrate	AB + n-LAB	Unrelated
20	M, 57	Double lung	36	3,10	Ulcerative TB	Probable	*A. terreus*	plaque	consolidation	voriconazole – n-LAB	No
21	F, 55	Double lung	121	5,10	Ulcerative TB	Probable	*A. fumigatus*	plaque	negative	voriconazole – n-LAB	Unrelated
22	F, 49	Single lung	137	5,9,10	Ulcerative TB	Probada	*A. fumigatus* + *A. terreus* + *A. niger*	plaque	negative	voriconazole – n-LAB	No

AB, ambisome (intravenous); CT, computed tomography; F, female; M, male; IA, invasive aspergillosis; IPA, invasive pulmonary aspergillosis; n-LAB, nebulized liposomial amphotericin; TB, tracheobronchitis.

Risk factors. 1: acute rejection, 2: induction immunosuppression, 3: overimmunosuppression, 4: CMV, 5: pretransplant *Aspergillus* spp. Colonization, 6: bronchial stenosis, 7: bronchial stent, 8: CLAD, 9: massive inhalation, 10: chronic gram negative bacteria colonization, 11: abandonment of prophylaxis.

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Table 4. Bronchoscopy findings in invasive aspergillosis (IA) and noninvasive aspergillosis (NIA).

	Ulcerations or pseudomembrane or plaques	Mucus	Erythema	Normal	Total
IA	13 (59%)	4 (18.2%)	2 (9.1%)	2 (9.1%)	21/22
NIA	0	32 (86.4%)	3 (8.1%)	2 (5.4%)	37/37

Treatment and mortality

IA patients were treated with various antifungals until successful therapy response was achieved. Then, antifungal therapy was interrupted and n-LAB prophylaxis was restarted lifetime. (Table 3), and 60% had a successful outcome; median time to cure was 178 days (IQR 32–872). Successful therapy response was similar IA caused by potentially amphotericin-resistant species and IA by other *Aspergillus* species (57.1% vs 50.0%, respectively; $P = 0.5$). All cases of simple tracheobronchitis had successful outcomes. Patients with a bronchial stent had a poorer prognosis, showing a high rate of therapy failures (7/9, 77.8%), persistent chronic infection, and development of simple tracheobronchitis (2/9, 22.2%) or IA (1/9, 11.1%), despite treatment.

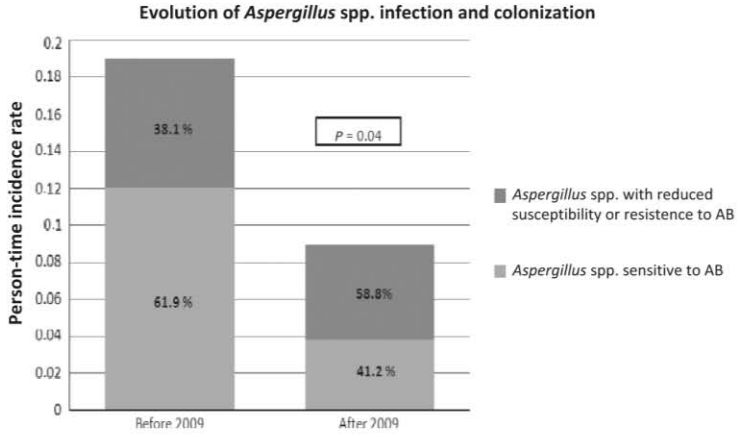
In total, 14 (63.6%) patients died after IA was detected, with 9 related (40.9%) and 5 unrelated (22.7%) deaths. Within IA forms, 8 of 15 (53.3%) patients with IPA and 1 of 7 (14.3%) with ulcerative TB presented a related death. Related mortality was 40.9% in invasive disease versus 3.2% in noninvasive forms (1 aspergilloma), and mortality was similar in IA caused by potentially amphotericin-resistant species and IA by other *Aspergillus* species (40.0% vs 42.9%, respectively; $P = 1.0$). Overall, 9 LTR (2.2%) died of *Aspergillus* spp. infection, accounting for 4.7% of the 188 deaths in these patients.

Discussion

The strengths of the present study reside in the large lung transplant population analyzed and the lengthy follow-up (compared to previous literature in this line) to reassess the outcome and tolerability of n-LAB. In addition, the long-term impact of prophylactic n-LAB use on the evolution of *Aspergillus* speciation and susceptibility to this drug was investigated.

In our LTR cohort, the overall incidence of *Aspergillus* spp. infection was 14.3% and IA incidence was 5.3%. Although it is difficult to compare *Aspergillus* spp. infection rates between studies, most publications in which universal or targeted *Aspergillus* spp. prophylaxis has been used in LTR have reported invasive disease rates ranging from 1.5% to 12.2% [17–19].

In terms of tolerability, our n-LAB prophylaxis strategy proved to be good [7,9,10] allowing lifetime maintenance.



Time/years	Person-time follow-up (days)	Rate	Incidence rate ratio	95% CI	P
2003–2008	214.53088	0.191	1.97	0.14-0.25	0.0003
2009–2014	857.06018	0.096	1.32	0.07-0.12	

Figure 2 Person-time incidence rates and incidence rate ratios of *Aspergillus* spp. infection and colonization, and evolution of *Aspergillus* spp. with reduced susceptibility or resistance (*A. flavus*, *A. terreus*, and *A. alliaceus*) to amphotericin from July 2003 to December 2008 (before 2009) and from January 2009 to July 2014 (after 2009). AB, amphotericin B.

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Table 5. Risk factors potentially associated with the development of 59 *Aspergillus* spp. infections in 53 lung transplant patients.

Risk factors	*Aspergillus* spp. infections *n* (%)
Chronic gram-negative bacterial colonization	38/59 (64.4)
Bronchial stenosis without stent	28/59 (47.5)
Chronic lung allograft dysfunction	21/59 (35.6)
Acute rejection	15/59 (25.4)
CMV disease	14/59 (23.7)
Bronchial stent	14/59 (23.7)
Pre-transplant *Aspergillus* spp. colonization	9/59 (15.2)
Massive inhalation	8/59 (13.6)
Overimmunosuppression	6/59 (10.2)
Abandonment of prophylaxis	4/59 (6.8)
Induction immunosuppression	3/59 (5.1)

In our series, prophylaxis with n-LAB was well tolerated, with only 2.9% of adverse effects and 1.7% of patients requiring treatment withdrawal for this cause. Similar tolerance has been reported in other studies [8,9]. One disadvantage of this therapy is local irritation with secondary effects such as bronchospasm (1.9%), but the use of salbutamol or halving the drug concentration can improve these symptoms. Inhaled n-LAB has the advantage that distribution is limited to the respiratory tract, there is no systemic absorption [7,9], and high levels of antifungal concentrations can be achieved in the lung without changes in respiratory function. Thus, the risk of nephrotoxicity is averted and the drug can be administered over lengthy periods. In comparison with azoles, n-LAB has a lower incidence of systemic side effects (especially hepatotoxicity) [17,20,21], and an absence of interactions with immunosuppressive drugs and glucocorticoids [7].

Aspergillus spp. infection was formerly considered an immediate post-transplantation complication, but recent evidence indicates that it can occur much later after lung transplantation [2,15,22]. The high proportion of late-onset *Aspergillus* spp. infection (84.7%) cases and median time to the first *Aspergillus* spp. infection (266 days) in our cohort concur with these findings. Probably changes in routine antifungal prophylaxis patterns against *Aspergillus* species appear to be shifting the occurrence of *Aspergillus* spp. infection later after transplantation. Other factors that may led to higher rate of late-onset infections could be age, overimmunosuppression or changes in immunosuppressive regimens (sirolimus use in correlation with tacrolimus), and chronic lung allograft dysfunction [2,15,22].These results are of particular interest considering that the duration of antifungal prophylaxis is usually limited to the first 3–6 months after transplantation [3,23]. Unfortunately, a consensus does not exist on what length of treatment should be.

Aspergillus spp. colonization and infection rates have decreased in the last 5 years in our center. In contrast to the findings from a recent epidemiologic study on *Aspergillus* species in Spain [24,25] and previous series in LTR [1,2], significant increases have occurred in colonization and infection by *Aspergillus* species with reduced susceptibility or resistance to AB. However, these species did not seem to be associated with lower successful outcome or higher mortality in our series. These data may have different interpretations. Probably these results are related to a more extensive n-LAB use over time in daily practice [6–10]. As has been reported [7], our protocol prescribes n-LAB use every 2 weeks starting from 6 months post-transplantation. It may be that inhaled n-LAB concentrations at this lengthy dosing interval are not high enough to inhibit growth of these potentially resistant *Aspergillus* species, and this would favor late-onset colonization and infection. Moreover, primary *in vitro* resistance to AB has been observed for *A. terreus*, which is intrinsically resistant to AB and *A. flavus*, in which resistance is 10–15% [26]. In addition, resistance development during AB treatment is rare, but isolates recovered from patients who previously received AB have shown higher MICs than those from patients without AB exposure [5,27]. Although the current knowledge regarding emergence of resistant organisms in patients receiving prophylactic n-LAB is poor [5,27,28], this situation may be a sentinel event that needs to be monitored and strictly surveilled. Although delayed occurrence of Aspergillus infection in LTR has relevant implications, these worrisome long-term consequences of indefinite treatment and the controversial use of lifetime prophylaxis in LTR do not support the use of universal long- term prophylaxis and highlight the need of individualizing. Probably it may be advisable to recommend universal prophylaxis post-transplant (6–12 months) and consider an extended course, mainly in targeted high-risk patients (acute and chronic rejection, augmented immunosuppression and CMV infection, fungal colonization). In this situation, the convenient administration schedule of n-LAB (every 2 weeks) would be a positive factor, conducive to adherence [7].

Post-transplant *Aspergillus* spp. colonization is a known risk factor for subsequent IA in LTR [29]. The incidence of *Aspergillus* spp. colonization in LTR receiving various antifungals and no prophylaxis varies from 4% to 28.1% [3]. Of note, our colonization rate was 14.8%, all but 6 episodes resolved, and there was only one subsequent case of IA. These findings seem to indicate that our standard practice of increasing n-LAB dose to 3 times weekly when *Aspergillus* spp. colonization is detected suffices to prevent the development of IA.

Previous studies have suggested that CLAD may be a risk factor for subsequent *Aspergillus* spp. Infection [13,30]. Although we did not statistically control for other effects (as it was not the main aim of our study), the results of our investigation support this notion. Considering that LTR

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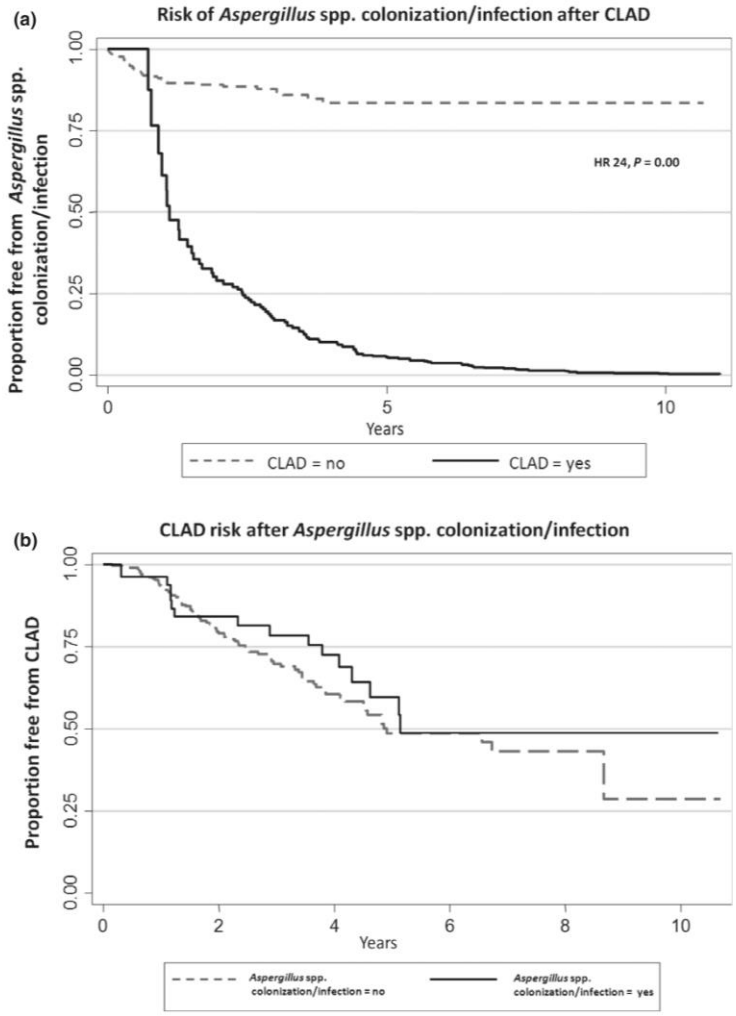

Figure 3 Time-dependent Cox-regression analysis: (a) development of *Aspergillus* spp. colonization or infection after chronic lung allograft dysfunction (CLAD); (b) Development of CLAD after *Aspergillus* spp. colonization or infection.

with CLAD may have a combination of overimmunosuppression and nonuniformly distributed restrictive and obstructive processes that can affect regional n-LAB deposition and favor *Aspergillus* spp. infection [31], it could be reasonable to intensify the frequency of n-LAB administration in these patients. Nonetheless, although previous studies have found an association between *Aspergillus* spp. colonization and infection [32,33] and posterior development of CLAD, our results did not confirm this relationship.

Although previous reports have alluded to a higher incidence of *Aspergillus* spp. infections in single lung transplant recipients [34], this difference was not observed in our cohort and the incidence of aspergilloma in native lung was low. It is likely that n-AB distribution occurs preferentially in the allograft, with unreliable distribution in the native lung, although sufficient to prevent *Aspergillus* spp. infection [31].

Of interest, chronic colonization by gram-negative bacteria in our series was often associated (57.4%) with *Aspergillus* spp. infection. The reason for this association is uncertain. It may be that LTR with CLAD may have a parenchymal disease that could advantage proliferation of multiple organisms. Moreover, the local lung milieu in

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patients colonized by gram-negative bacteria could favor proliferation of *Aspergillus* spp., as has been described in cystic fibrosis patients [13,35].

Bronchial stent infections have a poor prognosis, with very low cure rates. The presence of a foreign body, which can act as a fungal reservoir, may promote *Aspergillus* spp. biofilm formation, making antifungal penetration difficult [36].

The main limitation of this study is its observational and retrospective design and the necessary assumption of changes in the diagnosis and therapy of *Aspergillus* spp. infection to adapt to advances in the management of these patients over the lengthy study period. Lastly, in our cohort, incidence of cystic fibrosis (6.3%) is lower than reported in other series [3]. Therefore, it may need to be taken into account when comparing *Aspergillus* spp. infection and colonization rates with other studies, because it may be underestimated.

In conclusion, prophylaxis with n-LAB at the dose and frequency described has proved to be tolerable and can be used for preventing *Aspergillus* spp. infection in LTR. Over the last years, the incidence of *Aspergillus* spp. colonization and infection has decreased. Nevertheless, *Aspergillus* species with reduced susceptibility or resistance to AB are emerging but do not seem to be associated with lower successful outcome or higher mortality in our series. Emergence of resistant organisms in patients receiving prophylactic n-LAB may be a sentinel event that needs surveillance. CLAD is associated with the development of *Aspergillus* spp. colonization and infection, and n-LAB prophylaxis could be intensified in patients with this factor. A multicenter randomized controlled trial is warranted to assess the efficacy of *Aspergillus* spp. prophylaxis in LTR.

Authorship

MP: participated in the research design, in the performance of the research, in data analysis, and in the writing of the paper. VM: participated in the research design, in the performance of the research, in data analysis, and in the writing of the paper. MTM-G: participated in the performance of the research and in data collection and commented on the final version of the manuscript. IR-C: participated in the interpretation of the results, supervised the testing, and commented on the final version of the manuscript. CB: participated in the performance of the research and interpretation of the results and approved the final version of the manuscript. BS: participated in the performance of the research and interpretation of the results and approved the final version of the manuscript. JR: participated in the performance of the research and interpretation of the results and approved the final version of the manuscript. PU: participated in the interpretation of the results and commented on the final version of the manuscript. JS: participated in the interpretation of the results and commented on the final version of the manuscript. JG: participated in the interpretation of the results and approved the final version of the manuscript. AR: participated in the interpretation of the results, supervised the testing, and commented on the final version of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplemental material and methods

Figure S1. *Aspergillus* spp. infections and colonization.

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V.4.3. ARTÍCULO 3

Transplant Infectious Disease

Epidemiology of Invasive Respiratory Disease Caused by Emerging Non-Aspergillus molds in Lung Transplant Recipients

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Keywords:	molds, emerging molds, Invasive fungal infections, Fungal colonization, Emerging fungi, lung transplant

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TITLE PAGE

Title of article

Epidemiology of Invasive Respiratory Disease Caused by Emerging Non-*Aspergillus* molds in Lung Transplant Recipients

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For Review

Running title: emerging non-*Aspergillus* molds in lung transplant recipients

Abbreviations LTR, lung transplant recipients; AB, amphotericin B; n-LAB, nebulized liposomal amphotericin B; BAL, bronchoalveolar lavage; IFI, invasive fungal infection

Key words: Molds, Prophylaxis, Lung transplant, Invasive fungal infections, Amphotericin B, Fungal colonization, Emerging molds, Emerging fungi

Disclosure statement

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For Review

Authorship

- Maddalena Peghin participated in the research design, in the performance of the research, in data analysis and in the writing of the paper.
- Victor Monforte participated in the research design, in the performance of the research, in data analysis and in the writing of the paper.
- Maria Teresa Martin-Gomez participated in the performance of the research, in data collection and commented on the final version of the manuscript.
- Isabel Ruiz-Camps participated in the interpretation of the results, supervised the testing and commented on the final version of the manuscript.
- Cristina Berastegui participated in the performance of the research and approved the final version of the manuscript.
- Berta Saez participated in the performance of the research and approved the final version of the manuscript.
- Jordi Riera participated in the performance of the research and approved the final version of the manuscript.
- Juan Solé participated in the interpretation of the results and and approved the final version of the manuscript.
- Joan Gavaldá participated in the interpretation of the results and approved the final version of the manuscript.
- Antonio Roman partecipated in the interpretation of the results and commented on the final version of the manuscript.

For Review

ABSTRACT

OBJECTIVES: To assesses the impact of positive cultures for non-*Aspergillus* molds on the risk of progression to invasive fungal infection (IFI), and the effect of prophylactic nebulized liposomal amphotericin B (n-LAB) on these pathogens.

METHODS: Observational study (2003-2013) including LTRs undergoing lifetime n-LAB prophylaxis, in whom non-*Aspergillus* molds were isolated on respiratory culture before and after transplantation (minimum 1 year follow-up).

RESULTS: 412 patients, mean postoperative follow-up 2.56 years (IQR 1.01-4.65). Pre- and post-transplantation positive respiratory samples for non-*Aspergillus* molds were frequent (11.9% and 16.9% of LTR respectively). Post-transplantation, 10 (2.42%) patients developed non-*Aspergillus* mold infection (4 *Scedosporium* spp., 4 *Purpureocillium* spp., 1 *Penicillium* spp. and 1 *Scopulariopsis* spp.); 5 (1.21%) had IFI, with 60% IFI-related mortality. Non-*Aspergillus* molds with intrinsic amphotericin B (AB) resistance were more commonly isolated in bronchoscopy samples than AB-variably sensitive or AB-sensitive molds (54.5% vs 25%, p=0.04) and were associated with a higher risk of infection (56.3% vs 1.3%%, p=0.00).

CONCLUSIONS: In LTRs undergoing n-LAB prophylaxis, pre- and post-transplantation isolation of non-*Aspergillus* molds is frequent, but IFI incidence (1.21%) is low. *Purpureocillium* is an emerging mold. Amphotericin-resistant non-*Aspergillus* species were found more often in bronchoscopy samples and were associated with a higher risk of infection.

For Review

INTRODUCTION

Invasive fungal infections (IFIs) have long been recognized as a significant complication in immunosuppressed patients. Among lung transplant recipients (LTRs) *Aspergillus* species remain the most common cause of invasive disease (1),(2), but infections by non-*Aspergillus* molds have emerged in recent years as an important cause of death and disseminated disease, mainly in North American series (1, 2),(3). The spectrum and overall impact of these emerging fungal pathogens have not been not fully defined in LTRs, particularly in Europe.

Molds are ubiquitous in the environment and are easily aerosolized. Respiratory tract specimens often test positive to molds (1), but the clinical impact of positive status before and after lung transplantation has not been fully investigated. Patients considered for lung transplantation, especially those with cystic fibrosis (4), are often colonized by fungi. Pre-transplantation *Scedosporium* spp. colonization is a known risk factor for invasive disease following the procedure (5), but there is no available data on other non-*Aspergillus* molds. Post-transplantation *Aspergillus* colonization is a reported risk factor for IFI (6), but isolation of molds other than *Aspergillus* spp. did not seem to be associated with invasive disease in some studies (7), (8).

Recent reports have described a rise in non-*Aspergillus* mold infections, particularly mucormycosis, in persons receiving antifungal agents active against *Aspergillus* species. (9) Prophylactic nebulized amphotericin use is common practice in lung transplantation programs. Since 2003, our center has applied universal lifetime prophylaxis with nebulized liposomal amphotericin B (n-LAB). It is unknown whether amphotericin B (AB) exposure has an impact on the epidemiology of non-*Aspergillus* molds in lung transplantation.

The aim of this study was to describe the clinical impact of pre- and post-transplantation culture-positive status on the risk of progression to IFI, and to characterize the epidemiology of non-*Aspergillus* mold infections in LTRs under n-LAB prophylaxis.

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MATERIALS AND METHODS

Study setting and patient population

A single center, retrospective, observational study was conducted in Hospital Univeristari Vall d'Hebron (Barcelona, Spain). Consecutive adult patients (>18 years) undergoing lung transplantation from July 2003 to July 2013 were included. All patients were followed-up for ≥1 year (before and after transplantation) or up to death. We reviewed the results of all positive tests for non-*Aspergillus* molds before and after transplantation (sputum, tracheal aspirate, bronchoalveolar lavage, bronchial aspirate, and bronchial biopsy) from July 2002 to July 2014. Bronchoalveolar lavage (BAL), bronchial aspirate, and bronchial biopsy were classified as bronchoscopy specimens. Data were obtained from the General Hospital, Microbiology, and Histopathology databases, using a standardized protocol. The immunosuppressive regimen, perioperative antimicrobial regimen, and prophylaxis utilized have been described (10). The study protocol was approved by the Vall d'Hebron Ethics Committee for Clinical Research.

Prophylaxis for mold infection

Our center applies universal prophylaxis with n-LAB for the life of the patient. All LTRs receive 25 mg (6 mL) of n-LAB thrice weekly for the first 60 days, 25 mg once-weekly from 60 to 180 days, and 25 mg once every 2 weeks thereafter.

We do not routinely perform prophylaxis with azoles or echinocandins except in patients with pre-transplant colonization with AB resistant fungi (*A.terreus*, *Scedosporium* spp., *Purpureocillium* spp.) or with severe intolerance to inhaled n-LAB. In these patients, treatment is usually continued during the first 3-6 months after transplant and until negativity of respiratory samples. After transplant, in all patients with AB-susceptible molds colonization

and all high-risk patients (suture abnormalities, post-transplantation culture isolation of molds, cytomegalovirus disease, increased immunosuppression), the n-LAB dose is maintained or increased to thrice weekly. In all high-risk patients with AB-resistant molds colonization, prophylaxis is assessed individually.

Disease definitions

Single isolation, colonization, and infection by non-*Aspergillus* molds are defined in Table 1 (11), (12). Ulcerative tracheobronchitis and invasive pulmonary mold infection were considered IFIs, whereas simple tracheobronchitis and bronchial stent infection were classified as non-invasive fungal infections. The EORTC/MSG (11) and ISHLT (12) criteria were used to define cases of IFI, and only proven and probable cases were included. Mold infections were categorized into early-onset, occurring <90 days after transplantation, and late-onset, occurring >90 days after (13). Response to therapy was categorized as success or failure, as described elsewhere (14). Mortality was considered IFI-related if IFI was the cause or played a major role in the patient's death, and IFI-unrelated if IFI played a minor role or had no role in the patient's death.

A post-transplantation infection was considered related to a positive pre-transplantation culture if it was caused by the same non-*Aspergillus* mold species. A post-transplantation infection was considered related to a positive post-transplantation culture if it occurred after isolation of a non-*Aspergillus* mold and was caused by the same species.

Scedosporium spp., *Purpureocillium spp.* (formerly *Paecilomyces* spp.), and *Scopulariopsis* spp., were categorized as intrinsically AB-resistant molds, whereas *Penicillium* spp., *Fusarium* spp., Mucormycetes, dematiaceous fungi (*Alternaria* spp., *Curvularia* spp.), dimorphic fungi, and unspecified molds were considered AB-variably susceptible or susceptible molds.

Follow-up

Before transplantation, respiratory samples from recipient lungs were cultured for bacteria, mycobacteria, and fungi. On the day of the operation, donor and recipient samples were cultured the same way. After hospital discharge, patients were regularly followed up in our outpatient clinic, and respiratory samples were taken when the patient had sputum production or bronchoscopy was indicated. All patients underwent a single surveillance bronchoscopy examination 4 to 6 weeks after transplantation and, additionally, at any time in the postoperative period according to clinical criteria. Samples obtained by bronchoscopy included bronchial aspirate and BAL specimens for cell examination and bacterial, fungal, and mycobacterial cultures. Transbronchial biopsy specimens were taken for histopathologic assessment, immunohistochemical staining, and microbiological culture.

Bronchoscopy samples underwent routine microscopic examination before culture (Gram stain) and were inoculated onto solid and liquid media (Sabouraud agar supplemented with gentamicin and chloramphenicol, and brain-heart infusion supplemented with gentamicin and chloramphenicol), whereas sputum and tracheal aspirates were inoculated only onto Sabouraud agar supplemented with antibiotics. Samples were incubated at 25ºC in room atmosphere for at least 15 days, with extension of the incubation period when fungal infection was strongly suspected.

Statistics

A descriptive analysis was performed. Continuous variables were expressed as the median and range. All proportions were calculated as percentages of patients with available data. Categorical variables were analyzed using the chi-square test.

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We assessed the influence of non-*Aspergillus* mold colonization or infection on the time to subsequent development of chronic lung allograft dysfunction (CLAD) as well as the reverse relationship with Cox proportional hazards regression; onset of CLAD and non-*Aspergillus* mold colonization or infection were time-dependent covariates. Differences were considered significant at a p value of <0.05.

RESULTS

Patients and baseline characteristics

In total, 412 lung transplantation procedures (264 double, 148 single) were performed from July 2003 to July 2013 in our center. Mean (SD) age of patients was 49.9 (11.4) years, and 62.4% (257) were men. The main baseline diseases leading to lung transplantation were idiopathic pulmonary fibrosis (159, 38.6%), chronic obstructive pulmonary disease (152, 36.9%), cystic fibrosis (26, 6.3%), pulmonary hypertension (18, 4.4%), and bronchiectasis (17, 4.1%). Mean postoperative follow-up was 2.56 years (IQR 1.01-4.65).

Pre-transplantation non-*Aspergillus* mold isolation

Forty-nine (11.9%) of 412 patients tested positive to non-*Aspergillus* mold on at least one respiratory sample before transplantation, including 23 (5.6%) patients with a positive intra-operative respiratory sample from the explanted organ. Molds were isolated in 24 (45.32%)

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sputum, 23 (43.4%) tracheal aspirate, and 3 (5.7%) BAL/bronchial aspirate specimens, and in 3 (5.7%) bronchial biopsies. Ultimately, 39 of them (9.46%) were considered to have single isolations and 10 (2.42%) colonization. No patients presented pre-transplantation respiratory fungal infection. The most common non-*Aspergillus* molds isolated are shown in Table 2. *Penicillium* spp. were the main colonization agents (9 of 10 LTR); *Scedosporium apiospermum* complex was found in 1 patient.

Post-transplantation non-*Aspergillus* mold isolation

Ten (2.42%) of the 412 patients developed 10 non-*Aspergillus* spp. mold infections, including 3 (30%) cases of simple tracheobronchitis, 3 (30%) ulcerative tracheobronchitis, 2 (20%) bronchial stent infections, and 2 (20%) invasive pulmonary infections. Total incidence of IFI (ulcerative tracheobronchitis or invasive pulmonary infection) was 1.21% (5 of 412 patients), with a 1-year cumulative incidence of 0.97%. Median time from transplantation to the first non-*Aspergillus* mold infection was 420 days (128-1621 IQR). Based on the time infection was diagnosed after transplantation, 9 (90%) were classified as late onset. *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus)* (4, 40%) and *Scedosporium apiospermum* complex (4, 40%) were the main causative agents. Four of the 10 patients (40%) were co-infected with *Aspergillus* spp. and 3 of 10 (30%) had a bronchial stent. The clinical characteristics, microbiologic, bronchoscopy, and radiologic findings, treatment, and outcome of the 10 patients with non- *Aspergillus* mold infection are reported in Table 3.

Among 49 cases of pre-transplantation non-*Aspergillus* mold isolation or colonization, only one (2.04%) infection was caused by the same pathogen after transplantation (*Scedosporium apiospermum* complex), manifesting early (31 days) after the procedure, despite the use of prophylaxis with n-LAB and voriconazole (Figure 1).

Overall, respiratory samples tested positive for non-*Aspergillus* mold at some time point after transplantation in 70 (16.9%) of 412 patients. Molds were isolated in 47 (40.2%) sputum, 34 (29.1%) BAL/bronchial aspirate, and 31 (26.5%) tracheal aspirate specimens, and 5 (4.3%) bronchial biopsies. Twenty-four (5.8%) LTRs had post-transplantation colonization and 41 (9.9%) a single isolation. The most common isolates in post-transplantation samples are listed in Table 2. One patient presented 1 subsequent case of IFI (1 *Scopulariopsis*) after colonization and another had non-invasive infection after *P. lilacinum* colonization (Figure 1).

In bronchoscopy specimens, intrinsically AB-resistant non-*Aspergillus* molds were detected more often than AB-variably sensitive and -sensitive molds (54.5% vs 25%, p=0.04) (Table 4) and detection of resistant species was associated with a higher risk of infection (56.3% vs 1.3%, respectively, *p*=0.000

Risk associated with non-*Aspergillus* mold colonization and infection

On time-dependent Cox regression analysis, CLAD showed an association close to significance with development of non-*Aspergillus* mold colonization and infection (HR 3.23, 95% CI 0.82-12.73; *p*=0.09). However, there was no time-dependent relationship between colonization and infection by non-*Aspergillus* spp. and development of posterior CLAD (HR 0.60, 95% CI 0.23-1.56, p= 0.29).

Patients with a single-lung transplant were not at higher risk of post-transplantation non-*Aspergillus* mold colonization or infection than those receiving a double-lung transplant (8.3% vs 11.5%, *p*=0.6).

Treatment, outcome, and mortality

Once the diagnosis of non-*Aspergillus* spp. mold infection was suspected, patients received therapy according to the severity of infection and antifungal susceptibility of the mold (Table 3). Four of 5 (80%) patients died after IFI was detected, with 3 IFI-related (60%) deaths, despite antifungal treatment. These included 2 of 2 (100%) patients with invasive lung disease and 1 of 3 (33.3%) with ulcerative tracheobronchitis. Mortality was 60% in patients with invasive disease vs. 0% in non-invasive infections. Three of 412 LTRs (0.72%) died of non-*Aspergillus* mold infection, which accounted for 1.6% of the 188 deaths occurring in these patients.

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DISCUSSION

Patients undergoing lung transplantation are an emerging group at risk of non-*Aspergillus* mold infection. Previous articles have provided some data on non-*Aspergillus* molds in LTRs (3), (7), but to our knowledge, none have specifically focused on the epidemiology of these molds and the impact of their pre- and post-transplantation detection in LTRs receiving lifetime n-LAB prophylaxis.

The incidence of non-*Aspergillus* mold infection was 2.42% in our cohort, and the incidence of invasive infection was 1.21% (1-year cumulative incidence, 0.97%). Various studies in which universal or targeted antifungal prophylaxis has been used in LTRs have reported rates of 0.4% to 3% (7), (15), (3) ,(2). Our findings are also in keeping with those of some retrospective

epidemiologic studies reporting that these infections typically occur late (>90 days) after transplantation (1, 3).

Among our LTRs testing positive on preoperative respiratory culture, only 1 patient developed fatal invasive infection, caused by *S. apiospermum* complex. The indication for transplantation is controversial in patients in whom *Scedosporium* spp. are isolated preoperatively (especially *S. prolificans*). Although pre-transplantation *Scedosporium* spp. colonization is common, particularly in patients with advanced cystic fibrosis (16), previous studies (5),(17) have concluded that because of the low incidence of post-transplantation IFI, *Scedosporium* colonization, in itself, should not be considered a contraindication for transplantation. There is little available information (18),(19) on the significance of pre-transplantation colonization by non-*Aspergillus* molds other than *Scedosporium* spp. Based on previous articles (5),(17) and our experience, we believe that the implications of respiratory tract colonization with filamentous fungi should be assessed individually and not preclude lung transplantation.

Post-transplantation single isolation (9.9%) and colonization (5.8%) by non-*Aspergillus* molds were common findings, as has been described (range, 14%-23.6%)(7, 8) . Of note, all but one episode resolved, and there was only one subsequent case of invasive infection (1 *Scopulariopsis*). Our data support the results of a study by Silveira et al (7) suggesting that isolation of non-*Aspergillus* molds in BAL specimens may not be associated with development of IFI, regardless of the prophylaxis.

In contrast to previous reports citing mucormycosis (20), (21), (22) and fusariosis (23, 24) as emerging problems in solid organ transplant recipients, there were no cases of invasive disease due to these microorganisms in our LTRs. The epidemiology of non-*Aspergillus* molds in our population was likely the result of the Spanish setting and n-LAB prophylaxis, with emergence of infections caused by intrinsically AB-resistant non-*Aspergillus* molds (*Scedosporium* spp.,

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Purpureocillium spp.) rather than variably susceptible or sensitive ones (*Penicillium* spp., *Fusarium* spp., *Mucormycetes*).

There was a high prevalence of *Penicillium* species and dematiaceous molds, mainly in sputum and tracheal aspirate. Detection of these molds (with the notable exception of *P. marneffei*) possibly corresponded to normal contamination of laboratory samples, a single isolation, or respiratory tract colonization (8). Despite the high percentage of positive specimens, there was only one subsequent case of invasive disease (ulcerative tracheobronchitis caused by *Penicillium* spp.). Nevertheless, invasive infections caused by these fungi have been described previously (25), so it is important to exclude a true infection when they are detected.

Purpureocillium lilacinum and *Scedosporium apiospermum* complex were the main causes of infection in our cohort, and isolation of these molds in highly representative respiratory samples such as bronchoscopy specimens was associated with greater risk of infection compared to other non-*Aspergillus* molds.

In a study by Heats et al, *Scedosporium* spp. infection was documented in 36% of LTRs from whom *Scedosporium* spp. was recovered after transplantation (26). Based on these findings and our results, we believe that *Purpureocillium* spp. or *Scedosporium* spp. isolation is an important finding in this at-risk population that should prompt a diagnostic effort to recognize the possibility of true fungal infection.

In our cohort, *Purpureocillium* spp. emerged as an important cause of respiratory fungal infection. Surprisingly, this association has not been described in other studies focussing on IFI in immunosuppressed patients and LTRs (1, 2),(3),(7). *Purpureocillium* spp. is a saprophytic filamentous fungus with worldwide distribution, which, in the last years has been described as a cause of serious infections in immunocompromised patients (27),(3). *P. lilacinum,* one of the two *Purpureocillium* species associated with human infection (P. *variotii* and *P. lilacinum*) was the one most commonly isolated in our patients. The reported clinical manifestations of *P.*

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lilacinum are mainly oculomycosis and cutaneous and subcutaneous infections; it is rarely cited as a causal agent of disease in other parts of the body (28). *Purpureocillium* spp. have variable susceptibility to antifungal agents. Emergence of this mold in our LTR population may be related to low in-vitro susceptibility of clinical isolates to AB (27) Our patients were treated with a combination of voriconazole plus n-LAB. Previous studies have shown that triazoles (including posaconazole and ravuconazole) seem to be the most effective agents for treating *P. lilacinum* infection (27).

Scedosporiosis is a particular problem in Spain. The clinical manifestations of *Scedosporium* spp. infection in LTRs are diverse and range from asymptomatic colonization to severe invasive disease, which seems to predominate (26),(29). *S. apiospermum* complex has been more widely reported in LTRs than *S. prolificans,* and innate resistance or erratic susceptibility to AB is characteristic of both (30). Mortality caused by invasive *S. apiospermum* complex infection was very high (50%) in our cohort, as in previous studies (54%-78%) (30), despite the use of voriconazole, currently considered the treatment of choice (31).

Our *Scopulariopsis* spp. case adds to the growing literature on this mold as an important opportunistic pathogen in transplantation, with high fatality rates (32).

Of note, in 4 of the 10 patients (40%) with non-*Aspergillus* mold infection (20% of patients with IFI), *Aspergillus* spp. were also isolated. It is unknown whether co-habitation of these fungi has an enhancing effect on their individual virulence capacity or what antifungal therapy would be optimal in these cases (28).

Although there was a relatively low incidence non-*Aspergillus* IFI in our LTR cohort, these agents were major contributors to IFI mortality (60% of related deaths, 100% in pulmonary forms, 33.3% in ulcerative tracheobronchitis). Despite improvements in the antifungal armamentarium over the past decade, IFI-related mortality remains high in transplant recipients (21.7%-93% in disseminated forms) (3), (33).

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The limitations of our study include its retrospective, single center, observational nature, and the lengthy period studied (10 years), which implies some changes over time in the diagnosis and therapy of mold infections. The strengths of our study reside in the large lung transplant population analyzed and the lengthy follow-up. Beside in our cohort incidence of cystic fibrosis (6.3%) is lower than reported in the Registry of the International Society for Heart and Lung Transplantation (34) and therefore may need to be taken into account when comparing infection and colonization rates with other studies. Nevertheless in the study by Silveira et al. cystic fibrosis patients represented only the 6% of LTR with a positive BAL culture for non-Aspergillus mold (7). In addition, bronchoscopy protocols of our hospital differs from some other centers. Therefore, it may need to be taken into account when studying the relationship between molds and CLAD because fungal colonization could be underestimated.

In conclusion, pre- and post-transplantation isolation of non-*Aspergillus* molds in respiratory samples was high in LTRs undergoing n-LAB prophylaxis. There was a relatively low incidence of invasive disease, but it was associated with considerable related mortality. *Purpureocillium* spp. are emerging as a cause of respiratory infection in LTRs. Detection of intrinsically AB-resistant non-*Aspergillus* molds was more common in bronchoscopy samples. These strains were associated with a higher risk of infection than AB-variably susceptible or AB-sensitive molds.

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Single isolation	One positive sputum or tracheal aspirate culture for mold in asymptomatic patients with normal-appearing respiratory mucosa and absence of endobronchial lesions
Colonization	Single positive bronchoscopy specimen culture (bronchoalveolar lavage, bronchial aspirate or bronchial biopsy) or at least two positive sputum or tracheal aspirate cultures for mold in asymptomatic patients with normal-appearing respiratory mucosa and absence of endobronchial lesions
MOLD INFECTION	
Simple tracheobronchitis	Detection of mold spp. and clinical symptoms (eg, purulent sputum production) plus bronchoscopy findings of mucus and edematous red mucosa, with bacterial infection ruled out.
Bronchial stent infection	Detection of mold and clinical symptoms (eg, purulent sputum production) plus bronchoscopy findings of mucus and edematous red mucosa, with bacterial infection ruled out in patients with bronchial stent
Ulcerative/pseudomembranous tracheobronchitis	Detection of mold with bronchial biopsy and/or bronchoscopy findings of necrotic ulcers or pseudomembrane in the anastomosis or in the tracheobronchial tree that disappeared after treatment
Invasive pulmonary fungal infection	Detection of mold with evidence of tissue damage on lung histopathology or radiologic signs of invasive fungal infection

Table 1. Classification of mold single isolation, colonization, and infection modified (11) (12)

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Organism	Pre-transplantation positive respiratory samples n (%)	Post-transplantation positive respiratory samples n (%)
***Penicillum* spp.**	28 (52.8%)	53 (45.3%)
Unspecified molds	18 (33.3%)	12(10.2%)
***Fusarium* spp.**	2(3.7%)	11(9.4%)
Scedosporium apiospermum	1(1.9%)	12(10.2%)
***Purpurecillum* spp.**	1(1.9%)	19(16.2%)
Dematiaceous fungi	2 (3.7%)	6(5.1%)
***Trichoderma* spp.**	1 (1.9%)	0
***Scopulariopsis* spp.**	0	2(1.7%)
Mucor spp.	0	1(0.9%)
Acremomium spp.	0	1(0.9%)
Total	53	117

Table 2. Pre-transplantation non-*Aspergillus* molds isolated in 53 different respiratory samples from 49 lung transplant recipients and post-transplantation non-*Aspergillus* spp. molds isolatd in 117 different respiratory samples from 70 lung transplant recipients. Others: Respiratory samples: sputum, tracheal aspirate, BAS/BAL, biopsy. Abbreviations:BAL, bronchoalveolar lavage; BAS, bronchial aspirate.

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	Sex, Age	Year	Baseline disease	Type of transplant	Time elapsed (days)	Pre-transplant non-*Aspergillus* mold isolation/ colonization	Post-transplant non-*Aspergillus* mold isolation/ colonization	Risk factors infection	Type of infection	Histology	Infection's Mold (respiratory sample)	*Aspergillus* Co-infection	Bronchoscopy	Chest CT	Treatment and duration (days)	Death
1	F, 61	2005	Fibrosis	Single	31	*Scedosporium apiospermum*	No	AR	Ulcerative TBR	Proven (necropsy)	*Scedosporium apiospermum* (BAL, tracheal aspirate)	No	ND	ND	Voriconazole + n-LAB (46, death)	Related
2	M, 51	2006	COPD	Double	701	No	No	Stenosis GNB	Simple TBR	-	*Scedosporium apiospermum* (BAL)	No	Mucus plaque	Negative	Voriconazole + n-LAB (112)	Unrelated
3	F, 55	2009	COPD	Double	121	No	*Penicillium* spp.	GNB	Ulcerative TBR	Proven	*Scedosporium apiospermum* (biopsy)	*A. fumigatus*	Plaques	Negative	Voriconazole + n-LAB (180)	Unrelated
4	M, 23	2010	Burton	Double	139	No	*Scopulariopsis*	CMV, CLAD, GNB	Invasive pulmonary Infection-Disseminated	Proven (necropsy)	*Scopulariopsis* (BAL)	No	ND	ND	No	Related
5	F, 47	2011	Bronchiectasis	Double	132	No	No	GNB	Simple TBR	-	*Purpureocillium lilacinum* (BAL)	No	Mucus plaque	Negative	Voriconazole + n-LAB (136)	No
6	M, 59	2011	COPD	Double	1027	No	*Scedosporium prolificans*	AR	Simple TBR	-	*Scedosporium apiospermum* (BAL)	No	Mucus plaque	Negative	Voriconazole + n-LAB (110)	Unrelated
7	M, 63	2011	Fibrosis	Single	131	No	No	AR	Ulcerative TBR	Proven	*Penicillium* spp. (BAL)	No	Plaques	Negative	Voriconazole + n-LAB (139)	No
8	F, 50	2012	COPD	Double	1574	No	No	Stent, Stenosis, CLAD, GNB, no prophylaxis	Stent infection	-	*Purpureocillium lilacinum* (BAL, sputum)	*A. niger*	Mucus plaque	Negative	n-LAB (58)	No
9	M, 34	2013	Histiocitosis	Double	2089	*Fusarium* spp.	*Penicillium* spp.	Stent, Stenosis CLAD, GNB	Invasive pulmonary infection	Proven	*Purpureocillium lilacinum* (biopsy)	*A. flavus*	Plaques	Nodules	Voriconazole + n-LAB (81, death)	Related
10	F, 61	2013	Sarcoidosis	Single	1764	No	*Penicillium* spp. *Fusarium* spp. *Purpureocillium* spp.	Stent, Stenosis GNB	Stent infection	-	*Purpureocillium lilacinum* (biopsy)	*A. terreus* *A. flavus*	Mucus plaque	Negative	Voriconazole + n-LAB (60)	No

Table 3. Clinical characteristics, microbiological, bronchoscopy and radiologic findings, treatment, and outcome of patients with non-*Aspergillus* mold infection

Abbreviations: AR, acute rejection; BAL, bronchoalveolar lavage; GNB, chronic gram-negative bacterial colonization; CLAD, chronic lung allograft dysfunction; COPD, chronic obstructive pulmonary disease; n-LAB, nebulized liposomal amphotericin; ND, not done; TBR, tracheobronchitis

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Respiratory Samples	AB-resistant	
	No	Yes
Sputum	51.2%	12.1%
Tracheal aspirate	23.8%	33.4%
BAS/BAL	23.8%	42.4%
Biopsy	1.2%	12.1%

Table 4. Isolation of AB-resistant non-*Aspergillus* molds and AB-variably susceptible or sensitive non-*Aspergillus* molds in different respiratory samples. Abbreviations: AB, amphotericin B; BAL, bronchoalveolar lavage; BAS, bronchial aspirate

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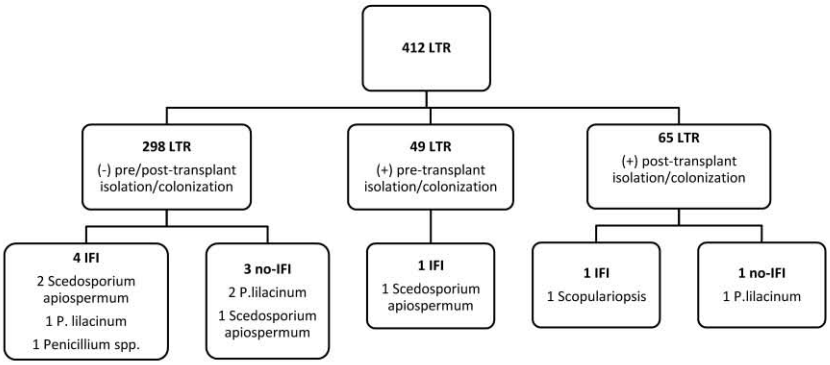

Figure 1. Distribution of invasive and no invasive fungal infections in LTR with negative (-) pre-transplant and post-transplant non-*Aspergillus* molds isolation or colonization; positive (+) pre-transplant and post-transplant non-*Aspergillus* molds isolation or colonization with fungal infection caused by the same previously isolated mold. Abbreviations: IFI, invasive fungal infection; no-IFI, no invasive fungal infection

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VI. DISCUSIÓN

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VI.1. Trabajo 1:"Unusual forms of subacute invasive pulmonary aspergilosis in patients with solid tumors"

En el primer estudio el 10% (18/180) de todos los pacientes con API presentaban un tumor sólido como factor de riesgo principal para API, como ha sido descrito en otras series (rango 2.4-10.3%) (103) (104). En el 77.8 % de estos pacientes (14/18) la infección se presentó en forma de APC (Tabla 3). Similares resultados han sido comunicados en otro estudio previo, que identificó la presencia de neoplasia pulmonar como un factor de riesgo de APC en el 10.3% de los casos (13/126) (30).

La importancia de este estudio está en la caracterización de la APC en los pacientes con neoplasia sólida, que son un nuevo grupo de riesgo para el desarrollo de esta entidad poco conocida y posiblemente infradiagnosticada por diferentes razones.

En primer lugar hemos evidenciado que en los pacientes oncológicos la presentación clínica de la APC es variable, inespecífica y los síntomas podrían estar solapados por la enfermedad de base o la presencia de coinfecciones bacterianas asociadas, como se ha comunicado también en otra serie (105).

En segundo lugar, hemos observado que los pacientes oncológicos pueden tener anomalías radiológicas (cavitaciones pulmonares o nódulos) causadas por su enfermedad neoplásica que pueden ser difíciles de interpretar y que presentan una evolución radiológica típica a lo largo del desarrollo de la APC (106). En nuestra serie, los hallazgos radiológicos más frecuentes en el TC torácico realizado antes del aislamiento de *Aspergillus* spp. eran masas o nódulos (66,7%), y masas cavitadas (33,3%). Tras el diagnóstico de APC, 8 de 12 pacientes (66,7%) mostraron en los TC de control la formación de cavidades nuevas o la expansión de una o más cavidades previas (Tabla 4). Estos patrones radiológicos estaban en concordancia con los descritos en estudios anteriores (29) (107).

En tercer lugar, observamos que en nuestros pacientes la primera línea de defensa local pulmonar estaba alterada por la presencia de la neoplasia primaria o metastásica, asociada a otros factores que comprometían la respuesta inmunológica local (EPOC, radioterapia, cirugía, neumotórax, TBC) y sistémica (quimioterapia, corticosteroides, otros inmunosupresores, alcoholismo, diabetes, cirrosis, VIH) (29) (30).

A destacar que todos los pacientes del estudio presentaban una enfermedad oncológica avanzada (estadio III o IV) o una respuesta limitada al tratamiento oncológico (enfermedad progresiva 57.1%, respuesta parcial 35.7%), sugiriendo que la enfermedad oncológica grave se asocia con una disminución de la inmunidad (Tabla 4). Aunque la neutropenia es un factor de riesgo conocido para la API en los pacientes hematológicos (19), sólo 2 pacientes de nuestra cohorte (7.1%) tenían neutropenia. La presencia de un recuento normal de leucocitos podría explicar el patrón radiológico y histológico de necrosis inflamatoria de la APC, en lugar del patrón angioinvasivo comúnmente visto en los pacientes hematológicos con neutropenia (108).

Otro de los hallazgos de este estudio es que, a pesar del uso del tratamiento considerado de elección con triazoles (109) (110) (111), la respuesta a la terapia a las 6 semanas de seguimiento era escasa (35.7%) y la mortalidad global era elevada (71.4%) en la población oncológica. Otros estudios en APC en poblaciones diferentes a los oncológicos han mostrado resultados más favorables de respuesta al tratamiento antifúngico (entre 43.5 y 83.1%) (110) (111). Posiblemente, este resultado se explica en nuestra cohorte por la gravedad de la enfermedad de base de nuestra población y por el tiempo de seguimiento limitado a causa de su enfermedad oncológica. Asimismo, sugiere que la evidencia de infección por *Aspergillus* spp. en los pacientes con neoplasia sólida se asocia a muy mal pronóstico. La mortalidad está probablemente relacionada con las comorbilidades subyacentes, el retraso en la introducción del tratamiento antifúngico y con la escasa penetración de los medicamentos en las zonas pulmonares desvitalizadas por la cirugía, radioterapia y necrosis tumoral. Posiblemente el

diagnóstico precoz y el tratamiento oportuno podrían tener un impacto beneficioso en tratamiento de esta entidad.

En resumen en los pacientes con neoplasia de pulmón primaria o metastásica, la presencia de cambios anatómicos pulmonares debidos a la neoplasia, asociados a alteraciones locales y sistémicas que comprometen la respuesta inmune, parecen ser factores que predisponen a la APC. En la actualidad, esta entidad poco conocida puede ser infradiagnosticada, debido a la enfermedad de base que puede enmascarar los signos y síntomas de la APC. El aislamiento de *Aspergillus* spp. en muestras respiratorias debe inducir un elevado nivel de sospecha de infección en estos pacientes vulnerables en los que la APC se asocia con un mal pronóstico.

Tabla 4. Características clínicas, microbiológicas, radiológicas, tratamiento y evolución de 18 pacientes con neoplasia primaria de pulmón y neoplasia con metástasis pulmonares complicadas con APC. Abreviaturas: DM, diabetes; EE, enfermedad estable; EP, enfermedad progresiva; ES, esteroides¸ QT, quimioterapia en el último mes; M, mujer; NSCLC, neoplasia de células no pequeñas; n-LAB, anfotericina B liposomal nebulizada; NTX, neumotórax; RP, respuesta parcial ; RT, radioterapia; SCLC, neoplasia de células pequeñas; V, varón ; TBC, tuberculosis

Sexo, edad	Neoplasia y estadio	Comorbilidades	Muestra	Tc toracico previo al diagnóstico de *Aspergillus*	Tc toracico al diagnóstico de *Aspergillus*	Tc toracico despues del diagnóstico de *Aspergillus*	Tratamiento	Exitus
V, 58	NSCLC primario, E-III, RP	EPOC, QT, RT, VIH	LBA: *A. fumigatus*	Masa	Masa cavitada y nódulos	Mejoria	Voriconazol + n-LAB.	No
V, 73	Neoplasia de sigma metastática, E- IV, RP	Alcoholismo, EPOC, NTX, RT, cirugia	LBA: *A. fumigatus*	Masa cavitada y consolidación	Expansión de cavidad existente y fomación de cavidades nuevas	Expansión de cavidad existente	Voriconazol	No
V, 53	Neoplasia de lengua mestástatica, E-IV, EP	Cirrosis, QT	LBA: *A. fumigatus*	Diagnóstico de neoplasia y APC superpuesto	Masa cavitada y nódulos	No TC	Voriconazol	Relacionada
V, 65	NSCLC primario, E-III, EP	Alcoholismo, EPOC, NTX	LBA: *A. flavus*	Nódulo	Masa	Mejoria	Voriconazol	No-relacionada
V, 56	Timoma metastático, E-IV, EP	miastenia gravis, QT RT, ST, tacrolimus	Esputo: *A. fumigatus*	Nódulo	Nódulo cavitado	Expansión de cavidad con formación de abscesos	Itraconazol	Relacionada
V, 65	SCLC primario, E-IV, EP	EPOC, DM, NTX, TBC	LBA: *A. fumigatus*	Diagnóstico de neoplasia y APC superpuesto	Masa	Consolidación estable, derrame pleural	Voriconazol	Relacionada
M, 55	NSCLC primario, E-IV, EP	erlotinib, ES	Punción pulmonar: *A. fumigatus*	Masa	Masa cavitada	Expansión de cavidad existente	Voriconazol	No-relacionada
V, 39	Timoma metastático, E-IV, EP	m-Tor, RT, cirugía	Esputo: *A. fumigatus*	Múltiples masas y nódulos	Formación de cavidades y abscesos nuevos	No TC	Voriconazol	Relacionada
M, 68	Neoplasia de cervix metastática, E-IV, EP	Alcoholismo, QT, ES	Biospia cutanea *A. flavus*	Diagnóstico de neoplasia y APC superpuesto	Nódulo cavitado con absceso	No TC	Voriconazol	No
V, 62	NSCLC primario, E-IV, EP	EPOC, ES	Esputo: *A. fumigatus*	Masa	Masa	Formación de cavidad y absceso	Itraconazol	Relacionada

V, 66	NSCLC primario, E-IV, EE	EPOC, QT	Esputo: *A. terreus*	Diagnóstico de neoplasia y APC superpuesto	Masa	Lobectomia	Voriconazol	No
V, 64	NSCLC primario, E-III, EP	QT	Esputo: *A. terreus*	Diagnóstico de neoplasia y APC superpuesto	Masa cavitada	Expansión de cavidad existente y formación de nuevas cavidades	Voriconazol	Relacionada
M, 46	NSCLC primario, E-III, EP	EPOC, alcoholismo, neutropenia, QT, cirugía	Esputo: *A. fumigatus*	Masa cavitada	Expansión de cavidad existente y formación de nuevas cavidades	Mejoria	Voriconazol	Relacionada
M, 76y	NSCLC primario, E- III, EP	ES, RT	LBA: *A. terreus*	Masa cavitada y nódulo	Expansión de cavidad con formación de abscesos	Expansión de cavidad con formación de abscesos	Voriconazol	Relacionada

VI.2. Trabajo 2: "10 years of prophylaxis with nebulized liposomal amphotericin B and the changing epidemiology of *Aspergillus* spp. infection in lung transplantation"

El segundo estudio realizado en el Hospital Vall d'Hebron, se centró en la evaluación de la eficacia, seguridad y tolerabilidad de la n-LAB y en los cambios en la epidemiología de la infección por *Aspergillus* spp. a lo largo del tiempo.

Se incluyeron todos los pacientes adultos consecutivos sometidos a trasplante de pulmón desde Julio de 2003 hasta Julio de 2013 (412 pacientes), en profilaxis de por vida con n-LAB.

Se recogieron un total de 59 infecciones en 53 TP (14.3%) y la incidencia global de API fue del 5.3%. Aunque es difícil comparar la tasas de infección por *Aspergillus* spp. entre estudios, la mayoría de las publicaciones en las que se ha utilizado profilaxis universal o dirigida, han descrito tasas de incidencia de API que varían entre 1.5% y 12.2% (112, 113)(114).

Nuestra estrategia de profilaxis n-LAB demostró ser bien tolerada (63, 65, 66), permitiendo realizar una pauta de por vida. Como ya había sido reportado en otros estudios (64, 65), en nuestra serie la profilaxis con n-LAB se asoció únicamente con un 2.9% de efectos adversos y con un 1.7% de suspensión del tratamiento por efectos secundarios. Una desventaja de esta terapia es la irritación local a nivel bronquial en forma de broncoespasmo (1.9%), pero el uso de salbutamol puede mejorar estos síntomas. La terapia inhalada tiene la ventaja de que distribuye la n-LAB en las vías respiratorias sin absorción sistémica (63, 65) y que se pueden conseguir concentraciones elevadas de antifúngico en el pulmón sin producirse cambios en la función respiratoria o en el surfactante pulmonar. Comparada con los azoles, la n-LAB, al no absorberse, tiene una menor incidencia de efectos adversos sistémicos (especialmente hepatotoxicidad y nefrotoxicidad) (112, 115, 116) y ausencia de interacciones con fármacos inmunosupresores y glucocorticoides (63).

Otro dato interesante de nuestra cohorte, es la alta proporción de aparición tardía de infección por *Aspergillus* spp. (84.7%) con una mediana prolongada de tiempo de 266 días. Estos

resultados son consistentes con otros trabajos publicados que indican que la infección por *Aspergillus* spp., que antes era considerada una complicación del postrasplante inmediato, ocurre mucho más tarde después del trasplante de pulmón (36, 38, 56). Estos resultados son de particular interés teniendo en cuenta que la duración de la profilaxis antimicótica se limita generalmente a los primeros 3-6 meses después del trasplante (57, 61). Basándonos en nuestra experiencia y los datos publicados, creemos que puede ser aconsejable extender la profilaxis durante más tiempo y que la pauta conveniente de administración de n-LAB (cada 2 semanas) podría ser un factor favorable para la adhesión de los pacientes trasplantados de pulmón (63).

Uno de los hallazgos más importantes de este estudio es sin duda la disminución de la incidencia de infección y colonización por *Aspergillus* spp. en los últimos 5 años (Figura 3). Sin embargo esta disminución se ha asociado a un aumento significativo de los casos causados por especies de *Aspergillus* spp. con susceptibilidad reducida o resistencia a la anfotericina (*A. flavus*, *A. terreus* y *A. alliaceus*) (2003-2008, 38.1 % vs 2009-2014, el 58.8%; p = 0.04) (Figura 3). Estos cambios observados contrastan con los datos de otros estudios epidemiológicos sobre infecciones por *Aspergillus* spp. realizados en España (117, 118) y en otras series de pacientes con TP (23, 56). En cualquier caso, en nuestro estudio no se ha evidenciado que las infecciones causadas por estas especies resistentes se asociaran a peor evolución o a mayor mortalidad. Son diversos los factores que pueden ayudar a explicar los cambios epidemiológicos observados en nuestro estudio. Posiblemente, estos resultados están relacionados con un uso más intensivo de n-LAB a lo largo del tiempo en la práctica diaria (62, 64-66). Como previamente se ha comentado (63), en nuestro protocolo a partir del sexto mes postrasplante, se realiza una pauta de n-LAB cada 2 semanas. Es posible que las concentraciones de n-LAB inhalada con este intervalo prolongado no sean suficientes para inhibir el crecimiento de estas especies de *Aspergillus* potencialmente resistentes y esto podría favorecer el desarrollo de colonización e infección tardía. Además se han observado resistencias primarias in vitro a AB

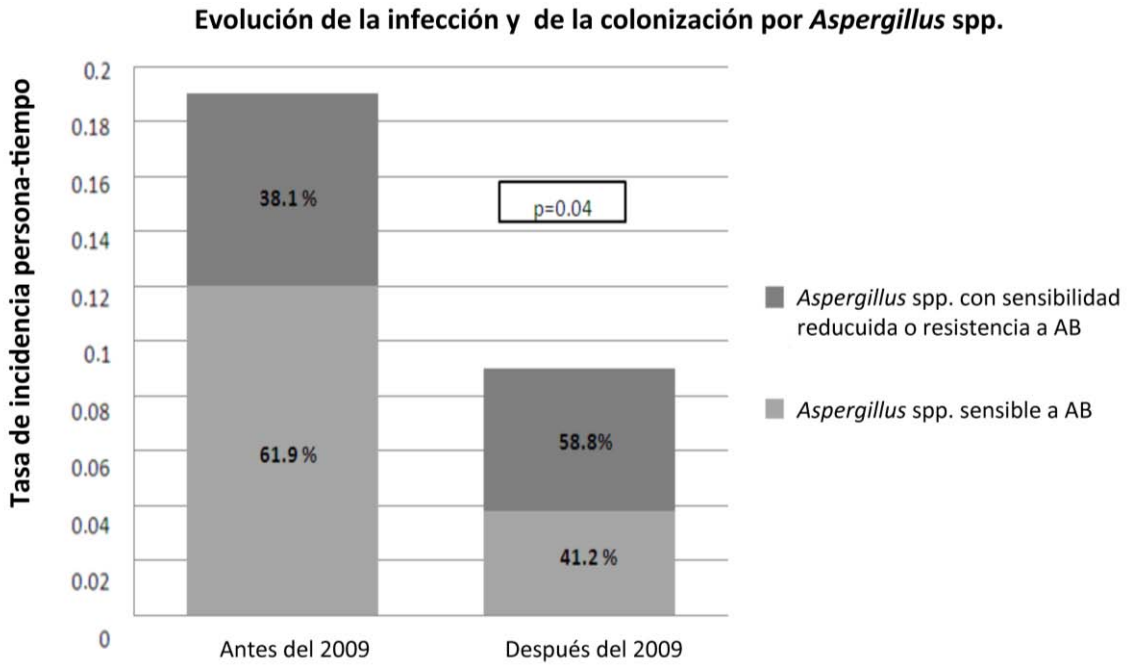
para *A. terreus*, que es intrínsecamente resistente a AB, y para *A. Flavus* en el 10%-15% de los casos (119). Finalmente, aunque el desarrollo de resistencias durante el tratamiento con AB es raro, estudios previos demostraron que el aislamiento de *Aspergillus* spp. en pacientes que previamente habían recibido AB mostraban una concentración mínima inhibitoria (CMI) a AB más elevada que la de los pacientes sin exposición (81, 82). Aunque el conocimiento actual con respecto a la aparición de microorganismos resistentes en los pacientes que reciben profilaxis n-LAB es pobre (81, 83), este hallazgo podría ser un evento centinela que necesita ser monitorizado y estrictamente vigilado.

Coincidiendo con estudios previos que sugerían que la DCI podía ser un factor de riesgo para el desarrollo de infección por *Aspergillus* spp. (120, 121), en nuestro estudio observamos que la DCI se asociaba a la posterior colonización e infección por *Aspergillus* spp. (HR 24.4; IC 95% 14.28-41.97; p = 0.00) (Figura 4). Dado que los TP con DCI tienen alteraciones ventilatorias restrictivas y obstructivas que puede afectar la distribución pulmonar de n-LAB (122), podría ser razonable intensificar la frecuencia de administración n-LAB en este subgrupo de TP. Por el contrario, aunque estudios previos han encontrado una asociación entre colonización e infección por *Aspergillus* spp. (123, 124)) y el desarrollo posterior de DCI, nuestros resultados no confirman esta relación (Figura 4).

En conclusión, este estudio muestra que la profilaxis con n-LAB a la dosis y la frecuencia descrita es bien tolerada y puede ser utilizada para la prevención de la infección por *Aspergillus* spp. en los TP. En los últimos años, la incidencia de la colonización e infección por *Aspergillus* spp. ha disminuido. Sin embargo, están emergiendo especies de *Aspergillus* con sensibilidad reducida o resistencia a la AB, pero no parecen estar asociadas con peor evolución o mayor mortalidad en nuestra serie. La aparición de microorganismos resistentes en los pacientes que recibieron profilaxis n-LAB puede ser un evento centinela que necesita vigilancia. La DCI se asocia con el desarrollo posterior de infección y colonización por

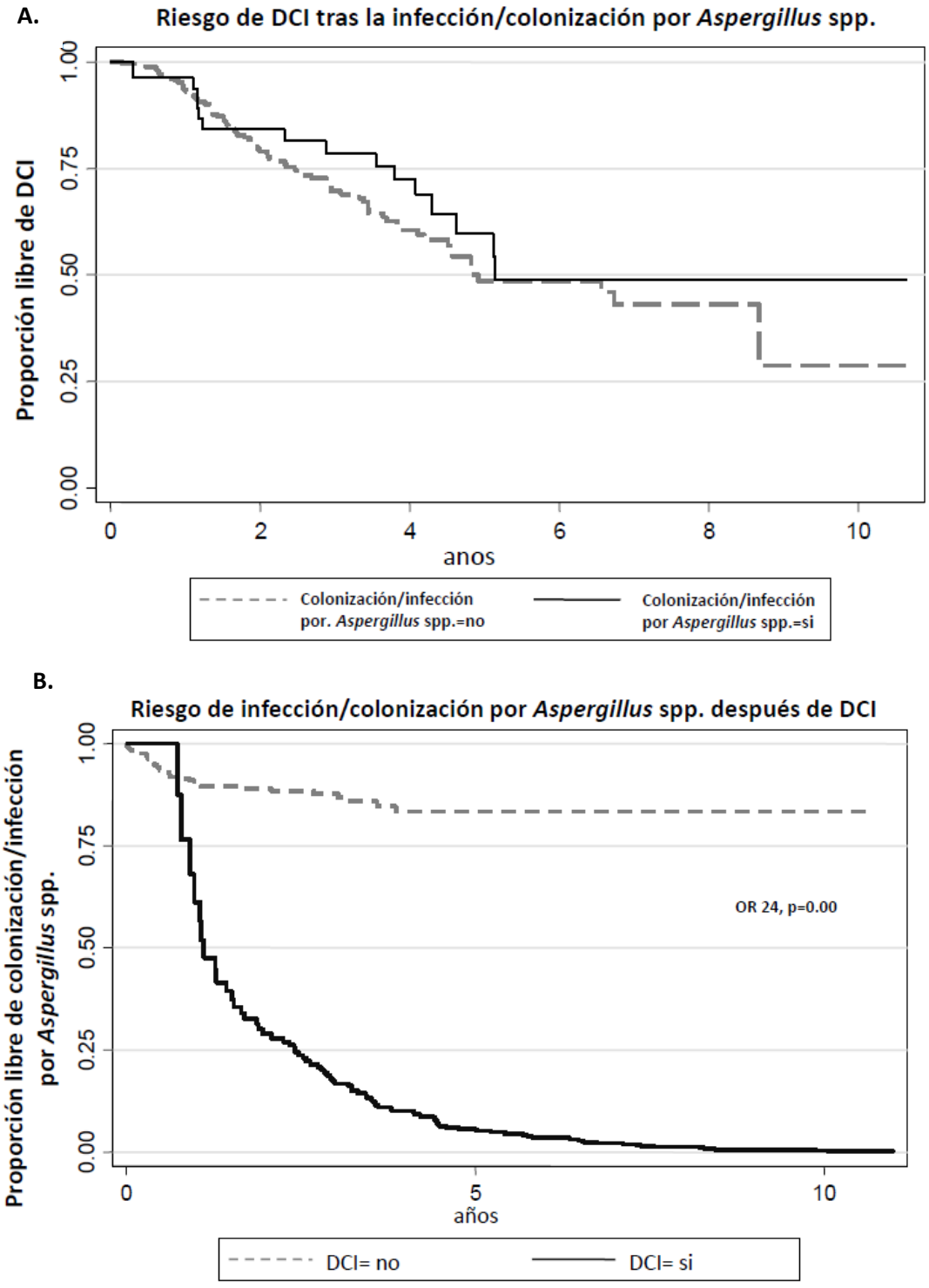
Aspergillus spp. y podría estar indicado intensificar la profilaxis con n-LAB en los pacientes con este factor de riesgo.

Figura 3. Tasa de incidencia tiempo-persona y razones de tasas de incidencia de infección y colonización por *Aspergillus* spp. y evolución de especies de *Aspergillus* spp. con susceptibilidad reducida o resistencia (*A. flavus*, *A. terreus* y *A. alliaceus*) a la anfotericina entre julio de 2003 diciembre de 2008 (antes de 2009) y desde enero 2009 a julio 2014 (después de 2009). Abreviaturas: AB, anfotericina B



Temporada (años)	TP	Seguimiento persona-tiempo (días)	Razón	Razón de incidencia	95% IC	p
2003-2008	43/177	214.53088	0.191	1.97	0.14-0.25	0.0003
2009-2014	84/235	857.06018	0.096	1.32	0.07-0.12	

Figura 4. Análisis de regresión de Cox tiempo-dependiente: (A) Desarrollo del DCI después de la colonización o infección por *Aspergillus* spp.; (B) Desarrollo de colonización y infección por *Aspergillus* spp. después de la disfunción crónica del injerto (DCI).



VI.3. Trabajo 3: "Epidemiology of Invasive Respiratory Disease Caused by Emerging Non-*Aspergillus* molds in Lung Transplant Recipients".

El tercer estudio realizado en el Hospital Vall d'Hebron, evaluó el impacto clínico de los cultivos respiratorios positivos en el pre y post-trasplante para el riesgo de progresión a enfermedad fúngica invasora y caracterizó la epidemiología de las infecciones causadas por hongos filamentosos no-*Aspergillus* spp. en los pacientes TP en profilaxis con n-LAB.

Se revisaron todos los cultivos respiratorios positivos para hongos filamentosos no-*Aspergillus* spp. antes (1 año) y después del trasplante de todos los TP adultos consecutivos desde Julio de 2003 hasta Julio de 2013 (412 pacientes).

Se recogieron un total de 10 infecciones (2.42%), y la incidencia global de enfermedad invasora (traqueobronquitis ulcerativa y infección pulmonar invasora) fue del 1.21% (5 de 412 pacientes). Estos resultados son consistentes con otros trabajos epidemiológicos publicados previamente en pacientes TP, con tasas de IFI causada por estos hongos entre el 0.4 y el 3% (125) (112) (100) (56).

Hay poca información disponible sobre el significado de la colonización del pre-trasplante por hongos filamentosos no-*Aspergillus* spp.(126, 127) y, por ejemplo, la indicación de trasplante es controvertida en pacientes con aislamiento previo de *Scedosporium* spp. (128, 129) . Entre los TP de nuestra cohorte con cultivos positivos pre-trasplante (49 de 412, 11.9%) (Tabla 5), sólo 1 paciente desarrolló una infección invasiva, causada por *S. apiospermum* (Figura 5). Por lo tanto, basándonos en nuestra experiencia y los datos publicados (128, 129), creemos que la colonización del tracto respiratorio por hongos filamentosos previa al trasplante se debe evaluar de forma individual y no es una contraindicación absoluta para la realización de mismo.

También durante el postrasplante (Tabla 4) evidenciamos la presencia de tasas elevadas de aislamientos respiratorios (9.9%) y de colonizaciones (5.8%). En cualquier caso, todos menos

un episodio se solucionaron y solo hubo un caso posterior de infección invasora (1 Scopulariopsis) (Figura 5). Nuestros datos apoyan los resultados de un estudio realizado por Silveira et al., que sugiere que el aislamiento de hongos no-*Aspergillus* spp. en muestras de LBA no parece estar asociado con el posterior desarrollo de IFI, independientemente del tipo de profilaxis seguida (125). Sin embargo esta afirmación dependerá de la especie de hongo aislado y de las características del paciente.

Uno de los hallazgos más importantes de este estudio, es sin duda la epidemiologia de las infecciones por estos hongos en nuestra población de TP. En contraste con estudios previos en los que la mucormicosis (130-132) y la fusariosis (133, 134) destacaban como problemas emergentes en los receptores de TOS, no hubo casos de enfermedad invasiva debida a estos microorganismos en nuestra cohorte. La epidemiología de los hongos no-*Aspergillus* spp. en nuestra población está condicionada posiblemente por el contexto geográfico español y por el uso de profilaxis con n-LAB, observándose la aparición de infecciones causadas por hongos intrínsecamente resistentes a AB (*Scedosporium* spp., *Purpureocillium* spp.) en lugar de por hongos con sensibilidad variable a la misma *(Penicillium* spp., *Fusarium* spp., Mucormycetos).

A destacar como *Purpureocillium* spp. y *Scedosporium* spp. fueron las principales causas (80%) de infección en nuestra cohorte. En nuestro estudio observamos como el aislamiento de estos hongos en muestras respiratorias de broncoscopia se asociaba con un mayor riesgo de infección, en comparación con otros hongos no-*Aspergillus* spp. (56.3% vs 1.3%, p=0.000). Basándonos en nuestros resultados y en otros datos publicados (135), creemos que el aislamiento de *Purpureocillium* spp. o *Scedosporium* spp. es un hallazgo importante en esta población de riesgo que obliga a la realización de un esfuerzo diagnóstico para descartar la presencia de una verdadera infección.

Es importante evidenciar que, aunque hubo una incidencia relativamente baja de IFI por hongos no-*Aspergillus* spp., estas se asociaban a una elevada mortalidad relacionada (100% en las formas pulmonares, 33.3% en la traqueobronquitis ulcerativas). Este dato confirma que, a

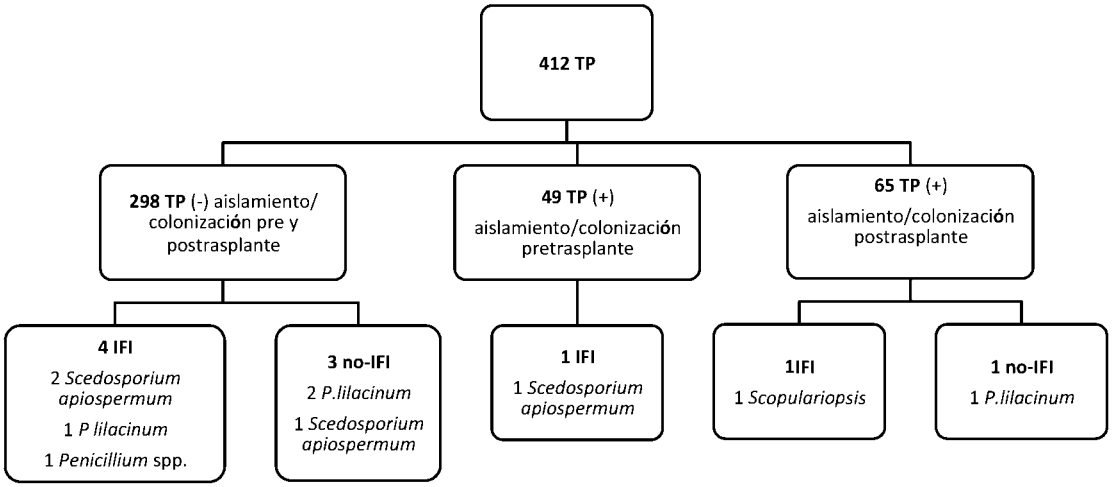
pesar de las mejoras en el armamentario antifúngico en la última década, la mortalidad relacionada con la IFI sigue siendo elevada en los receptores de TOS (21,7% -93% en las formas diseminadas) (100) (136).

En resumen, este trabajo evidencia que aunque el aislamiento de hongos filamentosos no-*Aspergillus* spp. antes y después del trasplante es elevado en los TP sometidos a profilaxis n-LAB, la incidencia de enfermedad invasiva es relativamente baja, pero asociada a una elevada mortalidad relacionada. *Purpureocillium* spp. es un hongo emergente como causa de infección respiratoria en los TP. Los hongos no-*Aspergillus* intrínsecamente resistentes a AB son más comúnmente aislados en las muestras de broncoscopia y se asocian con un mayor riesgo de infección comparados con los hongos sensibles o con sensibilidad variable a AB.

Tabla 5. Hongos filamentosos no-*Aspergillus* aislados en el pre-trasplante en 53 muestras respiratorias de 49 TP y aislados en el post-trapslante en 117 muestras respiratorias de 70 TP. Otros: Muestras respiratorias: esputo, aspirado traqueal, lavado broncoalveolar, broncoaspirado, biopsia

Hongo	Muestras respiratorias positivas pre-trasplante n (%)	Muestras respiratorias positivas post-trasplante n (%)
***Penicillum* spp.**	28 (52.8%)	53 (45.3%)
Hongo filamentoso no especificado	18 (33.3%)	12(10.2%)
***Fusarium* spp.**	2(3.7%)	11(9.4%)
Scedosporium apiospermum	1(1.9%)	12(10.2%)
***Purpurecillum* spp.**	1(1.9%)	19(16.2%)
Hongos dematiaceos	2 (3.7%)	6(5.1%)
***Trichoderma* spp.**	1 (1.9%)	0
***Scopulariopsis* spp.**	0	2(1.7%)
Mucormicosis spp.	0	1(0.9%)
Acremomium spp.	0	1(0.9%)
Total	53	117

Figura 5. Distribución de infección fúngica invasora y no invasora en pacientes TP con cultivos pretrasplante y postrasplante negativos (-) para hongos filamentosos no-*Aspergillus* y positivos (+) con infección fúngica causada por el mismo hongo previamente aislado.
Abreviaturas: IFI, infección fúngica invasora; no-IFI, infección fúngica no invasora; TP, trasplante pulmonar



VI.4. Perspectivas de futuro

Dada la continua introducción de nuevos tratamientos y el manejo de diferentes enfermedades que condicionan una alteración de la inmunidad secundaria, es probable que a lo largo del tiempo las poblaciones de riesgo para el desarrollo de IFI vayan aumentando y que las formas clínicas de presentación vayan cambiando en función del grado de inmunosupresión. Además, es probable que la aparición de nuevos fármacos y nuevos grupos de pacientes puedan condicionar la indicación de nuevos regímenes de profilaxis y de estrategias terapéuticas, que causaran nuevos patrones de resistencias, obligando a la realización estudios de vigilancia epidemiológica. Finalmente, es deseable que los métodos de aproximación diagnóstica a las micosis invasoras cambien, permitiendo un diagnóstico precoz, rápido y eficaz.

A la luz de lo ocurrido en la última década, es imposible no plantearse una serie de preguntas difíciles de responder y que quedaran pendientes para los próximos años. En este último apartado de la discusión, he querido hablar sobre varios de estos interrogantes.

¿Aumentará la incidencia de de aspergilosis pulmonar crónica en nuevas poblaciones de riesgo?

Es probable que en el futuro haya un aumento estimado de la incidencia de APC debido a un mejor diagnóstico de esta entidad, cuya nomenclatura y criterios diagnósticos han sido definidos sólo en los últimos años. Asimismo, la aparición en el futuro de nuevas poblaciones de riesgo con nuevos regímenes de inmunosupresión podrían favorecer el desarrollo de este grupo de enfermedades.

Ha despertado recientemente un gran interés, como problema de salud mundial, la aparición de la APC como secuela a lo largo plazo de enfermedades crónicas (31). Se ha estimado que esta complicación podría llegar a tener una elevada prevalencia como secuela de TBC (1.2

millones de personas en el mundo, de aspergilosis broncopulmonar alérgica (345.000 pacientes) y de sarcoidosis (71.900 personas) (31, 32, 137). Esta estimación puede variar según los registros locales y desafortunadamente en la actualidad no hay datos disponibles sobre enfermos con EPOC, uno de los subgrupos de pacientes más importantes, en los que podría incidir esta enfermedad en el futuro.

¿Cuáles son las nuevas estrategias de profilaxis antifúngica del futuro en los pacientes trasplantados de pulmón?

A pesar de varios estudios relativos a la eficacia de profilaxis antifúngica, siguen sin conocerse la utilidad real de la profilaxis, el agente óptimo y la duración de la terapia profiláctica postrasplante. A parte de la anfotericina B nebulizada, hay otros nuevos agentes antifúngicos nebulizables tales como voriconazol en aerosol, que han sido ensayado en modelos animales y que también podrían modificar en un futuro los regímenes de profilaxis (138). Asimismo, hay nuevos azoles (como isavuconazol, ravuconazol), candinas (aminocandina), asociaciones de antifúngicos con inmunomoduladores, que podrían cambiar también las recomendaciones (139, 140). Es evidente que es necesaria la realización de estudios clínicos prospectivos, multicéntricos para determinar mejor los factores de riesgo de infección fúngica y evaluar el enfoque profiláctico y terapéutico óptimo de la IFI, tanto en adultos como en niños TP.

¿Aumentarán las infecciones producidas por otros hongos a expensas de la reducción de la infección y colonización por *Aspergillus* spp.?

La aparición de hongos resistentes observada en nuestro estudio en pacientes en profilaxis con n-LAB y en otros programas de trasplante en pacientes sometidos a otras profilaxis sugiere el riesgo potencial asociado a esta práctica clínica. Es probable que futuros cambios en la elección del agente antifúngico así como las posibles tendencias hacia cursos más largos de

profilaxis, asociados al posible cambio de los regímenes de inmunosupresión, seguirán alterando los patrones observados de infección fúngica en los paciente con TP.

Para responder a estos interrogantes es imprescindible continuar con estudios de vigilancia epidemiológica que permitan detectar precozmente cambios entre microorganismos causantes de infecciones fúngicas invasoras, así como sus correspondientes implicaciones clínicas. Asimismo, es necesario utilizar estrategias terapéuticas eficaces basadas en el diagnóstico precoz de las micosis invasoras.

VII. LIMITACIONES

VII. LIMITACIONES

El trabajo que aquí se ha presentado tiene una serie de limitaciones que deben mencionarse.

La principal limitación de estos estudios es relativa al diseño retrospectivo. Otra limitación está relacionada con la asunción de los cambios en el diagnóstico y el tratamiento de la infección fúngica asociados a los avances en el manejo de estos pacientes durante el período de estudio.

Los pacientes de nuestra serie representan una población de pacientes frágiles y de elevado riesgo de infección fúngica, siendo esto un factor que hace difícil la realización de estudios clínicos prospectivos aleatorizados. Por otro lado, hemos intentado limitar estos sesgos realizando un estudio multicéntrico (primer estudio) y analizando una población de pacientes muy amplia con un seguimiento prolongado (segundo y tercer estudio).

Por último, los estudios se realizaron en una misma área geográfica (Barcelona), por lo que sus resultados pueden no ser representativos de otras regiones en las que la distribución de los hongos filamentosos puede diferir.

VIII. CONCLUSIONES

VIII. CONCLUSIONES

Como conclusión, este trabajo muestra que en la infección fúngica invasora causada por hongos filamentosos la relación entre patógeno y huésped se modifica constantemente debido a los cambios epidemiológicos y clínicos del propio paciente. Destaca la aparición de nuevas formas clínicas, en la frontera entre la enfermedad saprofítica e invasiva, como la aspergilosis pulmonar crónica, que puede afectar en nuevos grupos de riesgo, como son los pacientes con déficit inmunitario asociado a neoplasia sólida. Asimismo, resalta como en los grupos de pacientes con elevado riesgo de IFI como los trasplantados de pulmón la profilaxis antifúngica, con anfotericina B nebulizada es eficaz, segura y bien tolerada, pero se está observando una adaptación de los hongos filamentosos al nicho ecológico del huésped, con la aparición de cepas resistentes a la anfotericina B y de nuevas especies patógenas.

VIII.1. JUSTIFICACIÓN DEL ESTUDIO E HIPÓTESIS DE TRABAJO

VIII.1.1. Objetivos del primer trabajo

1. **Describir los factores predisponentes, la presentación clínica y la evolución de los pacientes con cáncer de pulmón primario o con metástasis pulmonares secundarias que presentan una aspergilosis pulmonar crónica.**

   En los pacientes con neoplasia de pulmón primaria o metastásica, la presencia de cambios anatómicos pulmonares debidos a la neoplasia, asociados a alteraciones locales y sistémicas que comprometen la respuesta inmune, parecen ser factores que predisponen a la aspergilosis pulmonar crónica. Estos pacientes presentan una clínica variable e inespecífica, muchas veces indistinguible de la enfermedad de base y presentan patrones radiológicos con una evolución típica a lo largo del desarrollo de la aspergilosis pulmonar crónica.

2. **Definir en qué pacientes el aislamiento de *Aspergillus* spp. en muestras respiratorias debe inducir un elevado nivel de sospecha de infección para un diagnóstico precoz.**

En los pacientes con neoplasia de pulmón primaria o metastásica, el aislamiento de *Aspergillus* spp. en muestras respiratorias debe inducir un elevado nivel de sospecha de infección. En estos pacientes vulnerables, la aspergilosis pulmonar crónica esta infradiagnosticada y se asocia con muy mal pronóstico, por lo que es necesario reconocer esta entidad, realizar un diagnóstico precoz y un tratamiento dirigido oportuno.

VIII.1.2. Objetivos del segundo trabajo

1. **Evaluar la eficacia, tolerabilidad y seguridad de la profilaxis con n-LAB tras 10 años de experiencia en los pacientes TP.**

   La profilaxis con n-LAB a la dosis y la frecuencia descrita es bien tolerada y puede ser utilizada para la prevención de la infección por *Aspergillus* spp. en los trasplantados de pulmón

2. **Investigar el impacto que puede tener la profilaxis con n-LAB a largo plazo sobre la evolución de la infección y/o colonización por *Aspergillus* spp. en los TP.**

   La incidencia de la colonización e infección por *Aspergillus* spp. ha disminuido. Sin embargo, están emergiendo especies de *Aspergillus* con sensibilidad reducida o resistencia a la anfotericina B, sin estar asociadas a un peor pronóstico o a mayor mortalidad. La aparición de microorganismos resistentes en los pacientes que reciben profilaxis n-LAB puede ser un evento centinela que necesita vigilancia.

3. **Definir si existe asociación entre infección por *Aspergillus* spp. y disfunción crónica del injerto.**

   La disfunción crónica del injerto se asocia con el desarrollo posterior de infección y colonización por *Aspergillus* spp. y la n-LAB podría intensificarse en los pacientes con este factor de riesgo.

VIII.1.3. Objetivos del tercer trabajo

1. **Describir el impacto clínico que puede tener el aislamiento de hongos filamentosos no-*Aspergillus* spp. pre y post-trasplante en el riesgo de progresión a IFI en los pacientes TP.**

   El aislamiento de hongos filamentosos no-*Aspergillus* spp. antes y después del trasplante es elevado en los trasplantados de pulmón sometidos a profilaxis con n-LAB. Hemos descrito una incidencia relativamente baja de enfermedad invasiva, pero con una elevada tasa de mortalidad asociada.

2. **Investigar la relación existente entre el uso de profilaxis con n-LAB y la infección por hongos filamentosos no-*Aspergillus.***

   En las muestras de broncoscopias de los pacientes trasplantados de pulmón en profilaxis con n-LAB se aíslan con mayor frecuencia hongos no-*Aspergillus* spp. intrínsecamente resistentes a anfotericina B. Estos hongos se asocian con un mayor riesgo de infección comparados con los hongos sensibles o con sensibilidad variable a anfotericina B.

   Purpureocillium spp. puede ser un hongo emergente como causa de infección respiratoria en los trasplantados de pulmón.

IX. ANEXOS

IX.1. ANEXO 1

ORIGINAL ARTICLE MYCOLOGY

Environmental variables associated with an increased risk of invasive aspergillosis

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Abstract

Information on the environmental variables that may affect the incidence of invasive aspergillosis (IA) is scarce. We sought to determine the relationship between airborne spore counts, climatic conditions and IA. We also examined whether circulating respiratory viruses predispose patients to IA in a multicentre cohort study of hospitalized adults with IA. Data on environmental mould spores, climatic conditions and circulating respiratory viruses were obtained from the Environmental Department of the Autonomous University of Barcelona, the Meteorological Service of Catalonia and the Acute Respiratory Infection Surveillance Project in Catalonia, respectively. Between 2008 and 2011, 165 patients with IA were identified. Diagnosis was based on one or more of the following: culture (125 cases), galactomannan antigen (98) and histology (34). One hundred and twenty-seven cases (77%) had criteria for probable IA and the remainder for proven IA. Environmental mould spore counts from the period 28–42 days preceding infection presented significant associations with admissions due to IA. None of the climatic conditions were associated with an increased risk of IA, but the presence of circulating respiratory viruses was associated with a higher risk of infection: the most strongly associated viruses were respiratory syncytial virus, influenza A(H1N1)pdm09 and adenovirus. In conclusion, the presence of high numbers of spores in the air increases the risk of admission due to IA. Circulating respiratory viruses appear to be associated with a higher risk of developing IA. Physicians should be aware of this association in order to optimize prevention and diagnosis strategies for IA during viral epidemic periods.

Keywords: Airborne mould counts, climatic conditions, environmental variables, invasive aspergillosis, respiratory viruses
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Introduction

Invasive aspergillosis (IA) is a life-threatening infection that affects mainly severely immunocompromised patients. Despite advances in diagnosis and treatment, mortality rates associated with IA range from 40 to 90% [1,2]. More detailed knowledge of factors contributing to the pathogenesis of invasive aspergillosis is needed in order to optimize the management of this infection and to improve prophylactic measures.

The development of IA is a multistage process that begins with the inhalation of conidia dispersed in the air and continues with the host response against infection [3,4]. Individual host factors that increase the risk of infection have been well defined [5–7]. However, our understanding of the natural history of infection, the inocula required and the incubation period of the disease are still poorly understood. In particular, information regarding the possible effect of certain environmental factors on the risk of IA is scarce. A previous study

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showed that climatic conditions may influence the airborne spore count in some geographical areas [8]. However, the relationship between climatic conditions, airborne spore counts and rates of admission due to IA is less clear. It is tempting to speculate that circulating respiratory viruses may hinder the host response to infection. It could be related to an increased risk of IA.

We sought to identify the relationship between airborne mould spore count in the community and hospital admissions due to IA. We also aimed to assess the effect of climatic variables on airborne spore counts and/or the risk of IA in our area, and whether circulating respiratory viruses predispose patients to IA.

Methods

Setting, patients and study design

We performed a retrospective multicentre study of all episodes of invasive aspergillosis occurring in hospitalized adult patients between January 2008 and December 2011 at three tertiary teaching institutions in Barcelona, Spain. Only outpatients hospitalized for newly developed signs or symptoms of IA have been included. All haematological severely immunocompromised patients stayed in rooms with strict HEPA filtration. Patients who developed IA were identified by assessment of clinical, microbiology and pathology records, and by review of the diagnostic codes on hospital discharge. Only patients with proven and probable IA in accordance with the definitions of the European Organization for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycosis Study Group (EORTC/MSG) [9] were included. The following information was carefully collected from medical records: demographic characteristics, clinical features and day of IA diagnosis (the day on which the first diagnostic test was performed). For patients whose diagnosis was obtained from post-mortem examination, the day of death was considered to be the day of diagnosis.

This observational study was approved by the Institutional Review Board.

Environmental data

Daily median airborne mould concentrations were calculated from environmental data collected from the Institute of Environmental Science and Technology at the Autonomous University of Barcelona. The estimations were based on continuous measurements taken in the centre of the city. Data on climatic conditions were obtained from the Catalan Meteorological Service and were based on continuous measurements taken at the weather station in our urban area. Daily median records for rainfall, measured in litres/m^2, humidity, wind and wind speed were used for the analysis. Data on circulating respiratory viruses were obtained from the acute respiratory infection surveillance project in Catalonia (PIRIDAC). The numbers of cases of influenza A, influenza B, adenovirus and respiratory syncytial virus (RSV) were used for the study.

Statistical analysis

Categorical variables were described using counts and percentages. Continuous variables were expressed as the mean and standard deviation or median and interquartile range depending on the Kolmogorov-Smirnov test.

Patients admitted for IA were recorded according to cases occurring during 2-week periods. The effect of environmental variables in each 2-week period was analysed. The Spearman ρ correlation was used to detect significant temporal associations between groups. For the analysis of quantitative associations between environmental mould spores and admissions due to IA in a 2-week period, a Poisson regression model was used. To assess potential confounding by environmental variables, we performed a multivariate analysis. A partial Spearman ρ correlation analysis and a Poisson relation with addition covariants were performed. The results were analysed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was established at $\alpha = 0.05$. All reported p-values are two-tailed.

Results

General features of the study population

During the 4 years of the study period, 165 patients with IA were hospitalized. The most frequent co-morbid conditions were haematological malignancies (67 cases; 40.6%, including 13 stem cell transplantations), solid organ transplantation (33 cases; 20%), solid cancer (14 cases; 8.4%) and AIDS (nine cases; 5.4%). Other factors predisposing to IA were neutropenia (49 cases; 29.7%), corticosteroid use (87 cases, 52.7%) and use of other immunosuppressive drugs (97 cases; 58.8%). The diagnosis of IA was established by using one or more of the following methods: culture (125 cases), galactomannan (98 cases) and histology (34 cases). One hundred and twenty-seven cases (77%) had criteria for probable IA. Most cases were caused by *Aspergillus fumigatus* (78.4%), followed by *A. flavus* (7.3%), *A. terreus* (4.2%) and *A. niger* (3.6%). Table 1 summarizes the major epidemiological and clinical characteristics of the patients.

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TABLE 1. Patient baseline characteristics, clinical features and diagnosis

Characteristic	Patients, *n* = 165	%
Age, median years (IQR)	61 (50–68)	–
Male sex	104	63
Underlying disease		
Haematological malignancy	67	40.6
Solid organ transplant	33	20
Haematopoietic stem cell transplant	13	7.9
Solid tumour	14	8.4
AIDS	9	5.4
Immunodeficiency disorder	5	3.0
Other[a]	12	7.3
Immunological risk[b]		
Neutropenia	49	29.7
Corticosteroid therapy	87	52.7
Any immunosuppressive therapy	97	58.8
Infection site		
Pulmonary only	150	90.9
Disseminated IA	15	9.1
Diagnosis[b]		
Culture[c]	125	75.7
Galactomannan	98	59.4
Biopsy or autopsy	34	20.6
Type of IA		
Proven	38	23.0
Probable	127	77.0

[a]Contains patients with severe immunosuppressive treatment, mainly high dose of corticosteroids.
[b]Patients could have >1 characteristic within a category.
[c]*A. fumigatus*, 78.4%; *A. niger*, 3.6%; *A. terreus*, 4.2%; *A. flavus*, 7.3%.

Relationship between admission due to IA and environmental airborne spore count in 2-week periods

The distribution of admissions for IA and the environmental airborne spore count during the study period are shown in Fig. 1. Environmental mould spore counts in the third 2-week period (from 28 to 42 days) preceding infection presented significant associations with the occurrence of IA (Spearman ρ correlation 0.241; p 0.014), as shown in Fig. 2. Table 2 shows the quantitative relation between airborne spore count and admission due to IA in the overall cohort. The presence of environmental airborne mould spores increased the risk of IA admission 6.021 times after the third 2-week period. This correlation was statistically significant. We explored confounding with multivariate analysis but no effect was observed.

Climatic conditions, spore counts and admission for IA

Fig. 3 shows the relationship between climatic conditions and environmental airborne spore counts. No associations were found between precipitation, humidity and wind and the increase or decrease in mould spore counts in our environment in the next four 2-week periods. A relationship was found between higher wind speed and increased environmental spore count after the second 2-week period (Spearman ρ correlation 0.217; p 0.027). A second analysis did not show any association between any climatic conditions and admissions for IA in the next four 2-week periods. We explored confounding with multivariate analysis but no effect was observed.

Circulating respiratory viruses, spore counts and admission for IA

The presence of circulating respiratory viruses was not associated with an increase or decrease in environmental airborne mould spore counts in the next four 2-week periods. However, the existence of circulating respiratory viruses was associated with an increase in admissions due to IA (Fig. 4). The relationship was particularly strong during circulating influenza A(H1N1)pdm09 virus, adenovirus and RSV. The circulation of influenza A(H1N1)pdm09 virus was associated with increased hospital admissions for IA from the beginning of the virus circulation, in the first 2-week period and until the second 2-week period (Spearman's ρ correlation 0.304, p 0.002; 0.386, p < 0.001; 0.286, p 0.003, respectively). Adenovirus was associated with an increase in hospital admissions due to IA during the circulation of the virus and the

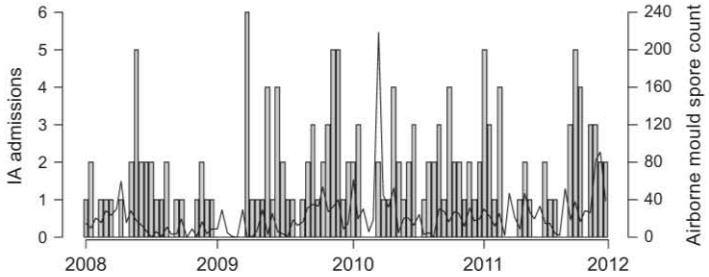

FIG. 1. Temporal trend of IA admission and airborne mould spore count during the study period. Cases occurring during each 2-week reported periods are shown in the bar graph, while the environmental airborne mould spore count is shown in the line graph. One hundred and four 2-week periods were analysed. The distribution of IA admissions by the 2-week periods was as follows: twenty-five 2-week periods had no admissions for IA, 33 periods had one admission for IA, 25 periods had two admissions, nine periods had three, six periods had four, five periods had five, and one period had six.

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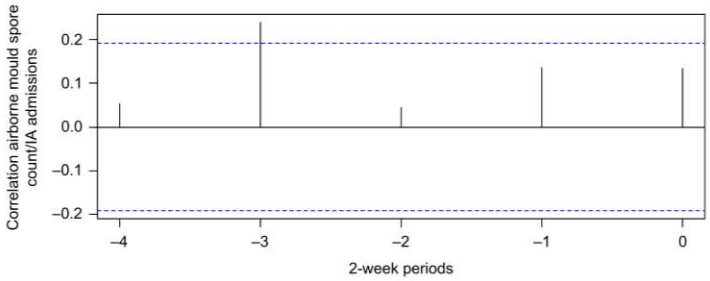

FIG. 2. Temporal relationship between median airborne spore count and invasive aspergillosis admission in 2-week periods. Day 0 was the day of invasive aspergillosis diagnosis. Correlation between median airborne spore count and invasive aspergillosis diagnosis at day 0 is shown in the vertical line graph. The dashed line shows the p value related to a statistically significant association ($p > 0.05$). The figure shows that environmental mould spore counts in the third 2-week period (from 28 to 42 days) preceding infection have significant association with the occurrence of IA (Spearman ρ correlation 0.241; p 0.014).

subsequent 2-week period (Spearman's ρ correlation 0.238, p 0.015, and 0.236, p 0.016). RSV was also associated with higher admission for IA in the current period of circulating virus (Spearman's ρ correlation 0.242, p 0.013). No temporal relationship was found between circulating influenza A (different than H1N1) or influenza B and admissions due to IA.

In a second analysis, we examined the quantitative relationship between airborne spore count and admission for IA in periods with circulating respiratory viruses. Table 2 shows the most significant results of a Poisson regression model. The higher the ratio, the fewer the spores required for infection. As a summary, the airborne spore load required to produce admission for IA was very low during the first 2-week period of circulation of respiratory viruses. This high correlation was also significant and strong during the first 2-week periods of influenza A, B and influenza A(H1N1)pdm09. This correlation

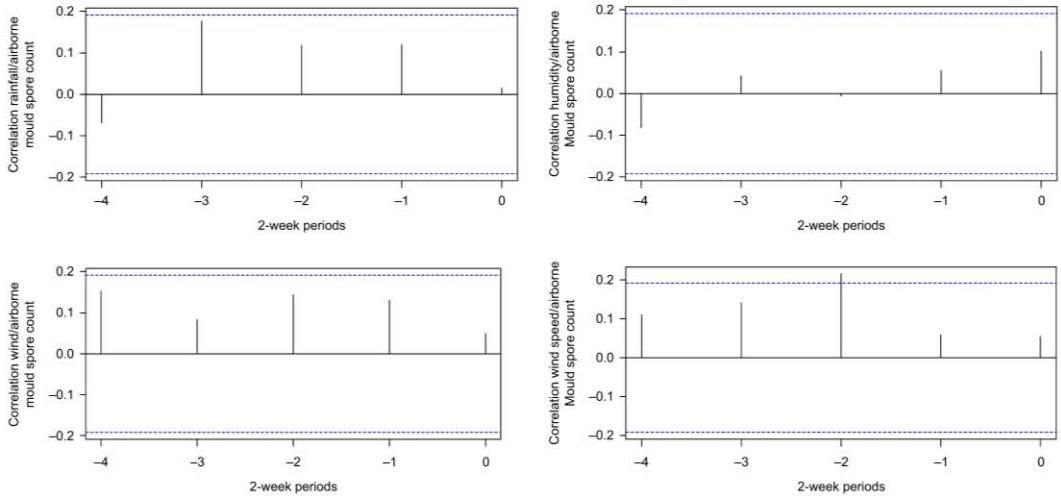

FIG. 3. Temporal relationship between climatic conditions (rainfall, humidity, wind and wind speed) and environmental airborne spore count in 2-week periods. Day 0 was the day of environmental airborne spore count determination. Correlation between climatic conditions and median airborne spore count on day 0 is shown in the vertical line graph. The dashed line shows the p value related to a statistically significant association ($p > 0.05$). The figure shows that there were not associations between precipitation, humidity and wind and the increase or decrease in mould spore counts in the environment in the next four 2-week periods. A relationship was found between higher wind speed and increased environmental spore count after the second 2-week period (Spearman ρ correlation 0.217; p 0.027); however, association between any climatic conditions and admissions for IA in the next four 2-week periods were not found.

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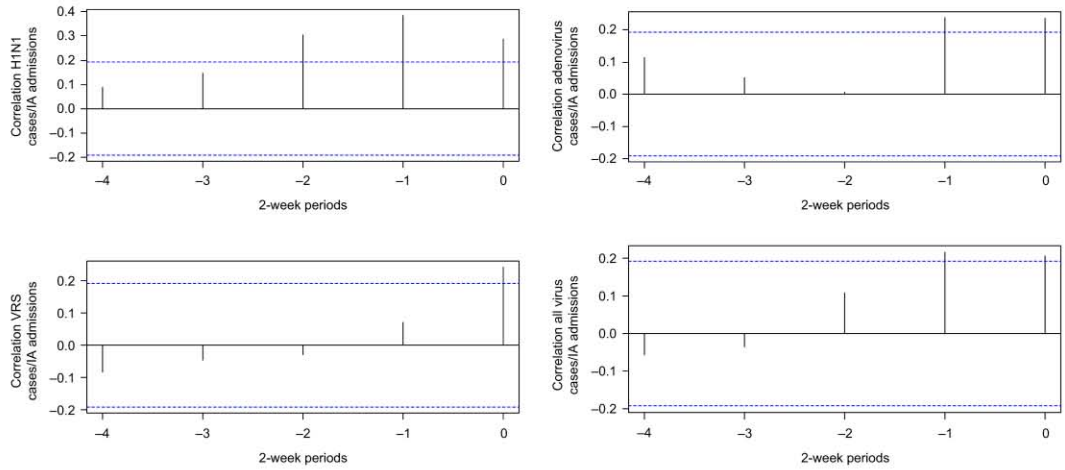

FIG. 4. Significant relationship between circulating respiratory viruses and invasive aspergillosis admission. Day 0 was the day of invasive aspergillosis diagnosis. Correlation between circulating respiratory viruses and invasive aspergillosis diagnosis on day 0 is shown in the vertical line graph. The dashed line shows the p value related to a statistically significant association ($p > 0.05$). The figure shows that the circulation of influenza A(H1N1)pdm09 virus was associated with increased hospital admissions for IA from the beginning of the virus circulation, in the first 2-week period and until the second 2-week period (Spearman's ρ correlation 0.304, p 0.002; 0.386, $p < 0.001$; 0.286, p 0.003, respectively). Adenovirus was associated with an increase in hospital admissions due to IA during the circulation of the virus and the subsequent 2-week period (Spearman's ρ correlation 0.238, p 0.015, and 0.236, p 0.016). RSV was also associated with higher admission for IA in the current period of circulating virus (Spearman's ρ correlation 0.242, p 0.013). No temporal relationship was found between circulating influenza A (different to H1N1) or influenza B and admissions due to IA.

was also observed in the third 2-week period after circulating adenovirus.

Discussion

This multicentre study of a large number of hospitalized adults with IA found a relationship between certain environmental factors and the risk of IA. Specifically, a high airborne mould spore count in the period from 28 to 42 days prior to infection was correlated with higher rates of IA diagnosis. We did not find an association between environmental spore count and climatic variables in our area, or between climatic conditions and IA admissions. Finally, we recorded a strong relationship between circulating respiratory viruses and risk of IA.

The results of our study coincide with those of previous studies that have documented the relationship between environmental mould spore counts and IA [8,10]. These findings support the notion that a substantial proportion of patients with IA acquired the infection outside the hospital. The incubation period of IA has not been well defined and is probably highly variable, depending on the inhaled inocula and host characteristics. In our cohort, which included a mix of patients with a highly varied immunity status, we found a strong relationship between IA admissions and high spore counts from 28 to 42 days before the diagnosis of infection. These data suggest that the interaction between *Aspergillus* and the host begins several days prior to the current diagnosis of IA. Establishing more prompt diagnosis of IA remains a challenge.

Climatic conditions may affect the epidemiology of bacterial, viral and fungal infections [11,12]. The relationship is affected by the impact of the weather on the prevalence or virulence of the pathogen, or by changes in host behaviours. Panackal *et al.* [8] reported that climatic conditions in Seattle affected spore counts and increased IA rates in a cohort of patients who received haematopoietic stem cell transplants. However, they were unable to demonstrate this association in Houston, an area with a very different climate. In our geographical area, climatic conditions were not associated with changes in airborne spore counts and/or with IA. This finding suggests that the pathogenesis of IA is complex and that geographical differences should be taken into consideration. Moreover, the relationship between climatic conditions and environmental spore counts may be biased by other factors such as air

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TABLE 2. Quantitative relationship between circulating viruses, airborne spore count and admission for IA

	Quantitative relationship between airborne spore count and IA admission	p
Overall cohort		
First 2-week period preceding infection	3.717	0.119
Second 2-week period preceding infection	1.522	0.593
Third 2-week period preceding infection	6.021	0.004
All circulating virus periods		
First 2-week period preceding infection	61.333	0.001
Second 2-week period preceding infection	−50.162	0.145
Third 2-week period preceding infection	−75.521	0.163
Circulating influenza A virus (non-H1N1)		
First 2-week period preceding infection	55.322	0.003
Second 2-week period preceding infection	−42.953	0.190
Third 2-week period preceding infection	−81.296	0.152
Circulating influenza A(H1N1)pdm09		
First 2-week period preceding infection	19.610	0.018
Second 2-week period preceding infection	3.066	0.734
Third 2-week period preceding infection	11.337	0.180
Circulating influenza B virus		
Current period of infection	56.880	0.038
First 2-week period preceding infection	−23.745	0.422
Second 2-week period preceding infection	12.368	0.668
Circulating adenovirus		
First 2-week period preceding infection	1.275	0.684
Second 2-week period preceding infection	−1.399	0.714
Third 2-week period preceding infection	4.789	0.047
Circulating respiratory syncytial virus period		
First 2-week period preceding infection	0.996	0.756
Second 2-week period preceding infection	−1.671	0.667
Third 2-week period preceding infection	4.529	0.063

Poisson regression coefficient. This table details that the presence of environmental airborne mould spores increases the risk of IA admission 6.021 times after the third 2-week period (from 28 to 42 days) in the overall cohort. This correlation is statistically significant. Moreover, the table also shows the number of spores required for infection. The higher the ratio, the fewer the spores required for infection. Remarkably, the airborne spore load required to produce admission for IA was very low during the first 2-week period of circulation of respiratory viruses. This high correlation was also significant and strong during the first 2-week periods of influenza A, B and pandemic A (H1N1). This correlation was also observed in the third 2-week period after circulating adenovirus.

pollution, land use and other modifying factors that are difficult to assess.

Interestingly, circulating respiratory viruses were associated with an increased risk of IA. During circulating respiratory virus periods we found that a lower airborne mould spore load was required for IA to occur. Moreover, time to report spore inhalation and IA was shorter, in spite of the absence of any relationship between circulating respiratory viruses and environmental airborne mould spore count. It is tempting to speculate that respiratory viruses produce a major variation in host susceptibility. It has been reported that current infection with parainfluenza 3, RSV or influenza increases the patient's risk of developing IA [5,13–15]. Respiratory viruses alter the bronchial mucosa, and the resulting epithelial disruption facilitates fungal invasion. Moreover, viruses may affect local and systemic host defences, producing a state of host adaptive immune deficiency and facilitating the pathogenesis of *Aspergillus* [3].

Our study suggests that the relationship between respiratory viral infection and IA is significant. Therefore, the implementation of effective diagnostic, treatment and prevention strategies for viral infections in severely immunosuppressed patients is of paramount importance. Primarily, a routine search for common respiratory viruses in all immunosuppressed patients complaining of symptoms of respiratory tract infection should be performed. Prompt treatment with oseltamivir or ribavirin when needed might help to diminish the incidence of IA. Finally, lifelong seasonal influenza vaccination with inactivated influenza vaccine appears to be the most effective strategy. However, the efficacy of vaccines may be compromised in this population due to insufficient antigenic response. Further information is needed in order to improve vaccine strategies in immunocompromised patients. Another important measure to reduce the viral load in the immunosuppressed host environment is the vaccination of healthcare personnel and family members against influenza early in the season [16,17]. Optimizing hospital or home isolation measures against viral infection, diminishing patient visits or increasing antifungal prophylaxis in more severe immunosuppressed patients during circulating respiratory virus periods should be considered.

The strengths of the current study include its inclusion of a large cohort of consecutive hospitalized patients with IA, the comprehensive data collection and the prospective collection of environmental variables. Nevertheless, there are several limitations that should be acknowledged. Firstly, this is an observational study and residual confounding cannot be ruled out. Moreover, due to its retrospective design, potential biases in the selection of patients can not totally be ruled out. Secondly, our study showed indirect evidence between viral infection and IA admissions. It should be noted, however, that we did not demonstrate a direct proof of a causal association between an acute viral disease of the respiratory tract and IA. To demonstrate the direct relationship between the virus and the IA, a prospective study of a cohort of patients with high risk of IA should be performed. All patients should be routinely tested for respiratory viruses in order to avoid under-diagnosis of viral infection. Multivariate analysis comparing environmental effects, host variables, underlying disease and time-dependent variables, including among others duration and dosage of steroid therapy, other immunosuppressive treatments, neutropenia, cytomegalovirus reactivation and presence of severe graft-vs-host disease, would be needed. To our knowledge, this study has not been performed yet. Thirdly, the study was carried out in a specific geographical area. As noted above, the impact of environmental variables on other areas may be different. Moreover, we did not find a relationship between climatic conditions and IA. It should be noted, however, that data from Seattle have demonstrated a high incidence of IA occurring just after seasonal periods of

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low precipitation and high temperatures, which coincide with high environmental spore counts. Fourthly, it is difficult to determine the impact of multiple variables that may affect the risk of IA, especially those related to host-immunity; our cohort of patients with IA had different host and prevention strategies applied. Finally, IA is a complex disease and it is difficult to interpret time-to-event. There may be wide variations in incidence or diagnosis depending on population, diagnostic approach or prophylactic measures.

In summary, a high airborne mould spore count in the environment increases the risk of IA admission in the following 28–42 days. This finding supports the idea that most IAs are acquired outside the hospital. Circulating respiratory viruses appear to be associated with an increased risk of developing IA. Physicians should be aware of this association in order to optimize the prevention and diagnosis strategies for IA during viral epidemic periods.

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Transparency Declaration

The authors have no conflicts of interest to declare.

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IX.2. ANEXO 2

PLOS | ONE

RESEARCH ARTICLE

Causes of Death in a Contemporary Cohort of Patients with Invasive Aspergillosis

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Abstract

Information regarding the processes leading to death in patients with invasive aspergillosis (IA) is lacking. We sought to determine the causes of death in these patients, the role that IA played in the cause, and the timing of death. The factors associated with IA-related mortality are also analyzed. We conducted a multicenter study (2008-2011) of cases of proven and probable IA. The causes of death and whether mortality was judged to be IA-related or IA-unrelated were determined by consensus using a six-member review panel. A multivariate analysis was performed to determine risk factors for IA-related death. Of 152 patients with IA, 92 (60.5%) died. Mortality was judged to be IA-related in 62 cases and IA-unrelated in 30. The most common cause of IA-related death was respiratory failure (50/62 patients), caused primarily by *Aspergillus* infection, although also by concomitant infections or severe comorbidities. Progression of underlying disease and bacteremic shock were the most frequent causes of IA-unrelated death. IA-related mortality accounted for 98% and 87% of deaths within the first 14 and 21 days, respectively. Liver disease (HR 4.54; 95% CI, 1.69-12.23) was independently associated with IA-related mortality, whereas voriconazole treatment was associated with reduced risk of death (HR 0.43; 95% CI, 0.20-0.93). In conclusion, better management of lung injury after IA diagnosis is the main challenge for physicians to improve IA outcomes. There are significant differences in causes and timing between IA-related and IA–unrelated mortality and these should be considered in future research to assess the quality of IA care.

Introduction

Invasive aspergillosis (IA) is a leading cause of infection-related death in immunocompromised patients [1–4]. Recent data suggest that the outcomes of this infection appear to be improving compared with observations in the 1990s, this being due to advances in diagnosis and the introduction of new antifungal agents [5–8].

Madrid, Spain. On behalf of Universitat Autònoma de Barcelona.

Competing Interests: The authors have declared that no competing interests exist.

However, knowledge regarding the cause of death in patients with IA remains scarce for a number of reasons. First, patients with IA are complex hosts with severe underlying diseases, aggressive treatments, coexisting infections, and/or treatment complications. Second, studies focusing specifically on the processes leading to death in these patients are lacking. Third, a multidisciplinary approach involving a large number of physicians is common in the management of these patients, and the final cause of death reported on the death certificate may depend on the experience of the signing physician. And fourth, there is no clear consensus about how to define the cause of a patient's death or the role that IA may have played in this event. This knowledge gap limits our understanding of optimal treatment strategies. It is not clear, therefore, whether IA-related mortality is caused by factors that could be modifiable through medical intervention.

Previous studies of IA prognosis have focused mainly on overall mortality, and they have assessed risk factors for all-cause mortality [7]. Moreover, definitions of IA-related mortality are vague [5, 6, 8]. A further aspect requiring elucidation is whether causes of death and factors associated with mortality within the first few days differ from those associated with mortality occurring later.

The present study sought 1) to identify the immediate causes of death in a contemporary cohort of hospitalized patients with IA, 2) to determine the role that IA played in the cause of death, and 3) to analyze the timing of death and risk factors associated with IA-related mortality in this cohort of patients.

Materials and Methods

Setting, patients, and study design

We conducted a retrospective multicenter study of all adults diagnosed with IA between 1 January 2008 and 31 December 2011 at three tertiary teaching hospitals in Barcelona, Spain. Patients who developed IA were identified by review of clinical records, of microbiology and pathology records, and of the diagnostic codes recorded on hospital discharge. The following information was carefully collected from medical records: demographic characteristics, underlying disease, use of immunosuppressive treatment, neutropenia, clinical features, diagnostic tools, infecting *Aspergillus* species, antifungal and adjunctive treatment, and outcomes. Informed consent was waived by the Clinical Research Ethics Committee because no intervention was involved and no patient identifying information was included. The study was approved by the Ethical Committee of the Hospital Universitari de Bellvitge.

Definitions

We included only patients with proven and probable IA according to the definitions published by the European Organization for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycosis Study Group (EORTC/MSG) [9]. Neutropenia was defined as an absolute neutrophil count of $<500/mm^3$. Disseminated IA was defined as evidence of infection in at least two noncontiguous sites or isolated CNS infection. The day of IA diagnosis was the day on which the first positive test was performed. For patients whose diagnosis was obtained from postmortem examination, the day of death was considered to be the day of diagnosis.

Assessment of mortality and the cause of death

Mortality was assessed at 90 days from day of diagnosis (overall mortality). Cause of death and the role of IA in causing death were reviewed by members of clinical review panel. This panel

was composed by 6 investigators. Data from each study's patient were independently reviewed by three of these investigators. Results were based on full consensus among the investigators. The investigators were blind to the patient's day of death. Five members of the clinical review panel were infectious disease specialists and one was an advanced fellow in infectious diseases. All reviewers had extensive clinical experience dealing with patients with IA. The reviewers were asked to assign the immediate causes of death based on World Health Organization criteria [10], and to assess the role that IA played in the patient's death.

The immediate cause of death was defined as the disease process, injury, or complication immediately preceding death. IA was considered the cause of death when the immediate cause of death was due to this infection. IA was judged to have played a major role if death would not have occurred had the patient not had IA, even though another condition was present that also contributed to death. IA was defined as playing a minor role if IA was not essential in explaining the patient's death but did play some role in the event. Mortality was classified as IA-related if IA was the cause of death or if it played a major role in the patient's death. Mortality was defined as IA-unrelated if IA played a minor role or had no role in the patient's death.

Statistical analysis

Categorical variables were described using counts and percentages. Continuous variables were expressed as the mean and standard deviation or median and interquartile range, depending on the result of the Kolmogorov-Smirnov test. To detect significant differences between causes of IA-related and IA-unrelated death we used the chi-square or Fisher's exact test for categorical variables and the Student's *t* test or Mann-Whitney *U* test for continuous variables, as appropriate. A multivariate analysis to determine independent risk factors for IA-related mortality was performed comparing patients with IA-related death versus all other patients. Variables shown to be significant in the univariate analysis and which were considered clinically important were entered into the multivariate analysis. The relative risks were expressed as hazard ratios (HR) and 95% confidence intervals (CI).

The results were analyzed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $\alpha = 0.05$. All reported p values are two-tailed.

Results

Study population and causes of death

A total of 152 patients with IA were enrolled in the study. Table 1 summarizes the characteristics of patients hospitalized for IA, as well as their clinical features, diagnosis, and treatment. Overall mortality (90 days) was 60.5% (92 of 152 patients). The median time to death after diagnosis was 16 days (IQR 6.25–33.5). The immediate causes of death are detailed in Table 2.

Mortality was judged to be IA-related in 62 cases (67.4% of deaths; 40.8% of patients). Of these, IA was the cause of death in 36 cases (39.1% of deaths; 23.7% of patients) and was judged to have played a major role in the patient's death in the remaining 26 cases (28.3% of deaths; 17.1% of patients). Mortality was defined as IA-unrelated in 30 cases (32.6% of deaths; 19.7% of patients): IA played a minor role in 18 cases (19.5% of deaths; 11.8% of patients) and had no role in the patient's death in 12 cases (13.0% of deaths; 7.9% of patients).

Table 2 shows the differences in cause of death between patients with IA-related versus IA-unrelated mortality. The most common cause of IA-related death was respiratory failure (50 of 62 patients; 80.6%), caused primarily by *Aspergillus* infection, although also by concomitant infections or severe lung comorbidities. Progression of underlying disease and septic shock caused by bloodstream infection were the most frequent causes of IA-unrelated death.

Table 1. Patient baseline characteristics, clinical features, diagnosis, and treatment.

Characteristics		Patients n = 152	%
Age, median years (IQR)		60 (49–67)	-
Male sex		93	61.2
Underlying disease			
	Hematologic malignancy	67	44.1
	Solid organ transplant	34	22.4
	Hematopoietic stem cell transplant	13	8.6
	Solid tumor	13	8.6
	AIDS	9	5.9
	Immunodeficiency disorder	5	3.3
	Other[&]	12	7.8
Immunologic risk[#]			
	Neutropenia	49	32.2
	Corticosteroid therapy	89	58.6
	Any immunosuppressive therapy	99	65.1
Infection site			
	Pulmonary only	136	89.5
	Disseminated IA	16	10.5
Diagnosis[#]			
	Culture[$]	113	74.3
	Galactomannan	95	62.5
	Biopsy or autopsy	34	22.4
Type of IA			
	Proven	38	25.0
	Probable	114	75.0
Primary antifungal therapy*			
	Voriconazole monotherapy	61	40.1
	Voriconazole-containing regimen	92	60.5
	Amphotericin B monotherapy[@]	19	16.8
	Combination therapy	25	16.4

[&] Contains patients with severe immunosuppressive treatment, mainly high dose of corticosteroids.

[#] Patients could have >1 characteristics within a category.

[$] *A. fumigatus*, 87 cases (76.9%); *A. niger*, 6 (5.3%); *A. terreus*, 6 (5.3%); *A. flavus*, 12 (10.6%) other, 6 (5.3%).

* Systemic antifungal therapy with anti-Aspergillus activity given for at least 5 consecutive days.

[@] Liposomal amphotericin B 13 (11.5%); Lipidic amphotericin B 6 (5.3%).

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Fifteen patients were diagnosed after death. Autopsyprovided the diagnosis of five patients in whom the diagnostic tests to rule out IA were not performed during hospitalization. Positive cultures and/or galactomannan results reported after death, plus necropsy results, allowed a diagnosis to be made in a further five patients, while positive cultures and/or galactomannan results reported after death enabled the remaining five patients to be diagnosed. Disseminated IA was documented in 6 of these 15 patients (40%). IA was judged to have been the cause or to have played a major role in the patient's death in all 15 of these cases.

Timing of IA-related and IA-unrelated mortality

Survival plots and frequency distributions of death by time for IA-related and IA-unrelated mortality are shown in Fig. 1. There were significantly different patterns in time to death for

Table 2. Immediate cause of death for patients with IA.

Cause of death[1]		IA-related death N = 62 (%)	IA-unrelated death N = 30 (%)	Total N = 92 (%)	p[2]
Respiratory failure		50 (80.6)	9 (30)	59 (64.1)	<.001
	- caused primarily by *Aspergillus* infection	24 (38.7)	0	24 (26.1)	<.001
	- caused by *Aspergillus* infection and concomitant lung infections[3]	14 (22.6)	6 (20)	20 (21.7)	.99
	- caused by *Aspergillus* infection and severe comorbidities[4]	12 (19.4)	3 (10)	15 (16.3)	.37
Underlying disease[5]		6 (9.7)	12 (40)	18 (19.6)	<.01
Septic shock caused by bloodstream infection[6]		5 (8.1)	11 (36.7)	14 (15.2)	<.01
Pulmonary hemorrhage[7]		10 (16.1)	0	10 (10.9)	.03
Neurological conditions[8]		7 (11.3)	1 (3.3)	8 (8.7)	.27
Multiorganic failure[9]		7 (11.3)	0	7 (7.6)	.09
Other[10]		2 (3.2)	12 (40)	14 (15.2)	<.001

[1] More than >1 cause of death was considered in 38 patients.

[2] Differences between IA-related and IA-related mortality.

[3] Cytomegalovirus, 7 patients; *Pseudomonas aeruginosa*, 5 patients; *Pneumocystis jirovecii*, 3 patients; Influenza A(H1N1)pdm09, 3 patients; *Streptococcus pneumoniae*, 2 patients; nocardiosis, 2 patients; *Rhodococcus equi*, 1 patient; respiratory syncytial virus, 1 patient; aspiration pneumonia, 1 patient. Three respiratory co-pathogens were found in 5 patients.

[4] Severe chronic obstructive pulmonary disease, 8 patients; GVHD, 3 patients; and in 1 patient each: cerebrovascular disease, lung cancer, pulmonary fibrosis, and acute pulmonary thromboembolism.

[5] Acute myeloid leukemia relapse, 6 patients; graft failure in organ solid recipients, 4 patients (two lung, one kidney, one liver); GVHD, 4 patients with allo-hematopoietic stem cell transplantation; cavum massive hemorrhage secondary to solid cancer, 1 patient; advanced lung cancer, 1 patient; intestinal obstruction in patient with metastatic cancer, 1 patient; severe aplasia after chemotherapy in one patient with chronic lymphocytic leukemia.

[6] Gram-negative bacilli, 6 patients; *Enterococcus spp*., 4 patients; *Listeria monocytogenes*, 1 patient; *Streptococcus pneumoniae*, 1 patient; Candida albicans, 1 patient; polymicrobial bacteremia with *Enterococcus faecim* and *Achromobacter dentrificans*, 1 patient.

[7] Necrotizing pneumonia caused by *Aspergillus* in patients with severe pancytopenia due to hematologic disease, 5 patients (in one case co-infection with *Pseudomonas aeruginosa* was found); necrotizing pneumonia caused by *Aspergillus* in patients with pancytopenia and/or coagulopathy due to liver disease, 3 patients; necrotizing pneumonia caused by *Aspergillus*, 1 case; necrotizing pneumonia caused by *Aspergillus* and *Pseudomonas aeruginosa*, 1 case.

[8] Brain herniation caused by focal lesion +/- cerebral hemorrhage caused by *Aspergillus* in central nervous system, 5 patients; cerebral ischemic event, 2 patients; primary brain hemorrhage, 1 patient.

[9] Disseminated invasive aspergillosis with multiorganic failure, 3 cases; multiorganic failure caused by respiratory failure due to *Aspergillus* and liver failure due to severe underlying liver disease, 3 patients; multiorganic failure caused by respiratory failure due to *Aspergillus* and heart failure after cardiac transplantation, 1 patient.

[10] *Clostridium difficile* infection, 4 patients (co-infection with CMV was found in 1 case); sudden cardiopulmonary arrest in patients with multifactorial encephalopathy, 2 patients; sudden cardiac arrest, 2 patients; acute myocardial ischemia, 1 patient; neutropenic colitis, 1 patient, intestinal ischemia and secondary peritonitis, 1 case; post-surgical esophageal perforation and secondary mediastinitis, 1 case; severe cachexia (adult/32 kg), 1 case; diabetic ketoacidosis, 1 case.

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patients with IA-related versus IA-unrelated mortality. Median survival days for patients with IA-related death was 10 (IQR 1.5–18.25), as compared with 43.5 (IQR 18.75–60.25) for patients with IA-unrelated death (p<.001).

IA-related mortality accounted for 97.6% of deaths within the first 14 days and 86.7% of deaths within the first 21 days. IA-unrelated mortality accounted for 58% of deaths from day 14 to day 90 and 68.6% of deaths from day 21 to day 90 (p<.001 for both comparisons). Of the IA-related deaths, 66.1% occurred within 14 days, and 83.9% within 21 days. No IA-related death was documented after 60 days of follow-up. The odds of an IA-related death occurring

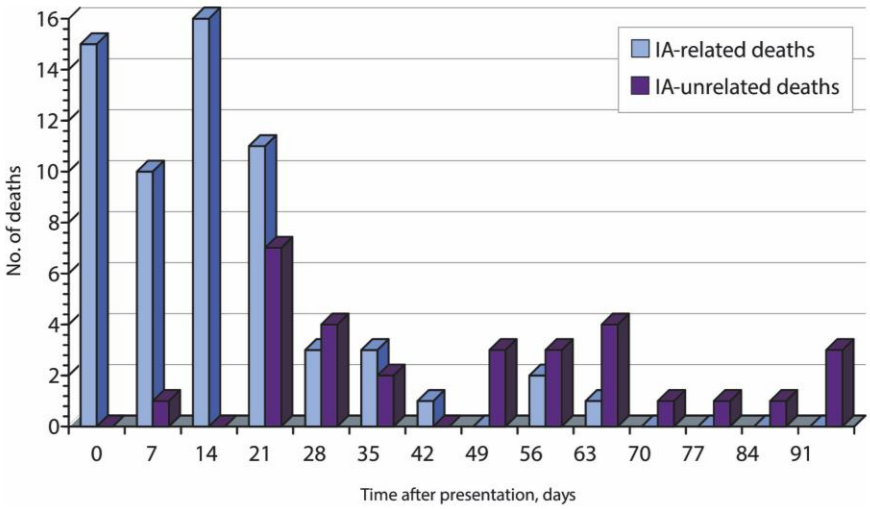

Fig 1. Frequency plot of IA-related and IA-unrelated mortality.

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within 14 and 21 days of presentation were 19.84-fold and 3.15-fold higher than that of an IA-unrelated death, respectively.

Factors associated with IA-related mortality

Table 3 shows the independent factors associated with IA-related mortality. After adjustment, chronic liver disease (HR 4.542; 95% CI, 1.69–12.23) was the only factor independently associated with IA-related mortality. Conversely, receipt of voriconazole was independently associated with reduced risk of IA-related death (HR 0.43; 95% CI, 0.20–0.93).

Table 3. Independent risk factors for IA-related death.

Variable	Adjusted	
	HR (95% CI)	p
Patient-related factors		
Chronic liver disease	4.54 (1.69–12.22)	.003
Severe impairment on PFT[1]	2.46 (0.90–6.77)	.081
Hematologic disease	0.99 (0.42–2.35)	.992
Corticosteroid treatment	1.37 (0.61–3.06)	.449
IA-related factors		
Disseminated IA	2.12 (0.58–7.69)	.253
Proven IA	2.23 (0.90–5.56)	.986
Voriconazole treatment[2]	0.04 (0.20–0.93)	.032

[1]Severe pulmonary function test abnormality.
[2] Voriconazole received for at least 5 days.

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Discussion

This multicenter study describes contemporary causes of death for adults with IA. Our cohort is representative of patients with IA and has similar patient characteristics, clinical presentation, and antifungal therapy to those reported by the PATH Alliance [11], the largest recent prospective description of IA patients. Previous information regarding the processes leading to death in adults with IA is scarce. In the present study, overall mortality for patients with IA at 90 days was 60.5%. This mortality rate is high, probably due to the heterogeneous population. IA in non-neutropenic, non-cancer patients still has a very poor prognosis, contrasting to the increasingly better survival in hematologic patients, likely due to strategies of early diagnosis allowing early appropriate treatment. Our mortality rate finding concurs with that of other investigators who reported rates ranging from 35.6% to 66% [5–8, 12]. Recently, Marr et al. [13] reported a mortality rate at 6-weeks close to 30% in patients who had an underlying hematologic malignance or hematopoietic cell trasplantation diagnosed with possible, probable, or proven IA. Mortality was judged to be IA-related in 62 of 152 cases (40.8%), accounting for 67.4% of deaths; in the remaining 32.6% of cases death was due to IA-unrelated causes. These data are difficult to compare with those obtained by other researchers, owing to differences in definitions and study populations. Importantly, we found substantial differences in causes and timing for IA-related and IA-unrelated mortality.

In our study the most frequent cause of IA-related death was respiratory failure. This was mostly a direct result of *Aspergillus* infection, although there were also cases of respiratory failure in patients with coexisting infections or severe comorbidities. Pulmonary hemorrhage was another frequent cause of IA-related death. These findings suggest the need for measures to improve the care of pulmonary function in the early management of IA. It is important to note, however, that IA is not a homogeneous disease. Its pathogenesis differs depending on the host's immune status [14–17], and the lung injury could occur through different pathways. Early rollout and treatment of coinfections, as well as optimized management of chronic pulmonary diseases, would also seem mandatory.

In our study the progression of underlying disease and septic shock caused by bacteremia were the most frequent causes of IA-unrelated death. The prognosis of patients beyond 21 days of IA diagnosis could be greatly influenced by improving the treatment of underlying diseases and/or preventing and managing bacterial infections.

Although our cohort of patients was recruited at a time of improved diagnostic tests such as chest CT and the galactomannan antigenemia assay, some cases of IA were still only diagnosed at autopsy or post-mortem. In line with previous reports [5] these patients were more likely to have disseminated disease. These results show that more prompt diagnosis of IA remains a challenge for physicians.

Most of the IA-related deaths (83.9%) occurred within the first 21 days after diagnosis. In fact, IA-related mortality accounted for 97.6% of deaths within the first 14 days. After this period the number of IA-related deaths diminished rapidly, with no IA-related death being documented after 60 days. By contrast, IA-unrelated mortality accounted for 68.6% of deaths from day 21 to day 90 after IA diagnosis. These results suggest that assessment of survival at 12 weeks after diagnosis (a criterion used by many researchers) [5, 6, 12] is an imprecise indicator of the efficacy of IA treatment. This finding concurs with that of other investigators [18]. Given that the 12-week period includes a large number of deaths due to competing causes that are not related to fungal infections, researchers interested in assessing treatment response in IA infection should therefore focus only on IA-related deaths. We believe that our study used clear and uniform definitions that were intended to be reproducible in future studies. Studies not using an independent clinical expert review committee to determine the cause of death for patients

should apply a cut-off of 14 days so as to provide relevant information only about deaths directly caused by IA. Twenty-one days (3 weeks) seems to be a good cut-off for a global evaluation of IA-related mortality. However, our data are retrospective and subject to limitations. Further prospective studies to assess the optimal cutt-off point to evaluate treatment response in IA infection should be conducted.

We found that the presence of liver disease was independently associated with an increased risk for IA-related mortality. The association between hepatic impairment and increased risk for mortality in patients with IA has been previously documented in hematopoietic cell transplant recipients [5].Importantly, we found that voriconazole treatment was independently associated with reduced risk of IA-related death. The better mortality outcomes of patients treated with voriconazole compared with amphotericin B therapy for IA has been demonstrated in observational studies [5, 6] and in a randomized trial [12]. Our study has several limitations. One potential weakness is that the validity of using a clinical review committee to determine the cause of death for patients with IA has not been previously established. Nevertheless, this method was chosen because it was the most practical in nature and is likely to provide more reliable data than would death certificates, most of which are signed by inexpert practitioners with limited experience in the management of these complicated patients. Similar clinical consensus methods have been used to classify mortality for many other conditions [19,20]. It is nonetheless difficult to avoid subjective points of view when establishing causes of death in these patients. We sought to address this by using precise definitions of IA-related and unrelated mortality that were applied in a uniform manner. Researchers were blind to the timing of mortality when determining the cause of death, and all decisions were agreed by consensus. A final limitation to consider is that an autopsy could not be performed for all the patients who died.

In conclusion, this study describes the immediate causes of death in a current cohort of patients with IA. The findings suggest that better management of lung injury within the first 21 days after IA diagnosis is the main challenge for physicians in terms of improving IA outcomes. Importantly, there were significant differences in causes and timing between IA-related and IA-unrelated mortality and these should be considered in future research in order to assess the quality of IA care.

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Author Contributions

Conceived and designed the experiments: CGV. Performed the experiments: CGV MP CC CG IR AM CR ER JP JA JC. Analyzed the data: CGV IR AM. Contributed reagents/materials/analysis tools: CR ER JP JA. Wrote the paper: CGV. Critical revision of the manuscript: CGV CC CG IR AM JC. Obtaining funding: CGV JC.

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IX.3. ANEXO 3

Micología

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Infecciones por hongos filamentosos en el paciente inmunosuprimido: profilaxis y tratamiento

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RESUMEN

Aunque ha disminuido la incidencia de aspergilosis invasora en pacientes hematológicos y en receptores de trasplante de órgano sólido con el uso de profilaxis, esta infección ha aumentado en otras poblaciones sometidas a tratamientos inmunosupresores donde la prevención no está bien definida. Además, en estos pacientes se presentan formas clínicas diferentes. Voriconazol constituye el tratamiento de elección de la aspergilosis invasora aunque la terapia combinada de voriconazol con anidulafungina podría tener su papel en las fases iniciales de la infección.

PALABRAS CLAVE: aspergilosis , profilaxis, tratamiento

Filamentous fungal infections in immunosuppressed patients: prophylaxis and treatment

ABSTRACT

Although the incidence of invasive aspergillosis has decreased in haematologic patients and solid organ transplant recipients due to the use of prophylaxis; aspergillosis has emerged in other populations undergoing immunosuppressive drugs where prophylaxis is not well defined presenting different clinical patterns. Voriconazole is the gold standard in the treatment of aspergillosis and probably combined therapy, with voriconazole plus anidulafungin, could have a role in the initial management of the infection.

KEY WORDS: aspergillosis, prophylaxis, treatment

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La infección fúngica invasora (IFI) por hongos filamentosos ha aumentado en frecuencia durante la última década, debido, por una parte, a la existencia de un mayor número de pacientes en riesgo, por someterse a tratamientos inmunosupresores o terapias invasivas y, por otra, a la mejora de los métodos diagnósticos microbiológicos y de las pruebas de imagen, especialmente la tomografía computarizada de alta resolución. En pacientes considerados de alto riesgo para IFI, como son los afectos de leucemia mieloide aguda (LMA) o sometidos a trasplante de progenitores hematopoyéticos (TPH) la incidencia de las IFI ha disminuido por debajo del 3% en aquellos que reciben profilaxis antifúngica con azoles de amplio espectro (voriconazol y posaconazol). Sin embargo, a pesar del diagnóstico más precoz y al uso de los nuevos antifúngicos, la IFI continúa asociándose con una elevada morbimortalidad, superior al 50% en algunos grupos de pacientes.

FACTORES DE RIESGO DE IFI

Los principales factores de riesgo asociados al desarrollo de aspergilosis invasora (AI) se pueden agrupar en: los que dependen del paciente y su situación clínica (factores del huésped), los relacionados con la inmunidad innata, los relacionados con el tratamiento recibido y comorbilidades del paciente, y los relativos a las condiciones medioambientales (figura 1). Así, pacientes que eran considerados de bajo riesgo para IFI, tales como pacientes con bronquitis crónica (BC), neoplasia sólida, vasculitis o pacientes de UCI al recibir inmunosupresores o al coexistir varios factores de riesgo pueden presentar una AI[1].

FORMAS CLÍNICAS DE ASPERGILOSIS INVASORA

El diferente grado de inmunosupresión que presenta el paciente condiciona las diferentes formas clínicas de la infección[2]. Aquellos con disfunción inmune severa como podrían ser los pacientes neutropénicos presentan la forma clásica de AI con angioinvasión e imagen radiológica típica con signo del halo y media luna y de corta duración (días-semanas). Los pacientes trasplantados de órgano sólido o con enfermedad de injerto contra receptor (EICR) o que reciben esteroides presentan for-

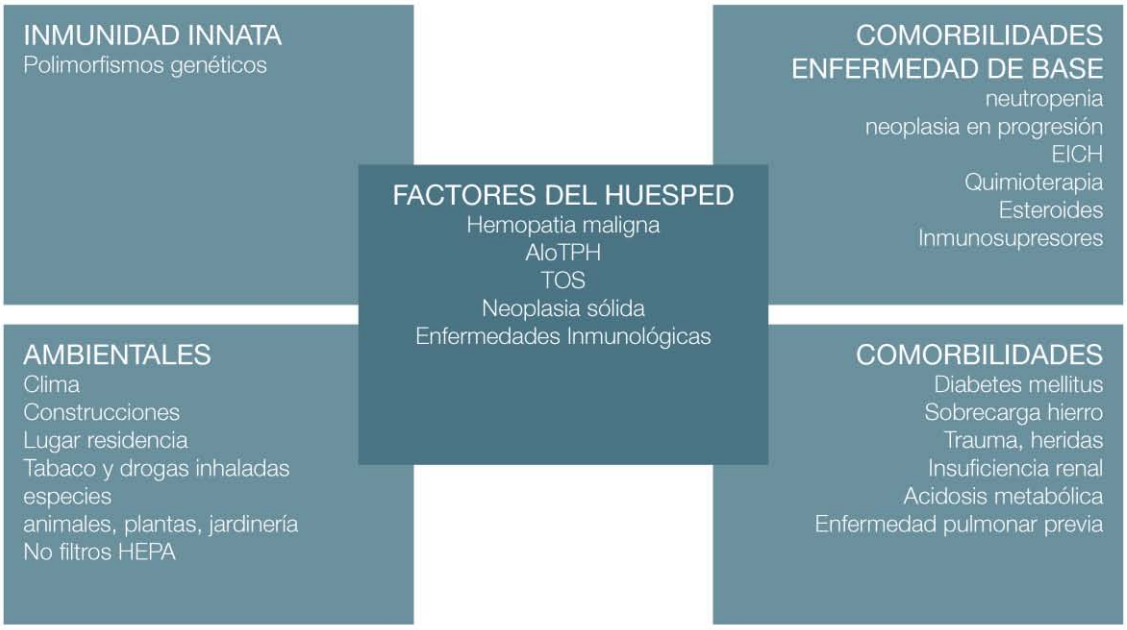

Figura 1 | **Factores de riesgo de IFI.**

Modificado de Herbrecht et al[11]. alo TPH: trasplante alogénico, TOS: trasplante de órgano sólido, EICH: enfermedad de injerto contra huésped

mas más bronquiales sin angioinvasión con imágenes pulmonares en "árbol en gemación", con duración de la infección que puede ser más prolongada. Y finalmente, aquellos con alteraciones estructurales pulmonares, como podrían ser los pacientes con neoplasias pulmonares o BC, presentan formas más crónicas con clínica de difícil diferenciación de su enfermedad de base y con imágenes radiológicas de consolidación o cavitación[3].

Esta diferenciación en ocasiones no es tan categórica y pacientes con formas sin angioinvasión pueden presentar afectación de vasos y formas crónicas pueden ser invasivas aunque no es lo más frecuente[4]. Este hecho implica la necesidad de pensar en la aspergilosis para diagnosticarla e iniciar el tratamiento de forma precoz.

PROFILAXIS DE LA AI

Se han establecido recomendaciones de profilaxis para pacientes hematológicos tales como LMA y trasplante de progenitores (tabla 1) y para trasplante de órgano sólido (tabla 2)[5]. Sin embargo, otras situaciones que también presentan riesgo de aspergilosis como son por ejemplo la leucemia linfoide aguda, no tienen recomendaciones claramente establecidas.

El disponer de diferentes antifúngicos en la actualidad permite realizar una profilaxis "a la carta" para cada paciente valorando, eficacia, tolerabilidad, e interacciones. De la elección de dicha profilaxis dependerá después el tratamiento dirigido en caso de que existiese una fungemia de brecha.

Un hecho a tener en cuenta es que los marcadores biológicos usados para diagnóstico precoz de la aspergilosis en el paciente hematológico pierden su sensibilidad con el uso de la profilaxis por lo que no deben usarse como método rutinario dos veces por semana y si como método diagnóstico ante una clínica sugestiva de aspergilosis[6].

TRATAMIENTO DE LA AI

Hoy por hoy, *Aspergillus fumigatus* es la especie más frecuentemente aislada en nuestro país[3,7] aunque parece ser que pueden estar en aumento las especies crípticas con un mayor porcentaje de resistencia a los antifúngicos habituales sobre todo en pacientes con formas crónicas de aspergilosis.

El tratamiento de elección de la aspergilosis (tabla 3) es voriconazol con un grado de evidencia AI en todas la guías terapéuticas publicadas. Voriconazol, en las diferentes series publicadas, constituye un factor protector de mortalidad y ha conseguido aumentar la supervivencia de estos pacientes. Si por toxicidad o porque el paciente estaba recibiendo previamente un azol en profilaxis, hasta disponer del antifungigrama, anfotericina B liposomal sería el tratamiento de elección[8,9].

Tabla 1 Profilaxis de la AI el paciente hematológico con su grado de evidencia

Patología	Antifúngico	dosis	Evidencia
LMA inducción	Posaconazol	300 mg/d (1º 300 mg/12h)	AI
	Fluconazol	50-400 mg/d	CI
	L-AmB inh+ fluconazol		BI
	Equinocandinas		¿?
	Itraconazol sol.	2.5 mg/Kg /12h	CI
	Polienos iv		CI
Alo-TPH-neutropenia	Fluconazol	400 mg/d	AI
	Itraconazol sol.	200 mg iv y después 200 mg 12h oral	CI
	Posaconazol	300 mg/d (1º 300 mg/12h)	ND
	Voriconazol	200 mg/12 h oral	AI
	L-AmB inh+ fluconazol		BII
	Micafungina	50 mg/d	CI
	Polienos iv		CI
Alo-TPH, EICH	Fluconazol	400mg/d	CI
	Itraconazol sol.	200 mg iv y después 200 mg 12h oral	BI
	Posaconazol	300 mg/d (1º 300 mg/12h)	AI
	Voriconazol	200 mg/12 h oral	AI
	L-AmB inh+ fluconazol		ND
	Equinocandinas iv		ND
	Polienos iv		CI

LMA = leucemia mieloide aguda; Alo-TPH = trasplante alogénico de progenitores hematopoyéticos; EICH= enfermedad de injerto contra huésped; L-AmB = anfotericina B liposomal

Aunque la terapia combinada no ha demostrado superioridad respecto a la monoterapia con voriconazol, un estudio randomizado publicado recientemente[10] ha demostrado que la combinación de voriconazol con anidulafungina reduce la mortalidad (de un 27,8% a un 19,5%) y esta disminución parece ser mayor (50% reducción de la mortalidad) en aquellos pacientes diagnosticados a través del antígeno de galactomanano, es decir que si la terapia combinada se establece de forma precoz podría disminuirse la mortalidad de nuestros pacientes. Lo que si parece claro en este momento es que en fases avanzadas de la enfermedad, con formas diseminadas la terapia combinada no aporta nada a la monoterapia.

Tabla 2 Profilaxis antifúngica en otras poblaciones de riesgo. Copiado de: Ruiz Camps et al[5]

Indicación	Población diana	Antifúngico	Duración	Observaciones
Trasplante pulmonar	Toda	1. Anfotericina B liposomal 25 mg o anfotericina B complejo lipídico 50 mg nebulizada 3 veces a la semana hasta resolución sutura, una vez semana del 2-6 mes y quincenalmente desde sexto mes	Indefinida	Broncoespasmo como efecto secundario
		2. Voriconazol 200 mg/12h oral	Determinada por presencia factores riesgo (mínimo 4 meses)	Monitorizar enzimas hepáticos
Otros trasplantes órgano sólido	Alto riesgo IFI precoz: depuración renal, CMV, insuficiencia hepática, fallo injerto, retrasplante	1. Anfotericina B formulación lipídica 2,5-5 mg/Kg iv	Determinada por presencia factores riesgo	Estudios realizados preferentemente en trasplante hepático
		2. Itraconazol 400 mg/d oral		Estudios en Trasplante cardíaco. Monitorizar
		3. Caspofungina 70 mg/d y después 50 mg/d		Estudios en trasplante hepático
	Alto riesgo tardío: rechazo crónico, recidiva hepatopatía VHC (trasplante hepático), técnica depuración renal insuficiencia hepática, fallo injerto, retrasplante 24	1. Anfotericina B liposomal 25 mg o anfotericina B complejo lipídico 50 mg nebulizada según pauta comentada		Broncoespasmo como efecto adverso
Enfermedad granulomatosa crónica	Mayores de 5 años	1. Itraconazol 200 mg/d via oral (100 mg/d <13 años o <50 Kg de peso)	Indefinida	

Tabla 3 Tratamiento de la aspergilosis invasora según las diferentes guías

	ECIL 2013	IDSA 2008	GUIA ALEMANA 2014	SEIMC 2011
Voriconazol	AI	AI	AI	AI
L-AmB	BI	AI	AII	AI
Caspofungina	CII		CII	CII
Micafungina			CII	
ABCL	BII			
Itraconazol	CIII			DIII
Posaconazol				
Voriconazol + anidulafungina	CI		CIII	
Combinaciones	CIII	BIII		CIII

L-AmB = anfotericina B liposomal; ABCL= anfotericina en complejo lípidico

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