TESIS DOCTORAL

COMPLICACIONES PULMONARES EN LOS PACIENTES CON INFECCIÓN POR EL VIRUS DE LA INMUNODEFICIENCIA HUMANA EN LA ERA DEL TRATAMIENTO ANTIRRETROVIRAL DE GRAN ACTIVIDAD. ESTUDIO EPIDEMIOLÓGICO Y PRONÓSTICO. DESCRIPCIÓN DEL PATRÓN DE RESPUESTA INFLAMATORIA EN EL HUÉSPED Y SU CORRELACIÓN CON LA ETIOLOGÍA Y EL PRONÓSTICO

Tesis presentada por

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para optar al Grado de Doctor en Medicina

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Septiembre de 2004

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CERTIFICAN:

Que la memoria titulada: **COMPLICACIONES PULMONARES EN LOS PACIENTES CON INFECCIÓN POR EL VIRUS DE LA INMUNODEFICIENCIA HUMANA EN LA ERA DEL TRATAMIENTO ANTIRRETROVIRAL DE GRAN ACTIVIDAD. ESTUDIO EPIDEMIOLÓGICO Y PRONÓSTICO. DESCRIPCIÓN DEL PATRÓN DE RESPUESTA INFLAMATORIA EN EL HUÉSPED Y SU CORRELACIÓN CON LA ETIOLOGÍA Y EL PRONÓSTICO**, presentada por María Natividad de Benito Hernández, ha sido realizada bajo su dirección y cumple los requisitos necesarios para ser leída delante del Tribunal correspondiente.

Firmado: Josep Maria Gatell Firmado: Asunción Moreno

Barcelona, 16 de Setiembre del 2004

PRESENTACIÓN

Esta tesis doctoral se estructura según las directrices de la normativa para la presentación de tesis doctorales como compendio de publicaciones aprobada por el Consejo del Departamento de Medicina el 18 de Noviembre de 1994 y ampliada el 17 de Marzo de 1999.

La presente memoria se basa fundamentalmente en dos artículos originales que pertenecen a una misma línea de trabajo: el estudio epidemiológico, diagnóstico y pronóstico de las complicaciones pulmonares en los pacientes con infección por el virus de la inmunodeficiencia humana (VIH) en la era del tratamiento antirretroviral de gran actividad.

El factor de impacto acumulado de los dos artículos originales sobre los que se centra esta tesis doctoral es de 5,49 según ISI® de 2003.

Esta tesis supone, desde la perspectiva investigadora, un abordaje del conocimiento de la epidemiología y el pronóstico de las complicaciones pulmonares en los pacientes con infección por el VIH tras la introducción de las combinaciones antirretrovirales más potentes, así como de la descripción de la respuesta inflamatoria que tiene lugar en el huésped durante estos eventos y su posible correlación con la etiología y el pronóstico. Constituye un intento de optimizar los esquemas diagnósticos y mejorar el pronóstico de estos eventos en los pacientes VIH. Se enmarca dentro de una línea de investigación más amplia de estudio de las complicaciones pulmonares en los pacientes inmunodeprimidos.

AGRADECIMIENTOS

A los Drs. Josep Maria Gatell y Asunción Moreno, directores de esta tesis, sin los que este estudio no habría podido llevarse a cabo.

A los compañeros del Servicio de Enfermedades Infecciosas y del Servicio de Microbiología, cuyo apoyo y colaboración han sido imprescindibles. Y particularmente a los becarios, con los que he compartido tantas horas de "despacho", que me han escuchado y alentado en muchas ocasiones, y con los que he compartido también muchos ratos de buen humor. A la Dra. Montserrat Luna que trabajó intensamente en la fase inicial de este trabajo.

A las enfermeras del Servicio de Infecciones que han estado siempre dispuestas a colaborar desinteresadamente en la realización de este estudio.

A los miembros del grupo de estudio de los "Infiltrados Pulmonares en Pacientes Inmunodeprimidos", que iniciaron y han mantenido esta línea de investigación a lo largo de varios años, haciendo posible la realización de los distintos trabajos, varios de ellos ya publicados, que se han llevado a cabo. La participación de los compañeros del Servicio de Microbiología, de Neumología y del Dr. Filella (del Servicio de Bioquímica), que pertenecen a este grupo de estudio, ha sido indispensable.

A mis padres. No es fácil expresar la gratitud que les debo y que siento por ellos. Por estar siempre ahí. También en la realización de este estudio, su apoyo ha sido fundamental.

A Juan Pablo, cuyo empeño inquebrantable en que esta tesis saliera adelante, ha sido un pilar clave en su consecución.

A Nerea, que con su sonrisa ilumina el mundo.

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INTRODUCCIÓN

Importancia del problema

El 5 de junio de 1981 se publicaba una comunicación sobre cinco pacientes jóvenes de Los Angeles, previamente sanos, con neumonía por *Pneumocystis carinii* * (NPC).[1,2] A partir de aquí se inició una investigación que llevó al descubrimiento del síndrome de inmunodeficiencia adquirida (SIDA) y su agente causal, el virus de la inmunodeficiencia humana (VIH). Desde la publicación de estos primeros casos de SIDA en Estados Unidos, se produjo un rápido aumento del número de casos y fallecimientos de personas con SIDA durante la década de los ochenta, seguido de un descenso en el número de casos y fallecimientos a partir de los últimos años de la década de los noventa.[3] En la actualidad, la infección por el VIH y el SIDA constituyen una pandemia, y uno de los mayores retos de la salud pública mundial.[4] Se estima que en el año 2003 cinco millones de personas se infectaron por el VIH en el mundo y que, en dicho periodo, la epidemia de VIH/SIDA se cobró la vida de más de tres millones de personas. Así, se calcula que hasta finales del año 2003 habían fallecido de SIDA veinte millones de personas en el mundo, y que la cifra de personas que viven con el virus se había elevado a 40 millones (34,6-42,3 millones) (Figura 1).[5,6] Durante el año 2004, el número de personas que viven con la infección por el VIH en el mundo continúa aumentando constantemente.[7]

* Recientemente se ha implementado el nombre de *Pneumocystis jiroveci* para el *Pneumocystis carinii*. No obstante, a lo largo de este trabajo se le seguirá denominando con la nomenclatura anterior, puesto que los artículos que sustentan este trabajo fueron realizados antes de la generalización del nuevo nombre.

En España se han diagnosticado desde 1981, 67466 casos de SIDA. Se estima que en el año 2003 se diagnosticaron 2126 casos nuevos de SIDA. Esto supone un descenso del 5,5% con respecto a los casos del año anterior. Aunque los nuevos casos continúan disminuyendo, en los últimos años el descenso es progresivamente menor, observándose una tendencia a la estabilización.[8] Los casos de SIDA se refieren a una consecuencia tardía de la infección por el VIH que, por lo tanto, no refleja la incidencia actual de nuevas infecciones. Aunque la fecha de la infección por VIH suele ser un dato desconocido, a través de modelizaciones matemáticas de la epidemia se ha podido reconstruir de forma aproximada la evolución en el número de nuevas infecciones anuales ocurridas en España. Los máximos niveles de transmisión del VIH se produjeron entre 1985 y 1988, y desde entonces el ritmo de nuevas infecciones ha sido predominantemente decreciente (Figura 2). Como corresponde al periodo de incubación que había en aquel momento, la incidencia de casos de SIDA alcanzó su máximo aproximadamente diez años después (Figura 2). Las cifras alcanzadas por la incidencia de SIDA y los fallecimientos son mucho menores que las de nuevas infecciones por el VIH (Figura 2), y en consecuencia, la mayoría de las personas infectadas permanecen vivas y sin SIDA. A finales de los ochenta y principios de los noventa la transmisión del VIH era muy intensa por lo que fueron aumentando rápidamente las personas vivas infectadas por el VIH hasta superar las 100.000. Posteriormente han disminuido mucho las nuevas infecciones por el VIH, pero el número de personas vivas infectadas ha quedado estabilizado entre 100.000 y 150.000 personas (Figura 3). A ello ha contribuido la mejoría de la supervivencia de las personas infectadas.[9]

Desde las descripciones iniciales del SIDA, el aparato respiratorio ha sido el área más frecuentemente afectada por la enfermedad.[10,11,12,13] Hasta el 70% de las personas infectadas por el VIH experimenta alguna complicación respiratoria durante el curso de su enfermedad, principalmente de etiología infecciosa.[14] En la necropsia, los pulmones son el órgano más frecuentemente afectado por procesos relacionados con el SIDA, con incidencias que han oscilado desde el 100% en los primeros años de la endemia hasta aproximadamente el 70% a finales de la década de los noventa.[15,16] Tanto en Estados Unidos, como en Europa y África, las neumonías son 25 veces más frecuentes en pacientes con VIH que en la población general, con tasas de hasta 90 casos por 1000 personas/año.[17] Estas cifras pueden dar una idea de la magnitud del problema que suponen las complicaciones pulmonares en los pacientes con infección por el VIH. Una de las formas de presentación clínica más frecuente son los infiltrados pulmonares radiológicos, acompañados generalmente, aunque no siempre, de sintomatología respiratoria.[18]

Por otra parte, el desarrollo de las complicaciones pulmonares más frecuentes en estos pacientes: neumonía bacteriana, NPC y TBC se han relacionado con una peor evolución de la infección por el VIH,[19,20,21,22] aunque no todos los estudios son concordantes en este sentido.[23]

Epidemiología

Pocas investigaciones han abordado el estudio de las enfermedades pulmonares de forma global.[24,25] La mayoría se han centrado en algunas de ellas, sobre todo la NPC. Por lo tanto, no se conoce cuál es el algoritmo diagnóstico más adecuado, que, además, debe tener en cuenta las

características epidemiológicas de cada área geográfica.[26,27] Un claro ejemplo es el de la tuberculosis (TBC), cuya incidencia en pacientes VIH varía ampliamente entre las distintas zonas, sobre todo en relación con la prevalencia de la enfermedad en la población general: en África constituye la primera causa de complicaciones pulmonares en pacientes VIH;[26] en la Europa del este era la causa más frecuente de enfermedad definitoria de SIDA en la década de los noventa, mientras que en el oeste lo era la NPC; sin embargo, dentro del oeste Europeo, la TBC era significativamente más frecuente en el sur que en el norte.[28] Otro ejemplo es el de la NPC, cuya frecuencia es mucho menor en el África subsahariana que en los países industrializados.[26,29,30,31,32,33]

Por otro lado, la epidemiología de las enfermedades pulmonares en los pacientes VIH ha experimentado cambios notables a lo largo del tiempo. Entre los factores que más han contribuido a estos cambios destaca el empleo generalizado de la profilaxis primaria frente a *P. carinii* a partir de 1989.[34] Varios estudios analizaron el impacto que supuso la introducción de esta profilaxis. La incidencia de NPC como enfermedad definitoria de SIDA había aumentado de forma creciente hasta entonces, demostrándose posteriormente un descenso significativo, mientras que la incidencia de otras enfermedades definitorias de SIDA continuó aumentando.[35] En un estudio la NPC dejó de constituir la principal causa de muerte de las personas con SIDA.[36] En cuanto a los patrones de ingresos hospitalarios, los debidos a infecciones por *P. carinii* se redujeron considerablemente.[37] Algunos estudios sugieren que la profilaxis primaria de la NPC con cotrimoxazol habría reducido la incidencia de infecciones bacterianas, incluidas las neumonías, en los pacientes VIH con menos de 200 CD4, aunque no todos los estudios son concordantes en este

sentido.[38,39,40,41,42] Más recientemente, la introducción de la terapia antirretroviral de gran actividad (TARGA) a partir de 1996, ha conllevado profundas modificaciones en el curso de la infección por el VIH. Así, la incidencia de infecciones oportunistas y el número de hospitalizaciones han disminuido, y se ha prolongado la supervivencia de estos pacientes.[43,44,45,46,47,48] En España, desde la introducción del TARGA en 1996, se produjo un rápido descenso en la incidencia de casos de SIDA, disminuyendo más de un 60% en cinco años.[8] Sin embargo, no se conoce cómo ha influido en la incidencia, etiología y pronóstico de las complicaciones pulmonares en estos pacientes, sobre todo las que no constituyen infecciones oportunistas.

Patrón de respuesta inflamatoria en el huésped

En los últimos años, el estudio de la respuesta inflamatoria del huésped frente a las infecciones ha recibido gran interés. Un componente importante de esta respuesta es la producción de citocinas. Son polipéptidos producidos por distintos tipos de células activadas, principalmente los macrófagos, los monocitos y los linfocitos, que intervienen como mediadores de comunicación intercelular en las respuestas inmunológica e inflamatoria.[49] También actúan como factores de crecimiento de diversas células y, de forma destacada, de las células hematopoyéticas. Una compleja red de citocinas controla la aparición y mantenimiento de las respuestas inmunológicas innatas y específicas. La respuesta inflamatoria no sólo tiene consecuencias locales, sino que puede desencadenar múltiples cambios en numerosos órganos y sistemas a distancia del lugar de origen. Los cambios sistémicos se han denominado “respuesta de

fase aguda" y son inducidos por un complejo sistema de señalización intercelular del que los principales constituyentes son las citocinas asociadas con la inflamación. Estas citocinas, que se producen durante el proceso inflamatorio, y participan en el mismo, son los principales estimuladores de la producción de proteínas "de fase aguda", como la proteína C reactiva (PCR). Las principales citocinas involucradas en los procesos inflamatorios son la interleuquina-1-beta (IL-1β), la interleuquina-6 (IL-6), la interleuquina-8 (IL-8), el factor de necrosis tumoral alfa (TNF-α) y el interferón gamma (IFN-γ)[50,51]. Otras citocinas, como la interleuquina-10 (IL-10) constituyen un mecanismo de autorregulación de la respuesta inflamatoria, mediante su inhibición (Tabla 1).

En las infecciones pulmonares, la liberación de citocinas, y otros mediadores de la inflamación, constituyen un mecanismo útil para eliminar los microorganismos que invaden el aparato respiratorio. Sin embargo, una producción excesiva puede tener efectos perjudiciales para el huésped, desencadenando una respuesta inflamatoria exagerada y, como consecuencia, una extensa lesión tisular, local (como el síndrome de "distress" respiratorio del adulto) y sistémica (shock, fallo multiorgánico), que en último término pongan en peligro la supervivencia del huésped. [52,53,70] Por lo tanto, se ha sugerido que la cuantificación de la respuesta inflamatoria puede tener implicaciones pronósticas[53]. Así se ha comprobado en algunos estudios que han mostrado una asociación entre los niveles plasmáticos de citocinas y la gravedad y el pronóstico de las infecciones, incluidas las neumonías.[54,55,56,57,67,68,69] En consecuencia, las mediciones seriadas podrían identificar cómo es la respuesta al tratamiento y detectar la necesidad de intervenciones o modificaciones terapéuticas[49].

Diversas investigaciones han mostrado también distintos patrones de producción de citocinas en diferentes enfermedades, lo que hace plausible que las determinaciones de citocinas puedan tener valor diagnóstico[49]. Por otra parte, el mejor conocimiento de la respuesta inflamatoria es el primer paso para intentar la modulación de la misma, lo que constituye una nueva posibilidad terapéutica que podría resultar útil como tratamiento adyuvante a la antibioterapia, especialmente en las infecciones graves.[70,58,59]

En el pulmón, los macrófagos y los neutrófilos polimorfonucleares (PMN) son las principales células implicadas en el aclaramiento de los microrganismos que alcanzan la vía aérea distal. En el pulmón normal, los macrófagos alveolares constituyen el 85% de las células del lavado broncoalvolar, mientras los PMN están prácticamente ausentes (menos del 1% de las células broncoalvolares de las personas que no fuman); sin embargo, en diversos procesos inflamatorios son reclutados desde los vasos sanguíneos al alveolo e intersticio pulmonares[60]. El macrófago alveolar (MA) juega un papel fundamental en el inicio y la regulación de las respuestas inflamatoria e inmune a la infección. Puede ser activado por diversos productos de los microrganismos, como endotoxinas (o lipopolisacáridos [LPS]) y componentes de la pared celular, y por el IFN-γ, producido por los linfocitos T de tipo Th1. El MA puede secretar múltiples productos implicados en la respuesta inflamatoria como citocinas, enzimas y otros tipos de proteínas, metabolitos del ácido araquidónico y radicales libres. En condiciones normales no secreta niveles significativos de citocinas; se produce la secreción cuando el MA es activado por un estímulo[58]. Las citocinas secretadas, como la IL-1, la IL-6, la IL-8 y el TNF-α, están implicadas en el reclutamiento de neutrófilos desde la circulación

pulmonar (que actuarán frente a la infección fundamentalmente mediante la fagocitosis), y en la regulación de la respuesta inmune de los linfocitos B y T[70]. Así, se ha comprobado que la liberación de endotoxinas -en las neumonías bacterianas por bacterias gramnegativas- estimula la producción de IL-1, TNF e IL-8, por el MA[52]. El TNF y la IL-1 se producen precozmente e inducen la liberación de otras citocinas, como la IL-6 y la IL-8. La IL-8 parece tener una potente actividad en la quimiotaxis y activación de los neutrófilos en el lugar de origen de la inflamación, y también podría actuar como mediador en la producción de proteínas de fase aguda por los hepatocitos[52,61]. También la IL-6 se ha implicado en la producción de proteínas de fase aguda (como la proteína C reactiva), así como en la regulación de la respuesta inmune de los linfocitos B y T[70]. Los linfocitos T cooperadores (*'helper'* en inglés) también pueden secretar citocinas. Se diferencian dos grupos, en función del patrón de citocinas que secretan: los linfocitos Th1 y los linfocitos Th2. Los primeros producen IFN-γ, IL-2 e IL-12, que tienen un papel fundamental en la respuesta inmune celular, de la que depende la resistencia a las infecciones por bacterias y parásitos intracelulares, hongos y ciertos virus[62]. Los linfocitos Th2 producen IL-4, IL-5, IL-6 e IL-10, con un importante papel en la respuesta humoral.

Recientes estudios indican que el MA contribuye significativamente a la respuesta del huésped frente a *P. carinii* a través de la liberación de TNF-α, y también se ha observado que, citocinas con papel quimiotáctico, como la IL-8, juegan un papel en el reclutamiento de células inflamatorias en los lugares de infección.[63,64] Los corticoides inhiben significativamente la liberación de IL-8, lo que podría explicar –al menos en parte- su papel en el tratamiento de la neumonía grave por *P. carinii*[64]. En el caso de los pacientes con TBC e

infección por el VIH, un estudio ha mostrado que las mediciones seriadas de TNF-α plasmático se correlacionan con la respuesta al tratamiento anti-tuberculoso[65]. En los últimos años se ha estudiado la respuesta inflamatoria local y sistémica que tiene lugar en las neumonías bacterianas, tanto en modelos animales como en pacientes inmunocompetentes.[53,66,67,68,69,70] Sin embargo, no se ha estudiado la respuesta inflamatoria en las neumonías bacterianas de los pacientes VIH. Por lo tanto, queda mucho por conocer sobre el papel de las citocinas en las distintas enfermedades pulmonares que presentan los pacientes con infección por el VIH a lo largo de su evolución.

Entre los productos que secreta el MA activado, en los pacientes VIH parecen estar incrementadas la IL-1, la IL-6, la IL-8 y el TNF-α.[71,72,73,74] Aunque se produce un aumento de la secreción en los pacientes con enfermedades pulmonares, también se observa en pacientes sin enfermedades pulmonares y asintomáticos desde el punto de vista de la infección VIH. Esto sugiere que sería consecuencia directa de la estimulación por el propio virus. Sin embargo, no se ha demostrado una correlación entre el estadio de la infección por VIH o la carga viral del VIH-1 en los macrófagos y la producción de estas citocinas.

Por otro lado hay que tener en cuenta que en la infección por el VIH existe una activación crónica del sistema inmune en respuesta directa al VIH o causada indirectamente por el virus, dando lugar a muchos de los datos patogénicos de la infección. Así, existe una clara disregulación de las citocinas: 1) Está incrementada la producción de citocinas proinflamatorias como el TNF-α, que puede activar directamente la replicación del VIH. 2) Hay una producción disminuida de las citocinas Th1 (IFN-γ, IL-2, IL-12) que tienen un papel fundamental en la respuesta inmune celular, de la que dependen la resistencia

a infecciones por bacterias y parásitos intracelulares, hongos y ciertos virus[75]. De hecho están en marcha estudios para evaluar el posible papel terapéutico de la IL-2 en el tratamiento de la infección por el VIH[76]. 3) Aumento de la producción de citocinas Th2 (IL-4, IL-6 e IL-10) que tienen efectos reguladores negativos, reduciendo la respuesta Th1[77]. Estas alteraciones se han implicado en la patogénesis de enfermedades relacionadas con el VIH como el síndrome de emanciación, la encefalopatía asociada al VIH o el sarcoma de Kaposi. Está situación habrá de tenerse en cuenta en un estudio sobre la respuesta inflamatoria a las complicaciones pulmonares en los pacientes con infección por el VIH.

HIPÓTESIS

1. La incidencia, etiología y pronóstico de las complicaciones pulmonares en los pacientes VIH ha cambiado tras la generalización del tratamiento antirretroviral de gran eficacia (TARGA), aunque siguen constituyendo una de las causas más frecuentes de morbimortalidad y de ingreso hospitalario en estos pacientes.
2. El mejor conocimiento, tanto de la epidemiología de las enfermedades pulmonares en nuestro medio, como del rendimiento de las distintas pruebas diagnósticas, permitirá definir cuál es la mejor estrategia diagnóstica.
3. La respuesta inflamatoria sistémica que se produce en las complicaciones pulmonares que presentan los pacientes con infección por el VIH (principalmente neumonías bacterianas, neumonía por *Pneumocystis carinii* y micobacteriosis) medida a través de la determinación de citocinas y proteínas de fase aguda como la proteína C reactiva, se correlaciona con la etiología de las mismas. El conocimiento del patrón de respuesta asociado a cada entidad, junto a otros datos clínicos, radiológicos y biológicos, podría permitir predecir la etiología (o grupo etiológico) más probable de una forma precoz y no invasiva.
4. Los niveles de citocinas en plasma pueden tener valor pronóstico, y sus determinaciones plasmáticas seriadas permitirían valorar la necesidad de modificar la estrategia terapéutica.

OBJETIVOS

1. Estudiar la incidencia, etiología y pronóstico de las complicaciones pulmonares en los pacientes VIH tras la introducción del tratamiento antirretroviral de gran eficacia (TARGA), mediante un estudio prospectivo observacional de pacientes consecutivos del Hospital Clínic de Barcelona con infiltrados pulmonares. Para ello se empleó en todos los casos un protocolo diagnóstico estandarizado.
2. Evaluar la rentabilidad de las técnicas diagnósticas utilizadas, según el protocolo establecido.
3. Estudiar el patrón de la respuesta inflamatoria, mediante medición plasmática de citocinas proinflamatorias y antiinflamatorias (IL-1β, IL-4, IL-6, IL-8, IL-10) y proteína C reactiva, en las complicaciones pulmonares más frecuentes que presenten los pacientes con infección por el VIH. Evaluar su correlación con la etiología y el pronóstico.

ARTÍCULOS

JAIDS Journal of Acquired Immune Deficiency Syndromes
27:35–43 © 2001 Lippincott Williams & Wilkins, Inc., Philadelphia

Pulmonary Infiltrates in HIV-Infected Patients in the Highly Active Antiretroviral Therapy Era in Spain

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Objective: To study the incidence, etiology, and outcome of pulmonary infiltrates (PIs) in HIV-infected patients and to evaluate the yield of diagnostic procedures.

Design: Prospective observational study of consecutive hospital admissions.

Setting: Tertiary hospital.

Patients: HIV-infected patients with new-onset radiologic PIs from April 1998 to March 1999.

Methods: The study protocol included chest radiography, blood and sputum cultures, serologic testing for "atypical" causes of pneumonia, testing for *Legionella* urinary antigen, testing for cytomegalovirus antigenemia, and bronchoscopy in case of diffuse or progressive PIs.

Results: One hundred two episodes in 92 patients were recorded. The incidence of PIs was 18 episodes per 100 hospital admission-years (95% confidence interval [CI]: 15–21). An etiologic diagnosis was achieved in 62 cases (61%). Bacterial pneumonia (BP), *Pneumocystis carinii* pneumonia (PCP), and mycobacteriosis were the main diagnoses. The incidences of BP and mycobacteriosis were not statistically different in highly active antiretroviral therapy (HAART) versus non-HAART patients. The incidence of PCP was lower in those receiving HAART ($p = .011$), however. Nine patients died (10%). Independent factors associated with higher mortality were mechanical ventilation (odds ratio [OR] = 83; CI: 4.2–1,682), age >50 years (OR = 23; CI: 2–283), and not having an etiologic diagnosis (OR = 22; CI: 1.6–293).

Conclusions: Pulmonary infiltrates are still a frequent cause of hospital admission in the HAART era, and BP is the main etiology. There was no difference in the rate of BP and mycobacteriosis in HAART and non-HAART patients. Not having an etiologic diagnosis is an independent factor associated with mortality.

Key Words: Acquired immunodeficiency syndrome—HIV infections—Lung diseases—Pneumonia—Pulmonary infiltrates.

Up to 70% of HIV patients may have a pulmonary complication during the evolution of the infection (1). According to results of autopsy findings, the lung is the organ most frequently affected by HIV-related processes, with an incidence ranging from 100% in the early period of the epidemic to 84% in the highly active antiretroviral therapy (HAART) era (2,3). Lung involvement usually appears as pulmonary infiltrates (PIs) on chest radiographs and is frequently (but not always) associated with clinical respiratory symptoms.

Few studies have systematically described the full spectrum of pulmonary disorders associated with HIV infection (4,5). Most investigators have focused on

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Presented in part at the XIII International AIDS Conference, Durban, South Africa, July 9–14, 2000 (abstract TuPeB3183).

Manuscript received November 15, 2000; accepted February 13, 2001.

pneumonias of specific etiologies such as those caused by bacteria or *Pneumocystis carinii*. Therefore, there is no consensus on any diagnostic algorithm of PIs in HIV patients. The decision tree should probably be different depending on the epidemiologic features in a specific geographic area (6,7).

There could be several reasons to explain the changes in the epidemiology of HIV-related pulmonary complications. General prescription of *P. carinii* primary prophylaxis since 1989 (8) is probably one of the main causes, and the use of HAART since 1996 may also be an underlying explanation. Highly active antiretroviral therapy has decreased the incidence of opportunistic infections and the number of hospital admissions, and it has increased the life expectancy of HIV patients (9–13). Nevertheless, its influence on the incidence, etiology, and prognosis of pulmonary complications, mainly on nonopportunistic infections, remains unknown.

The aims of this study are to analyze the incidence, etiology, and mortality prognostic factors of PIs in HIV patients in the HAART era and to evaluate the diagnostic yield of diagnostic procedures.

PATIENTS AND METHODS

A prospective study of consecutive hospital admissions of HIV-infected patients was carried out at Hospital Clinic Universitari, a 900-bed tertiary level hospital in Barcelona, Spain.

From April 1998 to March 1999, all HIV-infected patients with new-onset radiologic PIs on admission or with PIs that developed during hospitalization were analyzed. Patients were followed up until radiologic resolution of the infiltrates or death secondary to the pulmonary event.

The following variables were recorded: demographic features; HIV risk factors; antiretroviral therapy; CD4 lymphocyte count per cubic millimeter; HIV-1 plasma viral load (VL) before the pulmonary event; clinical, analytic, and radiologic data at admission; microbiologic results; final etiologic diagnosis; antimicrobial treatment; and outcome.

Two main groups were considered according to the characteristics of PIs shown on chest radiographs.

Unilateral Lobar Consolidation

Diagnostic procedures included the following:

Two sets of blood cultures

Sputum specimen (when possible) and bronchoaspirate (BAS) in case of mechanical ventilation. Sputum and BAS specimens were Gram and Ziehl-Neelsen stained and cultured for aerobic bacteria, fungi, and mycobacteria.

Induced sputum (if standard sputum specimen not available), which was additionally stained with Gomori's methenamine silver to detect *P. carinii*

Serologic tests for atypical pneumonias: *Legionella pneumophila* serogroups 1 through 6 (IgG determination by immunofluorescence [IF]), *Chlamydia pneumoniae* (IgM and IgG detection by IF), *Mycoplasma pneumoniae* (IgM and IgG determination by enzyme immunoassay [EIA] and complement fixation), and *Coxiella burnetii* (IgM and IgG detection by IF)

Serologic tests for respiratory viruses (IgG detection by complement fixation): adenovirus, respiratory syncytial virus, parainfluenza viruses 1 through 3, and influenza viruses A and B (at admission and 3 weeks later)

Urinary antigen test for detection of *L. pneumophila* serogroup 1 by EIA (Biotest *Legionella* Urine Antigen EIA, Biotest, Dreieich, Germany)

Pleural effusions were Gram and Ziehl-Neelsen stained and cultured for aerobic and anaerobic bacteria, fungi, and mycobacteria.

Multiple, Bilateral, or Diffuse Pulmonary Infiltrates and Initially Unilateral Localized Infiltrates in Patients Without Clinical or Radiologic Improvement After 3 Days of Treatment

Diagnostic procedures included the following:

Same investigations as for unilateral lobar consolidation

Bronchoalveolar lavage (BAL) and protected specimen brush (PSB) samples were retrieved by means of a fiberoptic bronchoscope. Samples obtained by PSB were Gram and May-Grünwald Giemsa stained and processed for aerobic and anaerobic bacteria and fungal cultures. Samples obtained by BAL were additionally Ziehl-Neelsen and Gomori's methenamine silver stained and cultured for mycobacteria. The latter samples were also processed for viral antigen detection and culture as well as cytologic study.

Nasopharyngeal wash sample for detection of respiratory viruses

Cytomegalovirus (CMV) antigenemia using monoclonal antibodies against CMV matrix protein pp65

If an etiologic diagnosis was not obtained and there was no clinical or radiologic improvement, a computed tomography–guided percutaneous needle biopsy was performed (mainly in case of peripheral infiltrates). These samples were submitted for pathologic and microbiologic studies. Microbiologic studies included Gram, Ziehl-Neelsen, and Gomori's methenamine silver stains as well as cultures for aerobic and anaerobic bacteria, mycobacteria, fungi, and viruses.

Media and Incubation Conditions

Media and incubation conditions were as follows:

For bacterial culture, secretions were plated on blood-sheep and chocolate agar and incubated at 37°C in a CO_2-enriched atmosphere for 2 days. *Legionella* buffer charcoal yeast extract agar was incubated for 10 days. Sputum and induced sputum cultures were qualitative, but all the other samples were quantitatively cultured for aerobic and anaerobic bacteria.

For fungal culture, secretions were inoculated on Sabouraud's agar and incubated at 30°C in aerobic conditions for 4 weeks.

For mycobacteria, Löwenstein-Jensen medium and radiometric BACTEC 12B broth culture bottles (Becton Dickinson Microbiology Systems, Sparks, MD, U.S.A.) were incubated at 37° in aerobic conditions for 6 weeks.

For direct viral identification, samples were inoculated in monolayers of MRC-5, A-549, Hep-2 years Madin-Darby canine kidney cell lines (Vircell, Granada, Spain). Cultures were maintained for 4 weeks and regularly examined for cytopathic effect. Viral growth was confirmed by IF staining with monoclonal antibodies (Respiratory Panel 1, Viral Screening and Identification Kit; Light Diagnostics, Temecula, CA, U.S.A). Detection of viral antigens was performed by direct fluorescent antibody staining with a pool of monoclonal antibodies against a panel of respiratory viruses

(influenza virus A and B, parainfluenza virus 1–3, respiratory syncytial virus, and adenovirus). In addition, CMV detection was performed by immunocytochemistry methods using a monoclonal antibody against the pp65-CMV protein (Dako, Glostrup, Denmark).

The etiologic diagnosis was considered as definitive if a pathogen such as *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, or *L. pneumophila* was cultured in a respiratory sample; a potential pathogen was cultured in a biopsy sample; blood or pleural effusion cultures were positive; urinary *L. pneumophila* antigen was detected; serologic change was confirmed; *P. carinii* was identified with Gomori's methenamine silver stain; CMV cellular inclusion bodies were reported in BAL; BAL cytologic testing or biopsy identified malignant cells; or the pathologic biopsy study was conclusive for the diagnosis of any defined condition.

The etiologic diagnosis was considered as probable in case of bacterial pneumonia (BP) when a bacterial pathogen was isolated from a sputum specimen included in group 4 or 5 of Murray and Washington's system (14) and Gram stain findings correlated with culture results or when a quantitative bacterial culture from PSB, BAL, or BAS, respectively, grew more than 10^3, 10^4, or 10^5 colony-forming units per milliliter of a bacterial pathogen. In case of viruses, the etiologic diagnosis was considered as probable when a cellular culture and/or antigen viral detection was positive and/or if serologic change was confirmed. In case of fungal infection, the etiologic diagnosis was considered as probable when a microorganism was isolated in two or more BAS, PSB, or BAL samples or when hyphae were found in Gram stain and a fungus was cultured in two or more sputum samples [group 4, 5, or 6 of Murray and Washington's system (14)].

A clinical assumption of BP was also accepted as a definitive diagnosis in patients who had a negative microbiologic analysis but who had clinical (fever and/or respiratory symptoms, including coughing, dyspnea, or pleuritic chest pain) and radiologic (PI lasting more than 24 hours) data suggestive of BP and a favorable outcome after empiric antibacterial treatment (except if this included an effective drug for *P. carinii*). All other cases were classified as "not diagnosed PIs."

All statistics were calculated with the SPSS statistical package (version 9.0; SPSS, Inc., Chicago, IL, U.S.A.). Continuous variables are summarized as means (and SD) or medians (interquartile range, range) depending on their homogeneity. Categoric variables were compared using the χ^2 test or Fisher exact test when appropriate. Associations are given as relative risks. Quantitative variables were compared with the Kruskal-Wallis (mean) or median nonparametric test (15). Analysis of factors independently associated with mortality was performed with logistic regression. Results are given as an odds ratio. The confidence interval was established at 95%. A probability value <.05 was considered to be significant.

RESULTS

There were 580 consecutive hospital admissions of adults with HIV infection during the study period. One hundred two episodes of PIs in 92 patients were recorded. Six patients had more than 1 episode. All patients were intravenous drug users, who had BPs (3 patients had 2 episodes and the other 3 had 3 episodes). The incidence was 18 episodes per 100 hospital admission-years (95% confidence interval [CI]: 15–21). Demographic features, HIV infection risk factors, immunologic (CD4 lymphocyte counts) status, and virologic status are shown in Table 1.

TABLE 1. *Demographic features, HIV infection risk factors, immunologic (CD4 lymphocyte counts) status, and viral load of HIV patients with pulmonary infiltrates*

Number of episodes/patients	102/92
Male patients	66 (72%)
Mean age (SD, range) (years)	37 (9.3, 22–73)
HIV risk factor	
Injecting drug user	58 (62%) patients (67 episodes)
Homosexual	15 (61%) patients
Heterosexual or unknown	20 (21%) patients
HIV diagnosis during pulmonary event	17 (18%)
CD4 cell count per cubic millimeter median (interquartile range, range)	106 (265, 2–882)
Episodes in patients with CD4 count <200 cells/mm^3	65 (64%)
Viral load median (interquartile range, range)	86,244 (276,045, <200–1,665,000)
Episodes in patients receiving highly active antiretroviral therapy	40 (39%)
Episodes in patients with viral load <200 copies	9 (23%)

An etiologic diagnosis was achieved in 62 episodes (61%) (Table 2). The three main diagnostic groups were "pyogenic" BP in 63 episodes (11 episodes per 100 admission-years [95% CI: 8–13]), 17 *P. carinii* pneumonia (PCP) cases (3 episodes per 100 admission-years [95% CI: 2–5]), and 11 cases of mycobacteriosis (*M. tuberculosis* and *M. kansasii*) (2 episodes per 100 admission-years [95% CI: 1–3]). A definitive diagnosis was not possible in 14 of the 102 episodes (14%). All episodes of BP were community-acquired pneumonia, except for 3 episodes of nosocomial pneumonia. A specific bacterial diagnosis was achieved in 36 episodes (58%) (see Table

TABLE 2. *Etiologic diagnosis of 102 episodes of pulmonary infiltrates in HIV-infected patients*

Etiology	Number of episodes (% of total etiologic diagnosis)
Infectious etiology	60 (97%)
Bacteria	36 (58%)
Streptococcus pneumoniae	23
Haemophylus influenzae	8
Staphylococcus aureus	2
Mycoplasma pneumoniae	1
Escherichia coli	1
Acinetobacter baumanii	1
Pneumocystis carinii	17 (27%)
Mycobacteriosis	11 (18%)
Mycobacterium tuberculosis	9
Mycobacterium kansasii	2
Viruses	4 (6%)
Influenza A virus	2
Parainfluenza 2 virus	1
Enterovirus	1
Cytomegalovirus	1
Aspergillus fumigatus	1 (2%)
Multiple organisms	9 (15%)
Noninfectious etiology	2 (3%)
Small cell lung carcinoma	2

2). Most *Streptococcus pneumoniae* strains were susceptible to penicillin (74%). Intermediate-level resistance (minimum inhibitory concentration: 0.25–1 mg/L) was found in 3 cases (13%), and high-level penicillin-resistant pneumococci (minimum inhibitory concentration: 2 mg/L) were also isolated in 3 cases (13%). Patients with PCP did not receive antipneumocystic prophylaxis or received it irregularly.

The HIV-1 VL distribution is shown in Figure 1, and CD4 counts per milliliter are shown in Figure 2. Median differences among the three main diagnostic groups (BP, mycobacteriosis, and PCP) were statistically significant.

Diagnostics in HAART and non-HAART patients are shown in Table 3. The number of patients who had a good viral response to HAART (VL <200 copies/ml) was too small to detect a statistically significant difference compared with the remaining patients. All PIs in patients with <200 copies/ml were due to BP (except for 1 case of mycobacteriosis).

Mortality was 10% (9 of 92 patients): 6 patients did not have an etiologic diagnosis, 2 had PCP together with pneumococcal pneumonia, and 1 had *Aspergillus fumigatus* infection. Four of 55 patients with BP (7.3%) (1 with nosocomial pneumonia) and 2 of 17 patients with PCP (11.8%) died during the episode. None of the patients with mycobacteriosis died. The relative risk of mortality associated with different variables is shown in Table 4. In the multivariate analyses, mechanical ventilation (OR = 83; CI: 4.2–1682), age >50 years (OR = 23; CI: 2–283), and not having an etiologic diagnosis (OR = 22; CI: 1.6–293) were factors independently associated with higher mortality in HIV patients with PIs.

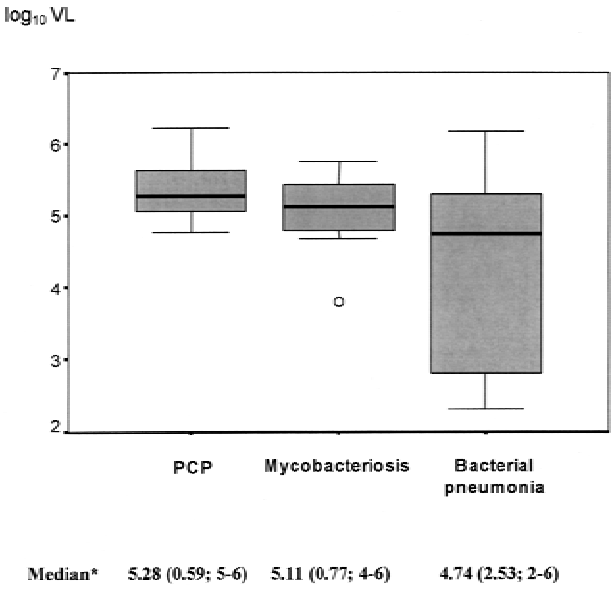

FIG. 1. Viral load $\log_{10}$ distribution in the main diagnostic groups: *Pneumocystis carinii* pneumonia (PCP, mycobacteriosis, and bacterial pneumonia.

*Median difference, assessed using nonparametric test of medians (15), were significant ($p = .002$).

The diagnostic yield of different techniques was evaluated. Spontaneous sputum sample analysis was performed in 85 cases; 55 samples (65%) were considered to be of good quality and were processed for culture. Sputum cultures yielded 31 positive results (56%) (isolation of ≥1 microorganism); definitive or probable infection was considered in 28 of these 31 cases (51%) (13 *Staphylococcus pneumoniae*, 7 *Haemophilus influenzae*, 7 *M. tuberculosis*, 2 *M. kansasii*, and 1 *Escherichia coli*). Induced sputum was positive in 2 of 22 cases (9%) (*P. carinii* and *M. kansasii*). Blood cultures were obtained in 92 episodes (92%); 15 were positive (16%) (11 *S. pneumoniae*, 2 *H. influenzae*, and 2 *S. aureus*). Atypical pneumonia serologic testing was carried out in 51 cases, and in 1 case (2%), it was positive for *M. pneumoniae*. Urinary antigen testing for *L. pneumophila* detection was performed in 80 cases, and in none of them was the test positive. Bronchoscopy was performed in 39 cases (38%). The diagnostic yield of BAL was 56%: a specific pathogen was identified in 22 episodes (16 *P. carinii*, 3 *M. tuberculosis*, 1 *Acinetobacter baumanii*, 1 *A. fumigatus*, and 1 enterovirus), and oat-cell carcinoma was diagnosed in 1 case by means of cytologic testing. A computed tomography–guided percutaneous needle biopsy was performed in 2 cases: 1 was an oat-cell carcinoma, and the other was inconclusive.

Sputum samples were obtained in 54 of 62 BP cases, and sputum culture was processed in 37 cases. Twenty-one of these cases (57%) provided the etiologic diagnosis. Blood cultures were positive in 15 of 58 (36%) BP cases (not obtained in 4 cases). Bronchoalveolar lavage analysis yielded *P. carinii* cysts in 3 of 12 cases (25%) in which bronchoscopy was performed. These 3 cases had a concomitant definitive pneumococcal diagnosis.

DISCUSSION

In this series, BP was the main etiologic diagnosis in HIV patients with PIs, followed by PCP and mycobacteriosis (*M. tuberculosis* and *M. kansasii*).

In early studies, PCP was the most frequent cause of PIs, accounting for 85% of cases (16–18). A majority of these cases were AIDS patients. In the last decade, the incidence of PCP has greatly decreased as a result of *P. carinii* primary prophylaxis. Whereas 30% to 50% of patients had PCP at the time of their AIDS diagnosis in the 1980s, only 10% to 20% of patients had PCP at the time of their AIDS diagnosis in the 1990s (19–23). Nevertheless, PCP remains one of the leading causes of

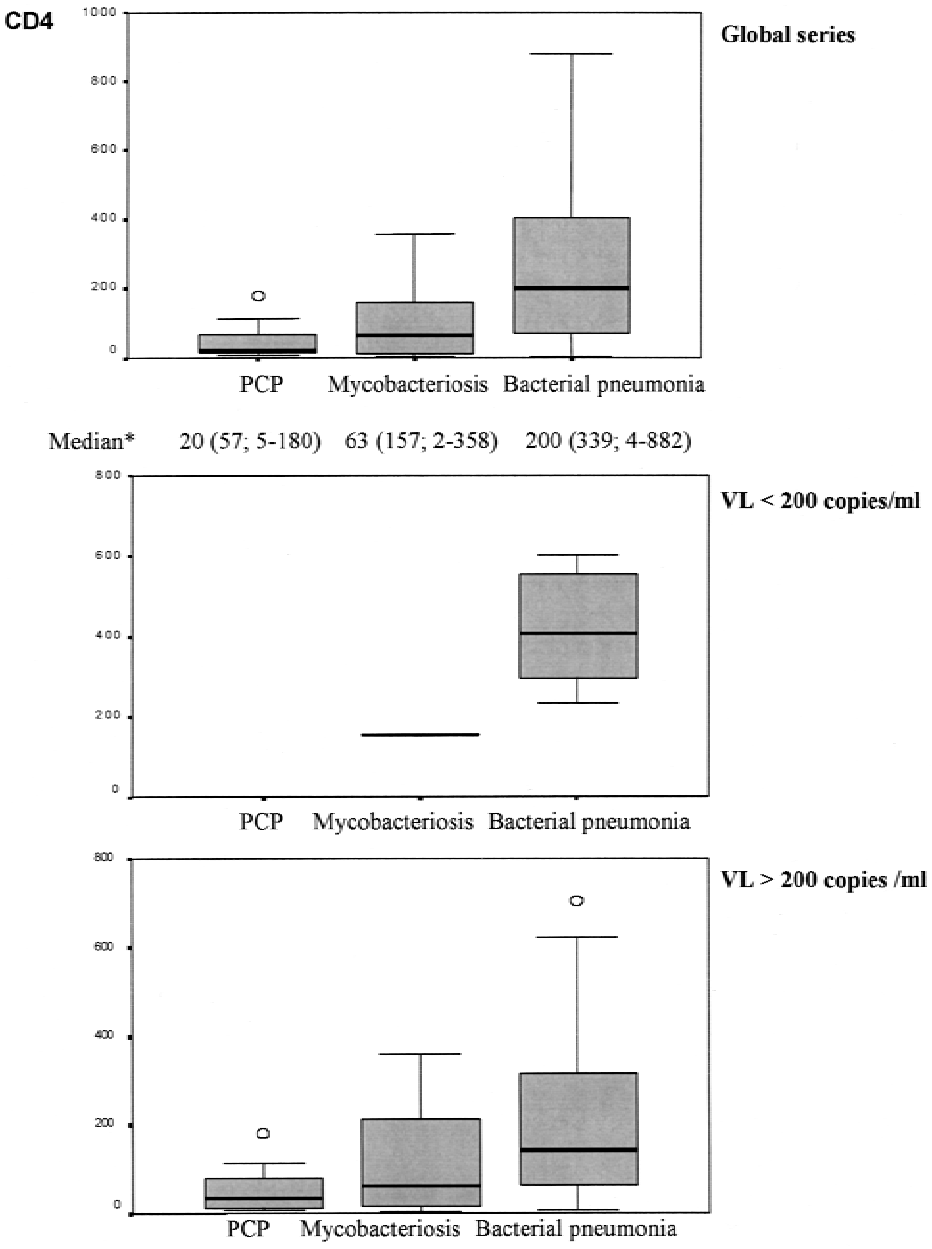

FIG. 2. $CD4^+$ distribution in the main diagnostic groups.
*Median difference, assessed using nonparametric test of medians (15), were significant ($p = .001$).

AIDS-related illness (22). In the current study, PCP was the second most frequent cause of PIs (17%; 3 episodes per 100 admission-years [CI: 2–5]); BP was the most frequent cause, with much higher frequency (61%; 11 episodes per 100 admission-years [CI: 8–13]). No patients who suffered from PCP in our series were receiving anti-*Pneumocystis* prophylaxis, and HIV infection was detected after the PCP diagnosis in 7 cases (41%). Only two of the previously known HIV-infected patients were on HAART, but compliance was poor. All these data supply further evidence regarding new epidemiologic features of PCP infection in the HAART and widespread prophylaxis era. *Pneumocystis carinii* pneumonia has become a complication for those patients who are not receiving effective HAART and for those who are not receiving anti-*Pneumocystis* prophylaxis. As reported, a significant percentage of these patients (41% in our data) were not previously known to be infected with HIV (24,25).

The median CD4 count in patients with PCP was 20 cells/mm^3 (maximum range, <200 cells/mm^3), which is similar to the findings in previous reports (see Fig. 2)

TABLE 3. *Main diagnostic groups in patients with or without previous highly active antiretroviral therapy*

Diagnostic group	Number of episodes (% refers to number of episodes in patients receiving highly active antiretroviral therapy)	Number of episodes (% refers to number of episodes in patients without previous highly active antiretroviral thearpy)	*p* value
Pneumocystis carinii pneumonia	2 (5%)	15 (24%)	.011
Mycobacteriosis[a]	5 (13%)	6 (10%)	NS
Bacterial pneumonia	25 (63%)	38 (61%)	NS

Differences between proportions have been assessed using the χ^2 statistic or Fisher exact test when appropriate.
[a] *Mycobacterium tuberculosis* and *Mycobacterium kansasii.*
NS, not significant.

(13,26,27). There is not much information about the relation between plasma VL and opportunistic infections (28). Before PCP prophylaxis, an increase in PCP risk was reported depending on high baseline VL among all CD4 count levels (≥350, 200–349, and <200 cells/mm^3) (28). In our data, the median VL for PCP cases was significantly higher than that of other diagnostic groups (BP and tuberculosis groups) ($p = .002$) (see Fig. 1). The VL was >50,000 copies/ml in all patients, and there was no case of PCP in patients with <200 copies/ml.

Our data confirm that BP is currently the most frequent pulmonary complication in HIV patients (4,29). Moreover, it is the most common admission diagnosis in this population (30,31). In the current study, BP was the cause of 62% of PIs, which is higher than the 20% to 46% rate reported in the late 1980s when PCP and other opportunistic processes were more common (32,33). Prevalence was even lower (2%) in early 1980s series, which only considered patients who met the criteria for AIDS (16). The incidence of BP in our study (11 episodes per 100 admission-years) was similar to that published in recent years before the introduction of HAART (12.5 episodes per 100 admission-years) (34,35). When comparing incidences of community-acquired pneumonia before and after introduction of HAART, there were no significant differences (10.4 vs. 8.2 episodes per 100 patients). The incidence of nosocomial episodes of BP have decreased in the HAART era, however (35). In our series, there was no difference in BP rates depending on HAART (see Table 3). Among HIV-infected persons, BP occurs with increased frequency at all CD4 lymphocyte counts, although the risk of developing BP increases as the CD4 lymphocyte count declines (5,29). In this study, the median CD4 lymphocyte count in BP cases was 200 cells/mm^3, which is significantly higher than that in tuberculosis and PCP cases. The increase in the risk of BP is already evident in early stages of cell-mediated immunity dysfunction (see Fig. 2). Because HAART delays the start of immunologic damage caused by HIV, the period of bacterial infection susceptibility could be increased. Although further analysis is necessary, this might explain the apparent ineffectiveness of

TABLE 4. *Prognostic factors of episodes of pulmonary infiltrates in HIV-infected patients: univariate analysis*

Risk factor	Number died/total (%)		*p* value	Relative risk (95% confidence interval)
	Present	Absent		
Age >50 years	3/9 (33)	6/93 (7)	.031	5 (1.5–17.2)
Sex (male)	9/70 (13)	0/32 (0)	.054	—
Injecting drug user	4/67 (6)	5/29 (17)	.238	1 (0.9–1.3)
Highly active antiretroviral therapy	5/40 (13)	4/62 (7)	.309	2 (0.6–6.8)
Prophylaxis with trimethoprim-sulfamethoxazole	6/84 (7)	3/18 (17)	.194	0.4 (0.1–1.6)
CD4 count <200 cells/mm^3[a]	8/65 (12)	0/33 (0)	.049	—
Viral load >50,000[b]	7/48 (15)	1/28 (4)	.245	4 (0.5–31.5)
Viral load <200[b]	1/11 (9)	7/65 (11)	1	0.8 (0.1–6.2)
HIV diagnosis during pulmonary event	3/17 (18)	6/85 (7)	.169	2.5 (0.7–1.1)
Without etiologic diagnosis	6/40 (15)	3/62 (5)	.149	3 (0.8–11.7)
Pneumocystis carinii pneumonia	2/17 (12)	3/71 (4)	.246	2.7 (0.5–15.4)
Bacterial pneumonia	4/63 (6)	1/25 (4)	1	1.6 (0.2–15.5)
Mycobacteriosis	0/11 (0)	5/77 (7)	1	—
Acute respiratory failure	6/29 (21)	3/71 (4)	.007	7.34 (1.6–34.3)
Mechanical ventilator	3/6 (50)	6/96 (6)	.009	8 (2.6–24.4)
Intensive care unit admission	5/10 (50)	4/92 (4)	<.0001	11.5 (3.7–36)

[a] CD4 lymphocyte count was available for 98 of the 102 pulmonary infiltrate episodes.
[b] Viral load (HIV plasma viral load in copies per milliliter) was available for 76 of the 102 pulmonary infiltrate episodes.

HAART in decreasing BP incidence. The median VL was also lower in BP cases when compared with other PI etiologies (see Fig. 1). Again, more data are needed to determine whether patients with an undetectable VL also have a higher risk of BP. With respect to the etiologic agents, *S. pneumoniae* and *H. influenzae* were the most common respiratory pathogens as previously reported (32,34).

In Europe, the incidence of tuberculosis in HIV-infected patients in the period from 1994 through 1999 was 0.8 case per 100 person-years of follow-up (36), which is lower than the incidence in the 1980s (3 cases per 100 person-years) (37), and at the same level as in the United State during the period from 1988 through 1994 (38). This marked decrease in Europe seems to be associated with the introduction of HAART (36). Remarkable regional differences are observed, however. The incidence of tuberculosis is 3.13 cases per 100 person-years in southwest Europe, which is four to seven times higher than in other regions (36). Similarly, in the current study, an incidence of 2 episodes per 100 admission-years was observed, and tuberculosis was the third most common cause of PIs in the HIV-infected patients studied (11% of cases). This high rate, together with the frequent association of mycobacteriosis with other pulmonary infections (3 of 11 cases in our series [27%]) supports the performance of routine *Mycobacterium* cultures in all HIV patients with PIs in our geographic area. Furthermore, the finding of 2 cases of *M. kansasii* infection, which requires a specific treatment, supports this diagnostic approach.

The 2 cases of oat-cell carcinoma found in a series of quite young patients (mean age, 37 years) are worthy of mention. It has been suggested that an increased incidence of pulmonary neoplasms can be seen in HIV patients, but data are poor and not conclusive (39,40).

Overall studies analyzing prognostic factors of PIs in HIV patients are scarce, limiting the possibility of comparing our results with those in the literature. In a previous series of HIV patients with PIs requiring intensive care hospitalization, PCP and mechanical ventilation were factors associated with higher mortality (41). Most investigators have focused on PCP, and 10% to 20% mortality has been reported (12% in our study) (42). In PCP, factors such as respiratory impairment and mechanical ventilation have consistently been related to fatal outcome; other factors include age >45 years, high lactate dehydrogenase levels (>800 IU/L), marked neutrophilia in BAL, malnourishment, and CD4 count <50 cells/mm^3 (43,44). In a recent study of BP, CD4 count $\leq$100 cells/mm^3, neutropenia, room air Pao_2 <70 mm Hg, and Karnofsky score $\leq$50 were predictive factors of mortality (45). Previous studies have indicated a variable mortality (5%–27%) for HIV-associated BP (18,29,34,45). The prognosis of BP in HIV patients is usually favorable, except for pneumonia caused by enterobacteria and *Pseudomonas aeruginosa.* In the current study, mortality was 7%, but 2 of the 55 patients with BP were also infected by *P. carinii.* If these 2 cases were excluded, mortality attributed to BP would be 3.6%. This low rate may be related to the low prevalence of pneumonia caused by enterobacteria and *P. aeruginosa* in our series. Mechanical ventilation, age >50 years, and not having an etiologic diagnosis were independent factors associated with higher mortality in HIV patients with PIs in our study. This last factor should probably be of special concern, because it focuses on the need for an improvement in the diagnostic yield of techniques.

There is no consensus on a diagnostic algorithm of PIs in HIV patients. Some investigators have recommended an empiric approach based on clinical features and local epidemiology. They have also suggested that diagnostic techniques should only be considered for patients in whom empiric therapy fails (6,46). Other authors think that a definitive diagnosis should be achieved initially by means of noninvasive specimens, followed by invasive techniques if these specimens are nondiagnostic (6). Our data would support the latter approach, because not having an etiologic diagnosis was associated with increased mortality in the current study. Moreover, polymicrobial etiology accounted for 9% of the cases. *Pneumocystis carinii* pneumonia and pneumococcal pneumonia coexisted in 3 patients in whom one of the two diagnoses was not previously suspected. The role of sputum sample microbiology is worthy of mention. The diagnostic yield of this noninvasive procedure was quite high (51%) in general and higher (67%) in BP. The microbiologic results of bronchoscopic samples were also quite encouraging, with a diagnostic yield of 56%.

Our proposed algorithm for the diagnosis of PIs in HIV patients can be considered effective, but more investigations are required and should probably focus on geographic differences in epidemiology. The identification of the etiologic agent in HIV patients with PIs is probably one of the cornerstones of the prognosis.

Acknowledgments: The authors thank María Pesqueira and Elisa García Vázquez for their assistance with English translation. During the study period, N. Benito was the recipient of a research grant from the Fundación Privada Máximo Soriano Jiménez.

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CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 2004, p. 608–614 Vol. 11, No. 3
1071-412X/04/$08.00+0 DOI: 10.1128/CDLI.11.3.608–614.2004

Inflammatory Responses in Blood Samples of Human Immunodeficiency Virus-Infected Patients with Pulmonary Infections

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Received 18 September 2003/Returned for modification 12 November 2003/Accepted 16 February 2004

We analyzed the characteristics of the inflammatory response occurring in blood during pulmonary infections in human immunodeficiency virus (HIV)-infected patients. A prospective study of consecutive hospital admissions of HIV-infected patients with new-onset radiologic pulmonary infiltrates was carried out in a tertiary university hospital from April 1998 to May 2001. Plasma cyclic AMP receptor protein (CRP), interleukin 1β (IL-1β), IL-6, IL-8, IL-10, and tumor necrosis factor alpha (TNF-α) levels were determined at the time of admission and 4, 5, and 6 days later. Patients were included in a protocol addressed to study etiology and outcome of disease. A total of 249 episodes of infection were included, with the main diagnoses being bacterial pneumonia (BP) (118 episodes), *Pneumocystis carinii* pneumonia (PCP) (41 episodes), and mycobacteriosis (36 episodes). For these three patient groups, at the time of admission the median CRP and cytokine levels were as follows: CRP, 10.2, 3.8 and 5 mg/dl, respectively ($P = 0.0001$); IL-8, 19, 3, and 2.9 pg/ml ($P = 0.045$); and TNF-α, 46.4, 44, and 75 pg/ml, respectively ($P = 0.029$). There were no significant differences in levels of IL-1β, IL-6, or IL-10 among the patient groups. A total of 23 patients died. At the time of admission, HIV-infected patients with BP had higher plasma CRP and IL-8 levels than did PCP and mycobacteriosis patients. TNF-α levels were higher in patients with mycobacteriosis. An elevated IL-8 level (>61 pg/ml) at the time of admission was an independent factor associated with higher mortality (odds ratio, 12; 95% confidence interval, 1.2 to 235.5).

Pulmonary infiltrates are a frequent cause of morbidity and hospital admission of human immunodeficiency virus (HIV)-infected patients in the highly active antiretroviral therapy era, with an incidence of ~20 episodes per 100 hospital admission-years (5). The main diagnostic groups are bacterial pneumonia (BP), *Pneumocystis carinii* pneumonia (PCP), and mycobacteriosis, in that order. BP is currently the most common admission diagnosis in this population (2, 26). Pulmonary infections usually appear as pulmonary infiltrates (PIs) on chest radiographs and are frequently (but not always) associated with respiratory symptoms.

In recent years, the study of the host inflammatory response to infections has received considerable interest. One component of the host response to infection is the release of cytokines, intercellular signaling polypeptides produced by activated cells (13). A complex network of cytokines controls the generation and maintenance of innate and specific immunological responses (9). The cytokines that are produced during and participate in inflammatory processes are the chief stimulators of the production of acute-phase proteins, such as C-reactive protein (CRP). In pulmonary infections, the release of cytokines and other inflammatory mediators serves as a useful mechanism for the elimination of invading pathogens. However, excessive release can be harmful to the host, leading to respiratory distress syndrome, shock, multiorgan failure, or death. Thus, it has been suggested that the quantitation of the inflammatory response may have prognostic implications (22). The knowledge thus obtained may eventually lead to the development of new strategies for therapy of infectious diseases. Additionally, reports of different patterns of cytokine responses in different diseases raise the possibility that cytokine determinations may have diagnostic value (13).

The aims of this study were to evaluate the role of plasma proinflammatory cytokines (interleukin 1β [IL-1β], IL-6, IL-8, and tumor necrosis factor alpha [TNF-α]), anti-inflammatory cytokines (IL-10), and CRP in the diagnosis and outcome of HIV-1-infected patients with pulmonary infections with different etiologies.

(Presented in part at the XIV International AIDS Conference, Barcelona, Spain, 7 to 12 July 2002 [N. Benito, A. Moreno, X. Filella, J. M. Miró, J. González, T. Pumarola, M. E. Valls, M. Luna, A. Rañó, A. Torres, and J. M. Gatell, Abstr. XIV Int. AIDS Conf., abstr. WePeA5800, 2002.])

MATERIALS AND METHODS

A prospective study of consecutive hospital admissions of HIV-infected patients was carried out at Hospital Clinic Universitari, a 700-bed tertiary-level hospital in Barcelona, Spain.

From April 1998 to March 2000, all HIV-infected patients with new-onset radiologic PIs on admission or with PIs that developed during hospitalization were included. Patients were followed up until radiologic resolution of the infiltrates or death secondary to the pulmonary event.

The following variables were recorded: demographic features; HIV risk factors; antiretroviral therapy; CD4 lymphocyte count per cubic millimeter and

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TABLE 1. Demographic features, HIV infection risk factors, immunologic status (CD4 lymphocyte count), and VL of HIV patients with PIs

Parameter	Value
No. of episodes/patients	249/220
No. of male patients	160 (73%)
Mean age (SD, range) (yr)	39 (10, 22–77)
No. with HIV risk factor	
Injecting drug user	118 (54%)
Heterosexual	45 (20%)
Homosexual	44 (20%)
Transfusion or unknown	13 (6%)
No. with HIV diagnosis during pulmonary event	21 (10%)
Median CD4 cell count per cubic millimeter (interquartile range)	139 (273)
No. of episodes in patients with CD4 count of <200 cells/mm^3	103 (41%)
Median VL of HIV (interquartile range)	66,921 (331,919.75)
No. of episodes in patients with VL of <200 copies	36 (15%)
No. of episodes in patients receiving highly active antirretroviral therapy	109 (44%)

HIV-1 plasma viral load (VL) before the pulmonary event; clinical, analytic and radiologic data at admission; microbiologic results; final etiologic diagnosis; antimicrobial treatment; and outcome.

All patients were included in a protocol that addressed studying the etiology and outcome of the pulmonary complication, as previously described (5). Definitions of definitive etiologic diagnosis, probable etiologic diagnosis, BP, and undiagnosed PI have also been previously reported (5).

In all these episodes, plasma CRP, IL-1β, IL-6, IL-8, IL-10, and TNF-α levels were determined at admission and 4 to 6 days later.

Plasma CRP levels were estimated by means of a nephelometric method (Dade Behring Inc., Newark, Del.). This assay uses monoclonal anti-CRP antibodies. Polystyrene particles are coated with the specific antibodies, which form a complex with CRP present in the measured study sample. The amount of scattered light is directly proportional to the size of the antigen-antibody complex and reflects the CRP concentration present in the study sample. The calibration range was 0.35 to 22 mg/dl. The lower limit of detection was 0.0175 mg/dl.

For assays to determine plasma IL-1β, IL-6, and TNF-α concentrations (Biosource Europe, Nivelles, Belgium), as well as IL-8 and IL-10 concentrations (Assay Designs, Ann Arbor, Mich.), blood samples were collected in sterile tubes not containing anticoagulants. The samples were centrifuged at 3,000 rpm for 10 min, and the plasma was stored at −70°C until it was processed. The Biosource tests are solid-phase enzyme-amplified sensitivity immunoassays performed on microtiter plates. These assays are based on an oligoclonal system in which mixtures of monoclonal antibodies directed against distinct epitopes of the corresponding cytokine are used. Standards used in the calibration curve and samples containing the cytokine react with capture monoclonal antibodies with which the microtiter well is coated. After incubation, excess antigen is removed by washing. A second monoclonal antibody labeled with horseradish peroxidase is then added. After incubation, the microtiter plate is washed and bound enzyme-labeled antibodies are measured through a chromogenic reaction. The reaction is stopped with the addition of H_2SO_4 as a stop solution. The Assay Designs tests use a monoclonal antibody directed against IL-8 or IL-10, with which the wells of a microtiter plate are precoated. IL-8 or IL-10 present in the standards or in the samples binds to the antibody on the coated well. After the cytokine is bound to the immobilized antibody, a second monoclonal antibody labeled with streptavidin is added to the wells and allowed to bind to a different epitope on the same cytokine. After the plates are washed, tetramethylbenzidine is added. A stop solution ends the reaction, and the absorbance is measured. In both cases, colorimetric determination was done by means of a polychromic reader (Vmax EASIA reader; Medgenix Diagnostics). Concentrations of cytokines from samples were determined by comparing the optical densities of the samples to the standard curves. The results are expressed in picograms per milliliter of serum. The sensitivity of the technique allows the detection of levels as low as 2 pg/ml for IL-1β and IL-6, 3 pg/ml for TNF-α and IL-10, and 8 pg/ml for IL-8.

All statistics were calculated with the SPSS statistical package (version 9.0; SPSS, Inc., Chicago, Ill.). Continuous variables are summarized as means (with standard deviation) or medians (with interquartile range), depending on their homogeneity. Quantitative variables were compared by the median nonparametric test (14). Graphic comparisons of quantitative data from two or more groups are shown by viewing box plots from the groups side by side. In the basic box-and-whisker plot, the central box represents the values from the 25th to the 75th percentile and therefore contains the middle half of the scores in the distribution. The numerical difference between the third quartile (75th percentile) and first quartile (25th percentile) is the interquartile range, a measure of the spread of the data. The middle line represents the median. Lines extend from the central box to the minimum and the maximum values, excluding outlier values, which are displayed as separate points: an outside value is defined as a value that is smaller than the first quartile minus 1.5 times the interquartile range or larger than the third quartile plus 1.5 times the interquartile range (inner fences). A far-out value is defined as a value that is smaller than the first quartile minus three times the interquartile range or larger than the third quartile plus three times the interquartile range (outer fences). (29). The cutoff of the studied CRP and cytokines that maximized the ability to make a correct diagnosis was established by means of receiver operating characteristic curves. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of this cutoff were calculated with the prevalence estimated in the sample (12). Linear correlations between quantitative variables were analyzed with the Spearman test. A multiple-regression analysis model was used to evaluate factors independently associated with quantitative variables. Analysis of factors independently associated with mortality was performed with logistic regression. The results are given as an odds ratio (OR). The confidence interval (CI) was established at 95%. A probability value of <0.05 was considered to be significant.

RESULTS

There were 1,016 consecutive hospital admissions of adults with HIV infection during the study period. Two hundred forty-nine episodes of PIs (25%) in 220 patients were diagnosed. Demographic features, HIV infection risk factors, immunologic status (CD4 lymphocyte counts), and virologic status are shown in Table 1. The three main diagnostic groups were pyogenic BP (118 episodes), PCP (41 episodes), and mycobacteriosis (36 cases) (Table 2).

The median levels of CRP and the studied cytokines for BP, PCP, and mycobacteriosis at admission are shown in Table 3. The median levels of plasma CRP and IL-8 at admission were significantly higher in BP (10.2 mg/dl and 19 pg/ml, respectively) than in mycobacteriosis (5.1 mg/dl and 0) and PCP (3.8 mg/dl and 3 pg/ml, respectively). The median level of plasma TNF-α in mycobacteriosis (75 pg/ml) was statistically higher than in the other two diagnostic groups (46.5 pg/ml in BP and 44 pg/ml in PCP). These differences in the medians of plasma levels of CRP and the studied cytokines at admission among the diagnostic groups remained when patients were classified according to the CD4 lymphocyte count (higher or lower than 200/mm^3) (Fig. 1). No other statistically significant differences were found in the studied cytokines among the three diagnostic groups at admission. There was no association between etiologies and plasma levels of cytokines and CRP determined on the fourth to sixth days after admission.

TABLE 2. Diagnosis of 249 episodes of pulmonary infiltrates in HIV-infected patients

Etiology	No. of episodes (% of total episodes)
Infectious etiology	199 (79.9)
Bacteria	118 (47.4)
Streptococcus pneumoniae	55
Haemophylus influenzae	8
Staphylococcus aureus	6
Legionella pneumophila	5
Escherichia coli	2
Acinetobacter baumanii	1
Without identification of etiology	41
Pneumocystis carinii	41 (16.5)
Mycobacteriosis	36 (14.5)
Mycobacterium tuberculosis	29
Mycobacterium kansasii	3
Mycobacterium avium complex	2
Mycobacterium fortuitum	1
Mycobacterium xenopi	1
Virus	9 (3.6)
Cytomegalovirus	4
Influenza A virus	2
Parainfluenza 2 virus	1
Enterovirus	1
Respiratory syncytial virus	1
Fungus	4 (1.6)
Cryptococcus	2
Aspergillus fumigatus	2
Parasite	1 (0.4)
Strongyloides stercolaris	1
Multiple organisms	14 (5.6)
Other	4
Bronchiectasies	3
Pulmonary abscess	1
Noninfectious etiology	6 (2.4)
Left heart failure	3
Carcinoma	3
Small-cell lung carcinoma	2
Other	1
Without diagnosis	44 (17.7)

The sensitivity, specificity, PPV, and NPV with a CRP level of ≥10 mg/dl and an IL-8 level of ≥20 pg/ml for the diagnosis of BP versus PCP and mycobacteriosis were 69, 83, 71, and 82%, respectively. A value for TNF-α of ≥60 pg/ml had a sensitivity, specificity, PPV, and NVP of 85, 70, 31, and 97%, respectively, for the mycobacteriosis diagnosis versus the other two diagnoses.

Linear correlations between CRP and the studied cytokines and the CD4 lymphocyte count, as well as between CRP and the studied cytokines and the HIV plasma VL were analyzed in order to evaluate the possible association among immunologic and virologic statuses of HIV patients and plasma levels of CRP-cytokines (Table 4). A positive linear correlation was found between plasma CRP and the CD4 lymphocyte count per cubic millimeter ($P = 0.007$), and a negative correlation was found between CRP and the HIV-1 plasma VL ($P < 0.001$). However, these associations were no longer significant when a current diagnosis of BP was also included in a multiple-regression analysis model. This can be explained by the fact that the CD4 lymphocyte median in the BP group (263/mm^3 [interquartile range, 320]) was significantly higher than the CD4 median in the patients with other diagnoses (46/mm^3) ($P < 0.001$). In fact, having BP was the only independent factor significantly associated with the levels of CRP ($P = 0.001$). Similarly, the HIV-1 plasma $\log_{10}$ VL was lower in the BP group (3.7 versus 5.3) ($P < 0.001$). A positive correlation was found between the HIV-1 VL and IL-10 ($P = 0.031$) and TNF-α ($P = 0.001$). There was a negative correlation between the CD4 lymphocyte count and TNF-α ($P = 0.033$). These associations remained significant when a current diagnosis of BP, a current diagnosis of mycobacteriosis, and a current diagnosis of PCP were also included in a multiple-regression analysis model. Mycobacteriosis did not seem to be a confounding factor in the correlation between TNF-α and the CD4 lymphocyte count, since the median CD4 lymphocyte count in mycobacteriosis cases (164 cells/mm^3 [interquartile range, 207]) was no different than that in the rest of the cases (156 cells/mm^3 [interquartile range, 287]) ($P = 0.780$). No others significant correlations were found between the studied cytokines and these variables showing the immunologic and virologic status of patients.

Twenty-three (10%) patients died: seven PCP patients, six patients without etiologic diagnoses, four with BP, one with mycobacteriosis, two with cytomegalovirus pneumonia, two patients with *Aspergillus fumigatus* infection, and one patient with

TABLE 3. Median levels and interquartile ranges at admission of CRP and cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) in main diagnostic groups

Parameter[a]	Value for diagnostic group[c]			P^b
	PCP ($n = 41$)	Mycobacteriosis ($n = 36$)	BP ($n = 118$)	
CRP (NV<0.8)	**3.8** (8.6)	**5.1** (6.9)	**10.2** (11.7)	<0.001
IL-1β (NV<15)	8 (31.5)	7.5 (25)	3 (11.3)	NS
IL-6 (NV<5)	29.5 (90)	35 (124)	45 (75.3)	NS
IL-8 (NV<10)	**3** (24.5)	**0** (8)	**19** (38)	0.012
IL-10 (NV<10)	8 (22)	5 (55.5)	6 (8.8)	NS
TNF-α (NV<20)	**44** (36.8)	**75** (29)	**46.5** (30.5)	0.016
CD4 lymphocyte count per mm^3	23 (43)	164 (207)	263 (320)	<0.001
HIV-1 plasma $\log_{10}$ VL	5.62 (0.76)	4.82 (1.68)	3.67 (2.56)	<0.001

[a] Median level of CRP (milligrams per deciliter) and cytokines (picograms per milliliter) (interquartile range) at admission, CD4 count, and HIV plasma VL. Differences among medians have been assessed using the nonparametric test of medians (7). NV, normal value.
[b] NS, not significant.
[c] Boldface type indicates differences that are statistically significant ($P < 0.05$).

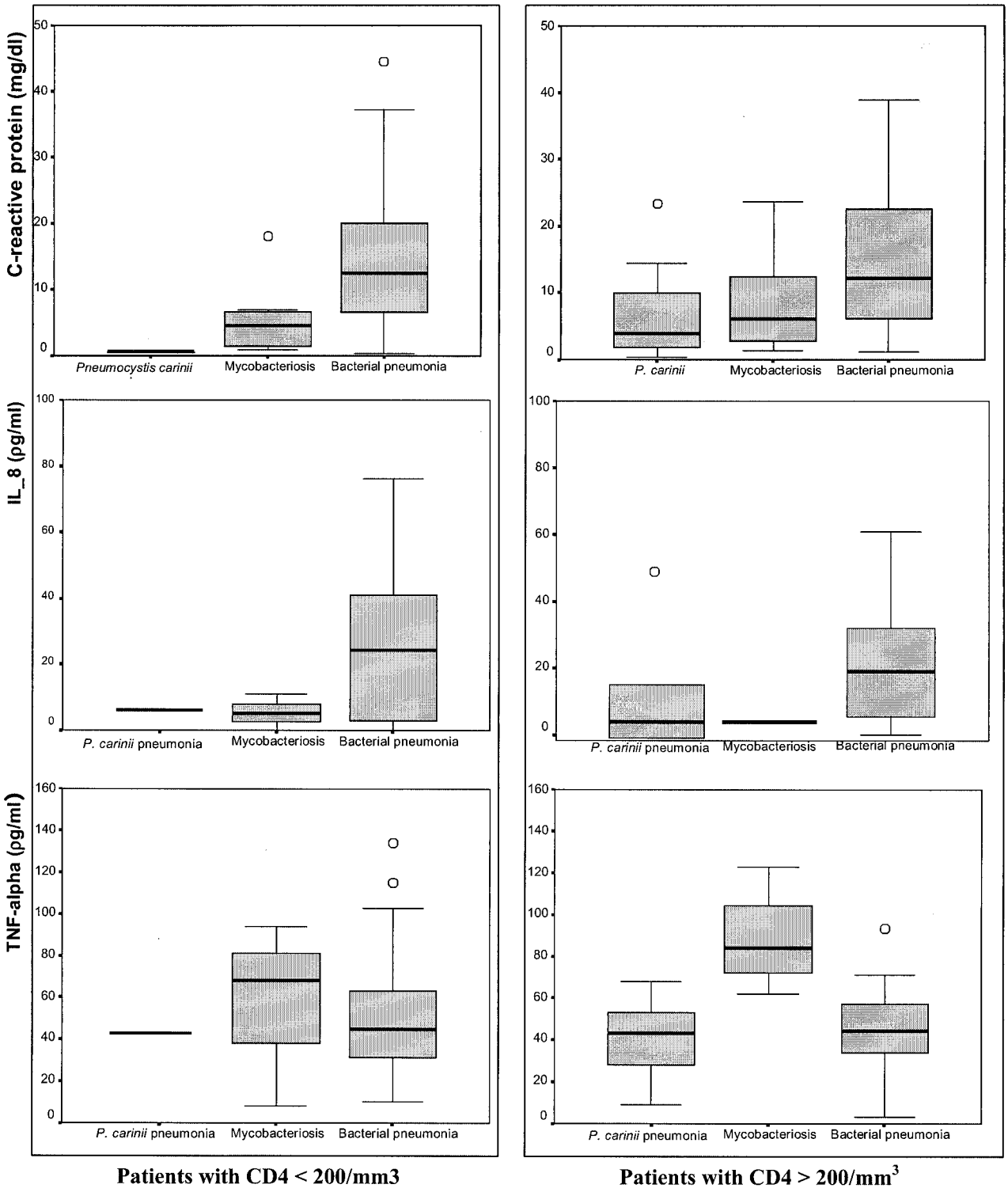

FIG. 1. Distribution of plasma CRP, IL-8, and IL-10 levels at admission in the main diagnostic groups (BP, PCP, and mycobacteriosis) in patients classified according to a CD4 lymphocyte count higher or lower than 200/mm^3. In the basic box-and-whisker plot, the central box represents the values from the 25th to the 75th percentile and therefore contains the middle half of the scores in the distribution. The numerical difference between the third quartile (75th percentile) and first quartile (25th percentile) is the interquartile range, a measure of the spread of the data. The middle line represents the median. Lines extend from the central box to the minimum and the maximum values, excluding outlier values, which are displayed as separate points: an outside value is defined as a value that is smaller than the first quartile minus 1.5 times the interquartile range or larger than the third quartile plus 1.5 times the interquartile range (inner fences). These values are plotted with a round marker. A far-out value is defined as a value that is smaller than the first quartile minus three times the interquartile range or larger than the third quartile plus three times the interquartile range (outer fences).

TABLE 4. Linear correlations between CRP and studied cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) and variables showing immunologic and virologic status of patients (CD4 lymphocyte count and HIV plasma VL)

CRP or cytokine	Linear correlation[a]		Confounding factors
	CD4 lymphocyte counts	HIV plasma VL	
CRP	Positive	Negative	BP
IL-10		Positive	None
TNF-α	Negative	Positive	None

[a] Only statistically significant linear correlations found between CRP and studied cytokines and variables showing the immunologic and virologic status of patients (CD4 lymphocyte count and HIV plasma VL) are shown.

Strongyloides stercoralis infection. Admission to the intensive care unit and mechanical ventilation were required in 5 out of 118 (4%) episodes and in 4 cases (3%) of BP, with 3% mortality. The levels of CRP and the studied cytokines at admission in patients who died and survivors are shown in Table 5. In the univariate analysis, elevated CRP and IL-6 values were associated with higher mortality, and there was an almost-significant association between higher plasma TNF-α levels and mortality. In a multivariate analysis, when CRP and all the studied cytokines were included—categorized as having a value higher or lower than the median value in the group of patients that died—an IL-8 level of >61 pg/ml (OR = 27; 95% CI, 2 to 384.8) and an IL-10 level of >22 pg/ml (OR = 11; 95% CI, 1.2 to 176.5) were the variables significantly associated with higher mortality in HIV-1-infected patients with PIs. When the CD4 lymphocyte count, the HIV-1 VL, and the diagnostic group were included in the model as potential confounding variables, an IL-8 level of >61 (OR = 12; 95% CI, 1.2 to 235.5) was the only variable independently associated with higher mortality. There was no association between mortality and plasma cytokines and CRP levels determined on the fourth to sixth days after admission.

DISCUSSION

The most significant findings of our study can be summarized as follows: (i) HIV patients with BP have higher plasma CRP and IL-8 levels at admission than patients with PCP and mycobacteriosis; (ii) the plasma TNF-α value is higher in HIV patients with mycobacteriosis than in HIV patients with BP or PCP; (iii) plasma IL-8 is an independent factor associated with mortality in HIV-infected patients with pulmonary infections.

The local and systemic inflammatory response in BP, with special emphasis on the patterns of cytokines and levels of CRP, has been studied in recent years in the immunocompetent population (20, 22, 23, 27). An increase in proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, as well as acute-phase proteins, such as CRP, has been described in BP (20, 27). IL-8 seems to be another inflammation-associated cytokine. It is a chemokine with neutrophil-activating capacity that has been shown to be increased in the pneumonic lung but not in the nonpneumonic lung (1, 9, 11, 24). IL-10 is an anti-inflammatory cytokine that inhibits the production of proinflammatory cytokines by monocytes and macrophages (9). In recent studies, a relationship between IL-10 levels and the severity of community-acquired pneumonia has been demonstrated (15). The inflammatory response in BP has not been studied in HIV-infected patients.

In our study, HIV patients with BP had elevated levels of the proinflammatory cytokines IL-6, TNF-α, and IL-8 in plasma. Additionally, the plasma CRP level was elevated. Overall, these results are similar to those found in the general population with BP, although we found neither high serum IL-1β levels nor high levels of the anti-inflammatory cytokine IL-10. Interestingly, although IL-8 in BP is usually compartmentalized in the involved lung and rarely spills over into the serum (6), in our study there were detectable plasma IL-8 levels in 75% of HIV-infected patients with BP. In a previous study of BP in the general population, serum IL-8 was detectable in only 25% of patients (6). In relation to the proinflammatory cytokines IL-1β, IL-6, and TNF-α, Dehoux et al. found that only levels of IL-6 were mildly increased in the sera of patients with BP in the general population, whereas the increased levels of the other cytokines were confined to the lungs alone (10). However, other studies have documented the presence of systemic inflammation in patients with more severe BP and found that serum IL-6 and TNF-α levels correlated with the severity of pneumonia and mortality (3, 22, 25, 27). Thus, in one study of consecutive patients with BP, serum IL-6 and TNF-α were

TABLE 5. Median levels (interquartile range) at admission of CRP and cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) in patients who died and survivors

Parameter[a]	Value for group[c]		P^b
	Survivors (n = 23)	Patients who died (n = 226)	
CRP (NV<0.8)	**6.8** (12.2)	**10.3** (12.3)	0.028
IL-1β (NV<15)	3 (14)	24.5 (56.8)	0.423 (NS)
IL-6 (NV<5)	**36** (67)	**95** (686)	0.016
IL-8 (NV<10)	8.5 (27.8)	61 (220.5)	0.620 (NS)
IL-10 (NV<10)	6 (8.25)	22 (197.5)	0.103 (NS)
TNF-α (NV<20)	**46** (35)	**70** (66)	0.068 (NS)
CD4 lymphocyte count per mm^3	180 (284)	20.5 (44.5)	0.004
HIV-1 plasma VL $\log_{10}$	4.68 (2.94)	5.22 (0.9)	<0.001

[a] Median levels of CRP (milligrams per deciliter) and cytokines (picograms per milliliter) (interquartile range) at admission, CD4 count, and HIV VL. Differences among medians have been assessed using the nonparametric test of medians (7). NV, normal value.
[b] NS, not significant.
[c] Boldface type indicates differences that are statistically significant ($P < 0.05$).

present only in 23 and 41% of patients, respectively, whereas in another study of patients with severe pneumonia requiring mechanical ventilation, IL-6 and TNF-α were detected in all patients (3, 23). In the present study, a cytokine pattern similar to that found in patients with severe pneumonia was observed in HIV-infected patients with BP: 99 and 100% of patients had detectable plasma IL-6 and TNF-α levels, respectively, However, our patients with BP did not show clinical criteria of severity: only 5 out of 118 (4%) required admission to the intensive care unit, and 4 (3%) required mechanical ventilation, with 3% mortality. In any case, the serum cytokine and CRP profile found in HIV patients with BP versus the other main diagnostic groups—PCP and mycobacteriosis—is quite characteristic and allows us to distinguish BP from the other processes with good sensitivity, specificity, and predictive values.

In this study, the plasma TNF-α levels were elevated in patients with BP, mycobacteriosis, and PCP. However, they were significantly higher in mycobacteriosis than in the other diagnostic groups (Table 3). Indeed, a TNF-α value of ≥60 pg/ml had a very high NPV for the diagnosis of mycobacteriosis (97%). It is known that TNF-α contributes to the host defense mechanisms in mycobacterial infection (8, 28, 30). Studies of mice have demonstrated that TNF-α is important in granuloma formation and in controlling the extent of mycobacteriosis (8, 28). In humans, the use of anti-TNF-α monoclonal antibodies, such as infliximab, has been associated with increased rates of tuberculosis reactivation (19). In immunocompetent tuberculosis patients, TNF-α production is present at the site of disease but is seldom found in the circulation (18, 30). Nevertheless, recent reports have indicated that the plasma TNF-α levels may be correlated with tuberculosis severity and activity (4). It has also been demonstrated that *Mycobacterium tuberculosis* phagocytosis induces greater TNF-α production in HIV-infected macrophages than in uninfected cells (17). In patients with HIV infection and active tuberculosis, serial measurement of plasma TNF-α levels correlated with the response to antituberculosis treatment (16). However, a possible role of plasma TNF-α levels in the diagnosis of mycobacteriosis in HIV-infected patients has not been reported. The high incidence of mycobacteriosis and the frequent association of mycobacteriosis with other pulmonary infections in HIV patients, as well as the epidemiological relevance of this diagnosis, support the importance of excluding mycobacteriosis at admission (5). However, direct microbiologic diagnosis is not always possible at admission and acid-fast smears in sputum are not always positive in mycobacteriosis patients. Since TNF-α has a very high NPV, it could be used as a tool to exclude the mycobacteriosis diagnosis in these patients.

Could some of the differences in cytokine levels observed in this study be due to different stages of HIV disease itself and not necessarily all be due to BP or mycobacterial infections? Immune dysregulation increases with more advanced HIV infection, even in the absence of opportunistic infections, and is associated with increased levels of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α. Additionally, decreased production of important Th1 inmunoregulatory cytokines (such as IL-2 and gamma interferon, which are critical for cell-mediated immune responses) and increased production of the Th2 cytokines, such as IL-10, which are important in humoral immunity, are observed in more advanced HIV infection (7, 21). However, we were able to demonstrate that a high level of plasma CRP at admission is associated with BP, independent of the immunologic and virologic status of HIV-infected patients. We observed that plasma levels of IL-10 and TNF-α have a linear correlation with CD4 lymphocyte counts, and therefore, confirmed that they are correlated with more advanced HIV infection. Nevertheless, TNF-α is also independently associated with mycobacterial infections, since there are no differences in CD4 lymphocyte counts between patients with and without mycobacterial infections.

There were not enough death events in each diagnostic group to analyze prognostic factors in each of them. In the overall group of HIV-infected patients with PIs, elevated plasma IL-8 levels (higher than 61 pg/ml) were independently associated with higher mortality. Although etiological diagnosis does not seem to be a factor related to this association, this cannot be asserted absolutely. In patients who died, all the studied serum cytokines and CRP were higher than in survivors (Table 5). Because of the profound cytokine dysregulation associated with HIV infection, some of these findings could actually be due to an advanced HIV status. However, when virologic and inmunologic statuses were also considered, IL-8 was independently related to higher mortality. This finding underlines the importance of the systemic inflammatory response of the host in the prognosis of pulmonary infections in this group of patients.

In conclusion, differences in the pattern of systemic inflammatory responses found in HIV-infected patients with PIs could be very useful noninvasive and early tools in the initial diagnosis of BP and mycobacteriosis. Thus, a CRP value of ≥10 mg/dl plus an IL-8 value of ≥20 pg/ml would allow the establishment of a diagnosis of BP versus PCP or mycobacteriosis with a PPV and NPV of 71 and 82%, respectively. Moreover, a TNF-α value of ≥60 pg/ml could be used for early exclusion of the mycobacteriosis diagnosis in these patients based on its high NVP (97%). Direct microbiologic diagnosis is not always possible at admission, and acid-fast smears in sputum are not always positive in patients with mycobacteriosis, which underlines the importance of having other quick and reliable diagnostic tools. The high incidence of mycobacteriosis and the frequent association of mycobacteriosis with other pulmonary infections in HIV patients, as well as the epidemiologic relevance of this diagnosis, support the importance of excluding mycobacteriosis at admission (5). More prospective studies are needed to evaluate the role of IL-8 in the outcome of each diagnostic group and to establish if the plasma IL-8 level could have value in the decision about initial treatment of PIs in HIV-infected patients.

ACKNOWLEDGMENTS

During the study period, N. Benito was the recipient of a research grant from the Fundación Privada Máximo Soriano Jiménez. J.M.M. was the recipient of a research grant from the Institut d'Investigacions Biomediques August Pi i Sunyer, Barcelona, Spain.

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ARTÍCULOS COMPLEMENTARIOS

Eur Respir J 2001; 17: 87–93
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European Respiratory Journal
ISSN 0903-1936

Reappraisal of the aetiology and prognostic factors of severe acute respiratory failure in HIV patients

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Reappraisal of the aetiology and prognostic factors of severe acute respiratory failure in HIV patients. C. Alves, J.M. Nicolás, J.M. Miró, A. Torres, C. Agustì, J. Gonzalez, A. Rañó, N. Benito, A. Moreno, F. Garcìa, J. Millá, J.M. Gatell. ©ERS Journals Ltd 2001.

ABSTRACT: The introduction of highly active antiretroviral therapy with protease inhibitors in 1996 has changed the morbidity and mortality of acquired immune deficiency syndrome patients. Therefore, the aetiologies and prognostic factors of human immunodeficiency virus (HIV)-infected patients with life-threatening respiratory failure requiring intensive care unit (ICU) admission need to be reassessed.

From 1993 to 1998, we prospectively evaluated 57 HIV patients (mean±SEM age 36.5±1.3 yrs) admitted to the ICU showing pulmonary infiltrates and acute respiratory failure.

A total of 21 and 30 patients were diagnosed as having *Pneumocystis carinii* and bacterial pneumonia, respectively, of whom 13 and eight died during their ICU stay (p=0.01). Both groups of patients had similar age, Acute Physiology and Chronic Health Evaluation (APACHE) II score, and severity in respiratory failure. The number of cases with bacterial pneumonia admitted to ICU decreased after 1996 (p=0.05). Logistic regression analysis showed that (APACHE) II score >17, serum albumin level <25 g·L^{-1}, and diagnosis of *P. carinii* pneumonia were the only factors at entry associated with ICU mortality (p=0.02).

Patients with bacterial pneumonia are less frequently admitted to the intensive care unit after the introduction of highly active antiretroviral therapy with protease inhibitors in 1996. Compared to the previous series, it was observed that the few *Pneumocystis carinii* pneumonia patients that need intensive care still have a bad prognosis.
Eur Respir J 2001; 17: 87–93.

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Keywords: Bacterial pneumonia, HIV, *Pneumocystis carinii*, prognosis, respiratory failure

Received: March 16 2000
Accepted after revision August 2 2000

This work was supported by grants from SEIMC/API and Fundaciûn Maximo Soriano Gimènez.

Patients with human immunodeficiency virus (HIV) infection, and especially acquired immunodeficiency syndrome (AIDS) patients, may suffer from episodes of pulmonary infiltrates accompanied by severe respiratory failure during the course of their illness [1]. In the previous decade, *Pneumocystis carinii* pneumonia was the main cause of severe respiratory failure in HIV patients admitted to the Hospital clinic of Barcelona Medical and Respiratory intensive care units (ICU), and was associated with a poor outcome when mechanical ventilation was required [2]. The introduction of *P. carinii* prophylaxis in the clinical practice in the late 1980s, usually with cotrimoxazole, was the first step in reducing the incidence of *P. carinii* pneumonia, as well as other bacterial infections of the upper respiratory tract [3]. An earlier treatment against *P. carinii* and the systematic use of steroids as co-adjuvant therapy has also been responsible for a decrease in severe episodes of *P. carinii* pneumonia [4, 5]. However, a manifest reduction in the morbidity and mortality of AIDS patients was not observed until the introduction of highly active antiretroviral therapy (HAART) with protease inhibitors in 1996 [6]. The immunological reconstitution, increasing the numerical and functional CD4 cell profile, produced by HAART, confers host protection against opportunistic infections [7], leading to a potential decrease in *P. carinii* as well as bacterial pneumonia [8, 9]. In some cases, this may result in a discontinuation in primary and secondary prophylaxis against *P. carinii* and other opportunistic infections [10].

The aim of the present study was therefore to reassess the aetiologies and prognostic factors of life-threatening respiratory failure in HIV patients requiring ICU admission. Patients admitted to the ICU from 1993–1998 were included in the study, taking into account the systematic use of *P. carinii* prophylaxis since the late 1980s and the introduction of HAART in 1996. Moreover, we have compared the current findings with those observed from 1985–1992 [2].

Patients and methods

Patients

All HIV patients showing pulmonary infiltrates and acute respiratory failure (arterial oxygen tension (P_{a,O_2})

<7 kPa breathing room air or P_{a,O_2}/inspiratory oxygen fraction (F_{I,O_2}) ratio <33 kPa) admitted to the Medical and Respiratory ICUs of the Hospital Clìnic of Barcelona from 1993–1998 were included. Sixty patients fulfilled inclusion criteria, although three subjects admitted for postoperative recovery were excluded from the study. Twenty-eight patients came directly from the Emergency Room, whereas twenty-nine patients had first been admitted to the Infectious Disease Ward with respiratory symptoms, and were thereafter transferred to the ICU because of impending respiratory failure. Patients were managed in the ICU by continuous positive airway pressure, Venturi masks, or masks with a reservoir, before invasive mechanical ventilation was applied.

Out of 57 patients, 47 (82%) fulfilled criteria for AIDS [11]. HIV infection was diagnosed in eight patients at the time of hospital admission. Nineteen out of 47 eligible subjects were receiving primary prophylaxis against *P. carinii* with cotrimoxazole. Twenty-five patients were on antiretroviral therapy before admission, eight of whom were on triple therapy with two nucleoside analogue transcriptase inverse inhibitors plus one protease inhibitor (HAART). Protease inhibitors were introduced in Spain in 1996, and as part of routine treatment in all HIV patients controlled by the HIV-outpatient clinic after February 1996. Antiretroviral therapy was discontinued while the patients remained in the ICU.

Hospital therapy for *P. carinii* included *i.v.* cotrimoxazole, with the maximum dosage for trimethoprim being 20 mg·kg^{-1} *q.d.*, and for sulphamethoxazole 100 mg·kg^{-1} divided in four doses; or *i.v.* pentamidine (4 mg·kg^{-1} once daily) according to the decision of the attending physician. Corticosteroids were routinely administered either on suspicion or diagnosis of *P. carinii* pneumonia in 48 patients at a dosage of methylprednisolone at 1 mg·kg^{-1} *q.d.* for 5 days, with the dosage later being gradually reduced. Antibiotic therapy against community-acquired bacterial pneumonia included a third generation cephalosporin (ceftriaxone or cefotaxime) plus a macrolide. The duration and type of antibiotic therapy against tuberculosis and other pulmonary pathogens was determined according to the recommended regimen [12].

Clinical and laboratory assessment

Epidemiological data and type of antiretroviral therapy were recorded. Chest radiograph examination and ventilatory parameters were recorded on hospital (retrospective) and ICU entry, and after the institution of mechanical ventilation. The parameters included pH, P_{a,O_2}, P_{a,CO_2}, $P_{a,O_2}/F_{I,O_2}$ ratio, and alveolar to arterial difference in oxygen tension ($P_{A\text{-}a,O_2}$). The presence of acute respiratory distress syndrome (ARDS) was considered according to the European Consensus score [13], these patients showing a Murrays score of $\geqslant 2.5$ [14].

Leukocyte and lymphocyte counts, recent CD4 count (from 40 routinely followed patients), serum creatinine, total cholesterol, total proteins, albumin and serum lactate dehydrogenase (LDH) were recorded upon ICU admission. The Acute Physiology and Chronic Health Evaluation score (APACHE II) [15] was calculated on hospital and ICU admission. The presence of systemic inflammatory response syndrome [16], and the sepsis-organ failure assessment [17] and multiple organ dysfunction syndrome scores [18] were recorded upon ICU admission. Bacterial pneumonia was classified as community-acquired or nosocomial according to the American Thoracic Society criteria [19].

Microbiological methods

Bronchoalveolar lavage (BAL) together with protected specimen brush (PSB) samples (BFW 1.0/70/90; Meditech Inc., Water-Town, MA, USA) were retrieved by means of a fibreoptic bronchoscope (Olympus BFT3R, New Hyde Park, NY, USA) exclusively used for HIV-infected patients. As part of a routine medical assessment, BAL with PSB was systematically performed in patients with interstitial pulmonary infiltrates and in those mechanically ventilated who did not promptly respond to antibiotic therapy (80%). Eleven patients with lobar infiltrates or not requiring mechanical ventilation, four of whom were finally diagnosed with pulmonary tuberculosis based on the presence of *Mycobacterium tuberculosis* in the sputum, did not undergo bronchoscopic microbiological studies.

BAL and PSB microbiological studies and sample processing were performed using standard methods, and thresholds for quantitative cultures of BAL 10^4 colony forming units (cfu)·mL^{-1} and PSB 10^3 cfu·mL^{-1} were applied, as described in detail previously [2]. In brief, serial dilutions of BAL and PSB samples were prepared in normal saline to obtain final concentrations of 10^{-1}, 10^{-2}, and 10^{-3}. Half of the specimen amount was inoculated into blood-agar, Wilkins-Chalgren, chocolate-agar, buffered charcoal yeast extract (BCYE)-α, and fungal media. The other half of the fluid obtained was centrifuged and the cell pellet was resuspended in phosphate-buffer solution. Smears were obtained by cytocentrifugation and were stained by the Papanicolau, Ziehl-Nielsen, Giemsa, periodic acid-Schiff (PAS), haematoxylin and eosin, Perls' and Grocott methenamine-silver methods. Staining for *Legionella pneumophila* was performed using the direct fluorescent antibody technique. Serological tests for respiratory virus (influenza, parainfluenza, adenovirus, respiratory syncytial virus), *Mycoplasma pneumoniae*, and *L. pneumophila* were also performed. The diagnosis of cytomegalovirus was considered only if cytopathic changes were found in BAL samples.

Statistical analysis

Standard statistical methods from the statistical package for the social sciences (SPSS) Statistical Analysis System V-9.0 (SPSS, Chicago, IL, USA) were used. Differences between groups were analysed using Chi-squared, Fisher's exact, Mann-Whitney U-, and two-tailed t-tests. In order to identify factors associated with risk of death in the different groups, univariate and forward-selection multivariate logistic

regression analysis were performed. Variables were categorized according to the population median and using enter criteria of p=0.05 and a removal criteria of p=0.10). Survival estimates of the different groups were compared by the Kaplan-Meier method (log-rank analysis) [20]. All variables are expressed as mean±SEM and significance was set at p=0.05.

Results

Clinical and microbiological data

The main characteristics of the population studied are summarized in table 1. After 1996, only eight (33%) of the patients were on HAART treatment, compared to about 80% of the HIV population controlled in the outpatient clinic. On ICU entry, chest radiograph disclosed bilateral interstitial pulmonary infiltrates in 35 patients, lobar consolidation in 16 subjects (half of them unilateral), and bilateral mixed infiltrates in the remaining six patients. On inclusion, 51 patients exhibited a $P_{a,O_2}/F_{I,O_2}$ ratio lower than 33 kPa and the remaining six subjects had $P_{a,O_2} < 7$ kPa breathing room air (table 1). The patients included from the Infectious Disease ward had been admitted to the ICU after a mean hospital stay of 10.5 days (range 2–32 days), and corresponded to a group of patients with a delayed diagnosis and/or poor response to conventional antimicrobial therapy. Obviously, these patients had significantly better pulmonary and illness scores on hospital admission (data not shown). Notwithstanding, when finally admitted to the ICU, patients from the Infectious Disease ward were in a poorer condition than subjects from the Emergency room (APACHE II score (17.9±0.8 *versus* 15.2±0.7, p=0.02); $P_{a,O_2}/F_{I,O_2}$ ratio (18.2±1.9 *versus* 26.1±3.2 kPa, p=0.04)).

The micro-organisms isolated and the diagnostic procedures used are shown in table 2. Twenty-one patients had *P. carinii* on the BAL, six with concurrent bacterial infections. These patients represented 6.8% of the 307 *P. carinii* pneumonia episodes diagnosed in our Hospital over the same period (1993–1998). As seen in figure 1, episodes of *P. carinii* pneumonia admitted to hospital markedly decreased after the introduction of HAART, although 2–5 cases of *P. carinii* pneumonia per year still required ICU admission. Most of these cases corresponded to subjects with undetected HIV infection, or who were not compliant with *P. carinii* prophylaxis and antiretroviral therapy. On the other hand, four patients were diagnosed with pulmonary tuberculosis and 30 with bacterial pneumonia, with microbiological confirmation in 40% of the latter (table 2). Bacterial pneumonia was considered community-acquired in 23 cases (77%) and nosocomial in 7 patients (23%). The remaining cases corresponded to one patient with endocarditis and pulmonary infiltrates and another subject with bronchopulmonary Kaposi's sarcoma.

The next step was to compare the characteristics between *P. carinii* pneumonia (all patients documented

Table 1. – Main epidemiological data on intensive care unit (ICU) admission of the population studied, and characteristics of patients with *Pneumocystis carinii* compared with those with bacterial pneumonia

	All patients n=57	*P. carinii* pneumonia n=21	Bacterial pneumonia n=30	Univariate p-value
Age yrs	36.5±1.3	39.6±2.3	34.8±1.6	0.10
Sex (M/F)	44/13	16/5	22/8	0.81
Admission (before/after 1996) n	33/24	10/11	21/9	0.05
AIDS definition (A3, B3, C3)	47 (82)	21 (100)	21 (70)	0.002
PCP prophylaxis* (n=47 eligible)	19 (39)	10 (47)	7 (23)	0.08
Prior antiretroviral treatment	25 (43)	10 (47)	13 (43)	0.76
HAART	8 (14)	4 (19)	3 (10)	0.42
Bilateral infiltrates	49 (85)	21 (100)	24 (80)	0.03
MV requirement	35 (61)	14 (66)	18 (60)	0.63
$P_{a,O_2}/F_{I,O_2}$ kPa	18.4±1.4	16.9±1.6	19.5±3.3	0.32
$P_{A\text{-}a,O_2}$ kPa	46.5±3.1	55.2±4.6	44.0±4.4	0.09
APACHE II score	16.6±0.6	16.3±1.1	16.7±0.8	0.74
SIRS	52 (91)	19 (90)	27 (90)	0.95
SOFA score	6.8±0.2	6.2±0.3	7.2±0.2	0.03
MODS score	5.5±0.2	5.0±0.2	5.9±0.1	0.05
Lymphocytes $10^9 \cdot L^{-1}$	1.01±0.09	0.78±0.13	1.0±0.28	0.47
CD4 lymphocytes $10^6 \cdot L^{-1\#}$ (n=40)	98±17	29±8	157±31	0.001
Serum LDH $IU \cdot L^{-1}$	970±91	1075±151	686±68	0.03
Serum LDH>1,000 $IU \cdot L^{-1}$	16 (28)	11 (52)	4 (13)	0.006
Cholesterol $mmol \cdot L^{-1}$	3.07±0.17	3.74±0.29	2.63±0.24	0.007
Serum albumin $g \cdot L^{-1}$	27.6±0.8	27.9±1.4	27.3±1.2	0.76

Data are expressed as mean±SEM or n (%), and refer to ICU admission unless indicated otherwise. p-values result from comparing the groups of *Pneumocystis carinii* and bacterial pneumonia (see *Methods*). HIV: human immunodeficiency virus; HAART: Highly active antiretroviral therapy; AIDS: Acquired immunodeficiency syndrome; PCP: *Pneumocystis carinii* pneumonia; MV: mechanical ventilation; P_{a,O_2}: arterial oxygen tension; F_{I,O_2}: inspiratory oxygen fraction; $P_{A\text{-}a,O_2}$: alveolar to arterial difference in oxygen tension; APACHE II: acute physiology and chronic health evaluation score; SIRS: systemic inflammatory response syndrome; SOFA: sepsis-related organ failure assessment score; MODS: multiple organ dysfunction syndrome; LDH: lactate dehydrogenase; *: n=47; #: n=40.

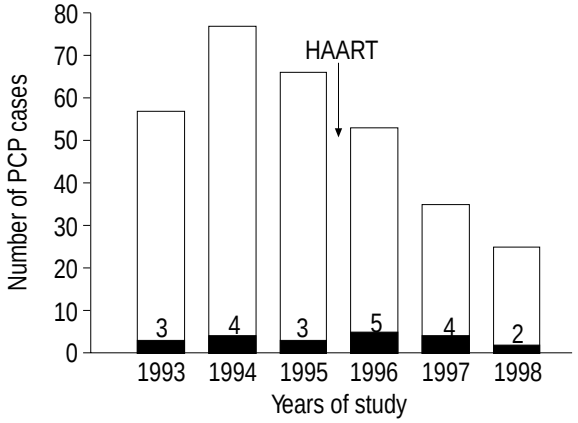

Fig. 1. – Episodes of *Pneumocystis carinii* pneumonia (PCP) admitted to hospital □ and the intensive care unit (ICU) ■. With the introduction of high antiretroviral therapy with protease inhibitors after 1996 there was a reduction in the amount of cases of *P. carinii* pneumonia. However, 2–5 patients *P. carinii* pneumonia per year still required ICU admission because of life-threatening respiratory failure.

microbiologically) and patients with bacterial pneumonia (pulmonary tuberculosis excluded), as shown in table 1. Age, APACHE II score, and severity of respiratory failure were similar in both groups of patients at ICU admission. Compared to subjects with bacterial pneumonia, patients with *P. carinii* pneumonia showed a worse immune status and had greater serum LDH concentrations than subjects with bacterial pneumonia. Curiously, similar to a previous series [2], serum cholesterol levels were significantly greater in patients with *P. carini* pneumonia. After 1996, a significant decrease in the number of bacterial pneumonia admitted to ICU was observed. No differences were observed in severity in the patients with bacterial pneumonia admitted before and after 1996.

Table 2. – Micro-organisms isolated from the patients

	Cases n	Diagnostic method
P. carinii along	15	BAL
P. carinii + *P. aeruginosa*	1	BAL
P. carinii + *Actinomyces Israelii*	1	BAL
P. carinii + CMV	1	BAL
P. carinii + *A. fumigatus* + CMV	1	BAL
P. carinii + *Enterobacter aerogenes*	1	BAL
P. carinii + *A. baumanii*	1	BAL
S. pneumoniae along	4	BAL+BC
S. pneumoniae + *E. faecalis*	1	BAL
S. pneumoniae + *H. influenzae*	1	BAL
H. influenzae + *Escherichia coli*	1	BAL
H. influenzae + *Varicella*	1	BAL
Coagulase negative staphylococci	1	BAL
P. aeruginosa + *S. maltophilia*	1	BAL
E. faecalis + *A. fumigatus*	1	BAL
E. faecalis + *Candida albicans*	1	BAL+BC (both)
Mycobacterium tuberculosis	4	Sputum
BAL and PSB negative	13	—
Unknown and BAL done	7	—

P. carinii: *Pneumocystis carinii*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *A. fumigatus*: *Aspergillus fumigatus*; *A. baumanii*: *Acinetobacter baumanii*; *S. pneumoniae*: *Streptococcus pneumoniae*; *E. faecalis*: *Enterococcus faecalis*; *H. influenzae*: *Haemophilus influenzae*; *S. maltophilia*: *Stenotrophomonas maltophilia*; BAL: broncoalveolar lavage. PSB: protected specimen brush. BC: blood cultures. CMV: cytomegalovirus.

Risk factors influencing intensive care unit outcome

Twenty-three (40%) HIV-infected patients died during their ICU stay, whereas 34 (60%) cases were discharged alive. Thirteen patients died from *P. carinii* pneumonia and eight patients who died had bacterial pneumonia at ICU admission. Univariate logistic regression analysis showed that patients with a low serum albumin levels and CD4 count, *P. carinii* pneumonia as the cause of respiratory failure, or a high APACHE II score on ICU entry, were at a higher risk for ICU mortality (p=0.05, table 3). Likewise, the need for mechanical ventilation, the development of ARDS, and the appearance of metabolic acidosis (arterial pH <7.35) during ICU stay were factors associated with death. In the multivariate forward-selection logistic analysis, an APACHE II score >17 (odds ratio (OR) 4.9 (1.2–19.9), p=0.02), a serum albumin $<25\ g{\cdot}L^{-1}$ (OR 6.0 (1.3–27.1), p=0.01), and *P. carinii* pneumonia diagnosis (OR 10.4 (2.1–50.1), p=0.003) remained as independent factors associated with death on ICU entry. Obviously, sicker patients required mechanical ventilation more frequently. Only one out of the 14 (7%) patients with *P. carinii* pneumonia receiving mechanical ventilation was weaned successfully, compared with ten of the 18 (55%) patients with bacterial pneumonia (p=0.007).

Table 3. – Factors influencing mortality in the intensive care unit (ICU)

Risk factor	Odds ratio (0.05–0.95 CI)	Univariate p-value
Age >33 yrs	2.69 (0.88–8.2)	0.08
Prior antiretroviral therapy	1.02 (0.35–2.98)	0.96
Prior HAART	2.25 (0.41–12.2)	0.34
AIDS diagnosis (A3, B3, C3)	2.72 (0.51–14.4)	0.24
PCP diagnosis	4.51 (1.40–14.4)	0.01
Corticosteroid therapy	0.81 (0.19–3.44)	0.78
MV requirement during ICU stay	19.2 (3.8–96.4)	0.0003
ARDS during ICU stay	14.0 (3.6–54.1)	0.001
$Pa,O_2/FI,O_2$ <19 kPa	2.56 (0.82–7.57)	0.09
Arterial pH <7.35 during ICU stay	3.62 (0.81–9.82)	0.05
APACHE II score >17	3.41 (1.13–10.42)	0.02
CD4 lymphocytes $<150{\cdot}10^6{\cdot}L^{-1}$	2.15 (0.12–4.22)	0.05
Serum LDH >1,000 $IU{\cdot}L^{-1}$	1.34 (0.45–3.99)	0.59
Serum albumin $<25\ g{\cdot}L^{-1}$	3.06 (0.96–9.5)	0.05

Data refer to ICU admission unless indicated otherwise. HAART: highly active antiretroviral therapy; AIDS: acquired immunodeficiency syndrome; PCP: *Pneumocystis carinii* pneumonia; MV: mechanical ventilation; ARDS: acute respiratory distress syndrome; Pa,O_2: arterial oxygen tension. FI,O_2: inspiratory oxygen fraction. APACHE II: acute physiology and chronic health evaluation score; LDH: lactate dehydrogenase.

Only eight out of 21 (38%) subjects with *P. carinii* pneumonia were discharged alive from ICU. Survivors had a lower APACHE II score (13.5±1.5 *versus* 18.0±1.2, p=0.04) and less severe respiratory failure ($P_{a,O_2}/F_{I,O_2}$ ratio: 23.7±3.8 *versus* 15.0±1.9 kPa, p=0.04) on ICU admission than nonsurvivors. Moreover, patients who died from *P carinii* pneumonia showed a worse nutritional status (p=0.03 for albumin) and tended to show greater serum cholesterol values (p=0.07) than survivors. In the multivariate stepwise regression analysis of these variables, the $P_{a,O_2}/F_{I,O_2}$ ratio on ICU admission was the only factor related to ICU mortality by *P. carinii* pneumonia (p=0.01). Bacterial co-infection, a very low CD4 count, and high serum LDH levels were not associated with increased mortality in these patients.

Regarding the patients bacterial pneumonia, those who died during their ICU stay (n=8) were older (39.7±3.1 *versus* 31.5±1.6 yrs, p=0.04), had a lower CD4 count (80±26 *versus* 235±52 $10^6 \cdot L^{-1}$, p=0.01) and developed ARDS more frequently (p=0.02) than survivors.

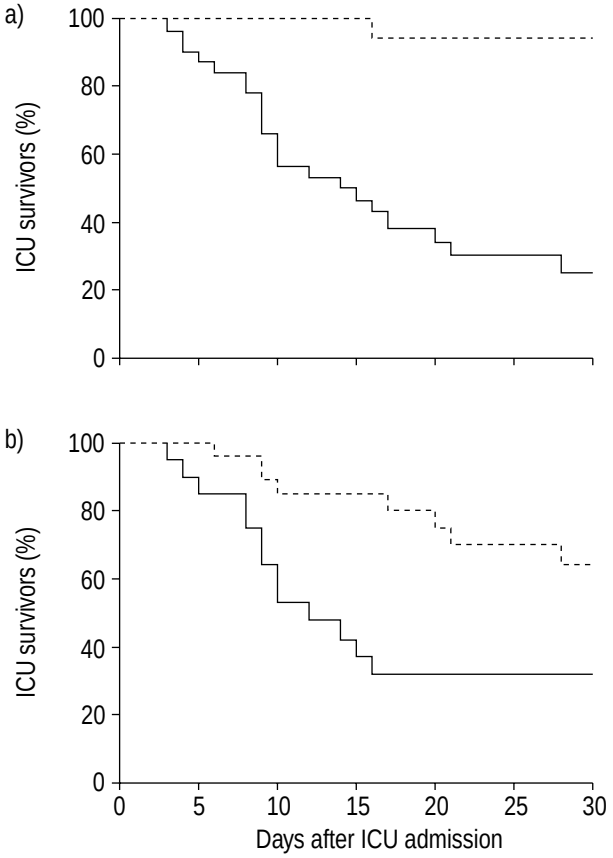

Fig. 2. – Kaplan-Meier estimates showed that a) survival was lower in human immunodeficiency virus patients that required mechanical ventilation ((MV; —) log rank 20.2, $p<0.001$), with a median survival of 15 days since intensive care unit (ICU) admission compared with no MV (- -). Additionally, b) subjects with bacterial pneumonia (- -) had a better outcome than those with *Pneumocystis carinii* pneumonia (—) (log rank 8.2, p=0.004). Patients with *P. carinii* pneumonia survived for a median period of 12 days after ICU entry. Follow-up truncated at 30 days because no deaths occurred in the ICU after this period.

Kaplan-Meier estimates showed that survival was associated with the need for mechanical ventilation (log-rank 20.2, $p<0.001$), with a median survival of 15 days since ICU admission for ventilated patients (fig. 2). Moreover, subjects with bacterial pneumonia had a greater survival rate than patients with *P. carinii* pneumonia (log-rank 8.2, p=0.004). Patients with *P. carinii* pneumonia survived for a median period of 12 days after ICU entry (fig. 2).

Follow-up of ICU survivors

Thirty-four (60%) patients were discharged alive from the ICU. However, three patients died in hospital within the week following ICU discharge and three more patients died within 10 days of leaving the hospital. Three patients had *P. carinii* pneumonia and one patient was from the bacterial pneumonia group. Death was due to other AIDS-related medical problems without respiratory involvement. The 28 patients that remained alive were followed for a median of 232 days (range, 15–968 days). The estimation of survival and outcome factors that influenced mortality were the same as those observed for ICU analysis (data not shown).

Discussion

In the present study a changing pattern in the aetiologies of life-threatening respiratory failure in HIV-infected patients admitted to the ICU was observed, with a decrease in the number of *P. carinii* pneumonia episodes compared with the previous decade. However, *P. carinii* pneumonia was still observed in people with unknown HIV infection and in those patients not compliant with the treatment or not undergoing prophylaxis. In addition, after the introduction of HAART, it was observed that few HIV patients with bacterial pneumonia were transferred to the ICU because of respiratory failure. The probability of survival was observed to be lower in cases of *P. carinii* compared to bacterial pneumonia, and in those subjects sick enough to require mechanical ventilation.

The aetiological pattern of life-threatening respiratory failure in HIV patients has evolved over the last few years [8, 9, 21, 22]. As a consequence of the systematic use of prophylaxis against *P. carinii* and steroids as co-adjuvant treatment, the number and severity of *P. carinii* pneumonia seemed to decline in the early 1990s, whereas no reduction was observed in the cases of bacterial pneumonia admitted to ICU [5, 24]. In the present study, only 6.8% of patients admitted to the authors' hospital because of *P. carinii* pneumonia required ICU admission, compared to 18.7% between 1986 and 1989 [23]. Moreover, *P. carinii* pneumonia was responsible for only 30% of the respiratory failures in HIV patients admitted to our ICU between 1993 and 1995, compared to 67% observed in a previous survey (1985–1992) [2].

However, the immunological reconstitution caused by HAART may have again changed the aetiological pattern of respiratory failure in these patients after 1996

[7, 10]. In the present study a further decrease in the number of patients with *P. carinii* pneumonia admitted to hospital after 1996 was observed. Interestingly, the number of cases of bacterial pneumonia requiring intensive care also decreased drastically. The few cases of severe *P. carinii* and bacterial pneumonia admitted to ICU after 1996 mainly corresponded to patients newly diagnosed with AIDS, and those who were not compliant with HAART or the recommended prophylaxis regimens [25, 26]. In addition, *P. carinii* prophylaxis may fail in cases with severe immunosuppression (<50 CD4 cells·μL^{-1}) [14] or because of the development of *P. carinii* dihydropteroate synthase mutations, which may be responsible for some cotrimoxazole failures [27].

Microbiological identification was established in 40% of the cases of bacterial pneumonia, similar to our previous series [2]. Because BAL was not performed in seven patients, the incidence of cases of *P. carinii* pneumonia may have been underestimated. However, this was not plausible as these patients exhibited lobar infiltrates and did not receive treatment with cotrimoxazole, but survived. Pulmonary infection was community-acquired in 23 cases, and *Streptococcus pneumoniae* was the most frequently involved micro-organism, similar to reports in the general population [28, 29]. Although HIV-infection was not a risk factor for severe community-acquired pneumonia in a recent case-control study [30], bacterial pneumonia is reported to appear more frequently in HIV patients with less than 200 CD4 cells·μL^{-1} [31]. Finally, *M. tuberculosis* infection was responsible for 7% of the ICU admissions, due to the high prevalence of tuberculosis in the Spanish HIV-infected population [32].

The clinical and epidemiological data observed in the present study are consistent with the literature [33]. Regarding radiographic findings, all patients with *P. carinii* pneumonia had bilateral interstitial infiltrates, while most of the bacterial pneumonia showed uni- and bilateral lobar infiltrates. APACHE II score, serum albumin level, and *P. carinii* pneumonia diagnosis were found as independent predictive factors of unfavorable outcome on ICU admission, in agreement with previous studies [2, 33, 34].

Due to the greater presence of patients with bacterial pneumonia, overall mortality of HIV-infected patients admitted to the ICU due to acute respiratory failure was 40%, slightly lower than in the 1985–1992 period (55%) [2]. However, the prognosis of the few remaining severe patients with *P. carinii* pneumonia that enter in the ICU has not changed over the last decade [2]. The degree of respiratory failure on ICU admission remains as the main prognostic factor in these patients. In addition, some subjects with prior *P. carinii* pneumonia died early after ICU discharge, as a consequence of other AIDS-related problems, confirming the defective immunological status of this population. Similarly, ICU survival was related to the degree of immunosuppression in the patients with bacterial pneumonia, an observation that has also been reported in HIV patients with infective endocarditis [35].

The convenience of intensive care unit admission of human immunodeficiency virus-infected patients with life-threatening respiratory failure has been a matter of debate in many centres, due to the poor prognosis of these patients [36]. Although the total number of human immunodeficiency virus-patients that require intensive care has declined, survival improvement is still not detected in patients with *Pneumocystis carinii* pneumonia requiring mechanical ventilation. Better defining of predictive variables on hospital admission, absence of delay in diagnostic measures and improvement in non invasive support methods may have a role in future research. Finally, it is important to ascertain the past antiretroviral therapy of the subjects, because currently naive patients or those with only one or two antiretroviral failures may still be able to use the highly active antiretroviral therapy, which, on relieving their cellular immunosupression, may influence the morbidity and mortality in this population.

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Pulmonary infiltrates in non-HIV immunocompromised patients: a diagnostic approach using non-invasive and bronchoscopic procedures

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Abstract

***Background*—The development of pulmonary infiltrates is a frequent life threatening complication in immunocompromised patients, requiring early diagnosis and specific treatment. In the present study non-invasive and bronchoscopic diagnostic techniques were applied in patients with different non-HIV immunocompromised conditions to determine the aetiology of the pulmonary infiltrates and to evaluate the impact of these methods on therapeutic decisions and outcome in this population.**

***Methods*—The non-invasive diagnostic methods included serological tests, blood antigen detection, and blood, nasopharyngeal wash (NPW), sputum and tracheobronchial aspirate (TBAS) cultures. Bronchoscopic techniques included fibrobronchial aspirate (FBAS), protected specimen brush (PSB), and bronchoalveolar lavage (BAL). Two hundred consecutive episodes of pulmonary infiltrates were prospectively evaluated during a 30 month period in 52 solid organ transplant recipients, 53 haematopoietic stem cell transplant (HSCT) recipients, 68 patients with haematological malignancies, and 27 patients requiring chronic treatment with corticosteroids and/or immunosuppressive drugs.**

***Results*—An aetiological diagnosis was obtained in 162 (81%) of the 200 patients. The aetiology of the pulmonary infiltrates was infectious in 125 (77%) and non-infectious in 37 (23%); 38 (19%) remained undiagnosed. The main infectious aetiologies were bacterial (48/125, 24%), fungal (33/125, 17%), and viral (20/125, 10%), and the most frequent pathogens were *Aspergillus fumigatus* (n=29), *Staphylococcus aureus* (n=17), and *Pseudomonas aeruginosa* (n=12). Among the non-infectious aetiologies, pulmonary oedema (16/37, 43%) and diffuse alveolar haemorrhage (10/37, 27%) were the most common causes. Non-invasive techniques led to the diagnosis of pulmonary infiltrates in 41% of the cases in which they were used; specifically, the diagnostic yield of blood cultures was 30/191 (16%); sputum cultures 27/88 (31%); NPW 9/50 (18%); and TBAS 35/55 (65%). Bronchoscopic techniques led to the diagnosis of pulmonary infiltrates in 59% of the cases in which they were used: FBAS 16/28 (57%), BAL 68/135 (51%), and PSB 30/125 (24%). The results obtained with the different techniques led to a change in antibiotic treatment in 93 cases (46%). Although changes in treatment did not have an impact on the overall mortality, patients with pulmonary infiltrates of an infectious aetiology in whom the change was made during the first 7 days had a better outcome (29% mortality) than those in whom treatment was changed later (71% mortality; p=0.001).**

***Conclusions*—Non-invasive and bronchoscopic procedures are useful techniques for the diagnosis of pulmonary infiltrates in immunocompromised patients. Bronchial aspirates (FBAS and TBAS) and BAL have the highest diagnostic yield and impact on therapeutic decisions.**

(*Thorax* 2001;**56**:379–387)

Keywords: immunocompromised; lung infection; bronchoalveolar lavage; nasopharyngeal wash; tracheobronchial aspirate; diagnosis

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Received 9 August 2000
Returned to authors
21 September 2000
Revised version received
19 January 2001
Accepted for publication
22 January 2001

The good results obtained with solid organ transplants and haematopoietic stem cell transplantation (HSCT) in recent years have led to an increase in the number of potential candidates.[1] One of the most important limiting factors for these treatments is the development of life threatening pulmonary complications.[2] Opportunistic and bacterial infections are common causes of pulmonary complications in immunocompromised patients including transplant recipients,[3 4] and must be distinguished from other conditions such as pulmonary oedema, diffuse alveolar haemorrhage, malignant diseases, and pulmonary drug reactions.[5–8] Accurate and prompt diagnosis of potentially treatable pulmonary complications is important in patients with severe immunosuppression.

In 1989 our group published the results of a protocol for the diagnosis of pulmonary infiltrates in a population of immunocompromised patients.[9] In this study it was suggested that bronchoalveolar lavage (BAL) and protected specimen brush (PSB) techniques used concurrently were useful for the diagnosis of pulmonary complications in these patients. Over the last decade significant developments

in diagnostic techniques have emerged.[10] Furthermore, the administration of new and effective treatments has changed the pattern of pulmonary complications in immunocompromised patients.[11–15] Recent publications have questioned the usefulness of bronchoscopic techniques in the diagnosis of pulmonary infiltrates in immunocompromised patients, suggesting that the information provided does not change treatment or improve survival and can cause significant complications.[16 17] In this study we have reappraised prospectively the current diagnostic usefulness and clinical impact of fibrobronchial aspiration (FBAS), BAL, and PSB for the study of pulmonary infiltrates in patients with different immunocompromised conditions (excluding HIV infected patients). We have also included other non-invasive procedures such as blood antigen detection, sputum, tracheobronchial aspirates (TBAS), and nasopharyngeal wash (NPW) to examine their diagnostic usefulness in this group of patients.

Methods

PATIENTS

We prospectively evaluated episodes of pulmonary infiltrates in 200 consecutive patients. All of the immunosuppressed patients with pulmonary infiltrates seen at a tertiary hospital between February 1998 and June 2000 were included (table 1). Patients included in the study belonged to four different groups—group 1: solid organ transplant (21 renal, 11 cardiac, 14 liver, and six pancreaticorenal) recipients; group 2: haematopoietic stem cell transplant (HSCT) recipients (n=53); group 3: patients with haematological malignancies treated with chemotherapy (n=68); and group 4: patients requiring chronic treatment with corticosteroids (minimum 30 mg prednisone daily for the previous 30 days before inclusion) or immunosuppressive agents (azathioprine or cyclophosphamide; n=27). The underlying haematological malignancies of groups 2 and 3 were acute myeloid leukaemia (n=30, 25%), acute lymphoblastic leukaemia (n=15, 13%), Hodgkin's lymphoma (n=14, 12%), chronic lymphoid leukaemia (n=12, 10%), chronic myeloid leukaemia (n=11, 10%), and others (n=39, 30%). Fifty nine of the 200 infiltrates (30%) were evaluated while the patients were on mechanical ventilation.

DATA COLLECTION

The following variables were recorded: age, sex, underlying immunosuppressed condition, leucocyte and platelet counts, serum biochemistry, arterial oxygen tension (Pa_{O_2})/inspiratory oxygen fraction (Fi_{O_2}) ratio at inclusion, and APACHE II score (Acute Physiology and Chronic Health Evaluation) at the time of admission to hospital. The requirement for mechanical ventilation was also registered and survival was defined as hospital discharge.

DIAGNOSTIC PROCEDURES

Within the first 24–48 hours after the identification of the pulmonary infiltrates, samples of blood were drawn for blood cultures and serological and antigen testing. A sample of spontaneous or induced sputum (after nebulisation with 3% saline solution) was obtained. A Gram stain was performed to assess the quality of the sample.[18] A sample of NPW was taken for viral detection and tissue culture. In patients under mechanical ventilation, a sample of TBAS was obtained via an orotracheal tube under sterile conditions using a 22 inch 14 Fr suction catheter and collected into a mucus reservoir (Mocstrap; Proclinics, Barcelona, Spain). Antigen detection of *Streptococcus pneumoniae* (urine), *Aspergillus* sp (serum), and *Chlamydia pneumoniae* (pharyngeal swab), and DNA detection of *Mycobacterium tuberculosis* by ligase chain reaction (respiratory samples) were included once the study had been started.

Fibreoptic bronchoscopy (FOB) was performed before antibiotic treatment was started in 14 cases (9% of total FOB) and 48–96 hours after starting antibiotic treatment in the remaining 136. Patients were not eligible for bronchoscopic exploration if arterial Pa_{O_2} was less than 7.3 kPa with oxygen administration or if the platelet count was less than $50 \times 10^6/\mu l$. The bronchoscope was passed transnasally or transorally into the trachea after topical nasal anaesthesia was instilled. In mechanically ventilated patients the bronchoscope was passed into the trachea through the endotracheal tube. A PSB sample (Microbiology Brush; Mill-Rose Laboratory Inc, Mentor, Ohio, USA)[19] was first obtained in 125 cases (62%). Respiratory secretions were aspirated through the suction channel of the bronchoscope in 28 patients. Bronchoalveolar lavage was carried out with 150 ml of sterile saline solution in three 50 ml

Table 1 Underlying immunosuppressed condition and clinical characteristics of the 200 patients studied

	HSCT	*Haematological malignancies*	*Solid organ transplantation*	*CIS*	*Total*
No. (%)	53 (27%)	68 (34%)	52 (26%)	27 (13%)	200
Mean (SD) age (years)	40 (12)	54 (17)	52 (16)	60 (14)	50 (17)
M/F	33/20	36/32	43/9	13/14	125/75
Neutropenia, n (%)	21 (40%)	28 (41%)	3 (6%)	1 (2%)	53 (27%)
Mean (SD) APACHE II score	18 (7)	19 (8)	20 (8)	20 (8)	19 (8)
Mean (SD) Pa_{O_2}/Fi_{O_2} at inclusion	269 (102)	266 (95)	221 (126)	222 (113)	249 (109)
Mechanical ventilation requirement, n (%)	24 (45%)	23 (34%)	33 (63%)	15 (56%)	95 (47%)
Mortality, n (%)	26 (49%)	21 (31%)	20 (38%)	14 (52%)	81 (40%)

HSCT = haematopoietic stem cell transplantation; CIS = chronic immunosuppressive treatment; APACHE II = Acute Physiology and Chronic Health Evaluation II score at admission.
Underlying diseases: chronic obstructive pulmonary disease (n=6), scleroderma (n=3), idiopathic pulmonary fibrosis (n=4), systemic vasculitis (n=3), rheumatoid arthritis (n=2), glomerulonephritis (n=2), and one each of systemic lupus erythematosus, relapsing polychondritis, dermatomyositis, mixed cryoglobulinaemia, Good-Pasteur syndrome, Beçhet syndrome, and primary biliary cirrhosis.
Neutropenia defined as a neutrophil count $<1 \times 10^9$ cells/l.

aliquots in 135 patients (70%). The lavage was performed in the involved lobe in patients with localised pulmonary infiltrates and in the middle lobe or lingula in patients with diffuse pulmonary infiltrates.

Although the primary intention was to perform all the different diagnostic procedures, the ultimate decision as to which of the procedures should be performed was always determined by the clinical state of the patient and the criteria of the physician in charge.

OTHER DIAGNOSTIC TECHNIQUES

Transbronchial (TBB) and bronchial biopsy specimens were obtained in 11 and three cases, respectively, and analysed pathologically and microbiologically. Videothoracoscopic (VTC) lung biopsy was carried out in two cases in which no diagnosis was reached by bronchoscopic examination. Necroscopic examination was performed in 19 of the 81 patients who died.

SAMPLE PROCESSING

Staining methods

Viral antigens in NPW and BAL samples were detected by a direct immunofluorescent staining method using a monoclonal antibody pool (influenza viruses A and B, parainfluenza viruses 1, 2 and 3, respiratory syncytial virus, adenovirus, virus herpes simplex, and enterovirus). Sputum, FBAS and TBAS specimens were directly stained using Gram and Ziehl-Neelsen methods. PSB and BAL smears were Gram and Giemsa stained for intracellular or extracellular bacterial and fungal detection and cellular differential count. In BAL samples additional stains were used: Ziehl-Neelsen staining for mycobacteria; Gomori staining for *Pneumocystis carinii*; PAS, haematoxylin-eosin, and Papanicolau for cell atypia; Pearls' staining for hemosiderophages.

Cultures

Sputum samples were qualitatively cultured for bacterial pathogens, fungi, and mycobacteria. Bronchoscopic samples were homogenised and processed for quantitative culture by serial dilutions (1:1 and 1:10, inoculating 0.1 ml/plate) for bacterial pathogens. Culture media for *Legionella* spp and fungi were plated undiluted. FBAS, TBAS, and BAL specimens were also cultured for mycobacteria, and NPW and BAL samples were cultured for viruses on monolayers of Hep-2, A-549, MRC-5, and MDCK cell lines (Vircell, Granada, Spain). Cultures were maintained for 4 weeks at 37°C and examined regularly for a cytopathic effect. Viral growth was confirmed by immunofluorescence staining with monoclonal antibodies (Respiratory Pannel 1, Viral Screening & Identification Kit, Light Diagnostics, Temecula, USA; Clonab CMV, Biotest, Germany).

Negative bacterial cultures were discarded after 3 days, *Legionella* after 10 days, mycobacteria after 6 weeks, and fungal cultures after 4 weeks. Positive bacterial cultures were counted as colony forming units per ml (cfu/ml), and identification and susceptibility tests were performed according to standard methods.

Rapid diagnostic tests

The urinary antigen test for detection of *L pneumophila* serogroup 1 was performed using an enzyme immunoassay (EIA) method (Biotest Legionella Urine Antigen EIA, Biotest, Germany) and *S pneumoniae* was detected using an immunochromatography method (Binax Now *S pneumoniae* Urinary Antigen Test, Binax, USA). Detection of the pp65 cytomegalovirus (CMV) antigen in BAL fluid and serum was performed using a specific monoclonal antibody (Clonab CMV, Biotest, Germany) by means of an immunocytochemistry method (APAAP; Dako, Denmark). Detection of *Aspergillus* spp galactomannan antigen in the serum was performed using an EIA method (Platelia Aspergillus, Sanofi Diagnostics Pasteur, France) and *Mycobacterium tuberculosis* DNA in respiratory samples was studied using a ligase chain reaction method (Abbot LCX *Mycobacterium tuberculosis* assay, Abbot Laboratories, Abbot Park, USA). *C pneumoniae* DNA was detected in nasopharyngeal swabs by a PCR-EIA method (Diasorin; Italy).

DIAGNOSTIC DEFINITIONS

The aetiology of pulmonary infiltrates was established based on the results of the different diagnostic procedures performed, the response to the specific treatment, and the histopathological evaluation of biopsy samples when available.

The aetiology of pulmonary infiltrates was defined as follows: (1) infectious: when there was a clinical suspicion of lower respiratory tract infection and a microbial agent was isolated in respiratory and/or non-respiratory samples; (2) non-infectious: when clinical data did not suggest an infectious aetiology, no microbiological agents were isolated in any processed sample, and the clinical course and response to treatment were in accordance with an alternative non-infectious aetiology; (3) undetermined (undiagnosed): when the pulmonary infiltrates did not meet criteria for infectious or non-infectious aetiologies.

Diagnostic criteria for specific microorganisms

Bacterial pneumonia was diagnosed whenever blood or sputum samples grew pathogenic bacteria. Similarly, when a quantitative bacterial culture from FBAS or TBAS, BAL or PSB specimens grew more than 10^5, 10^4, or 10^3 cfu/ml, respectively, the diagnosis of bacterial pneumonia was also established. Any growth of facultative pathogenic bacteria such as *Streptococcus viridans* and coagulase negative *Staphylococcus* was considered irrelevant.

Cytomegalovirus and other viruses were considered to be pathogens if they were isolated by cell culture from NPW or BAL specimens or when inclusion bodies were present on histopathological evaluation.

P carinii pneumonia (PCP) was diagnosed by a positive Gomori methenamine silver stain. The identification of *Legionella* spp and mycobacteria was accepted as a definite diagnosis regardless of colony counts. Fungal pneumonia was diagnosed in the presence of a compatible clinical and radiographic pattern if fungal

hyphae were identified by culture or by cytological evaluation of a Gram stain in at least two different respiratory samples, or when there was histological evidence for fungal pneumonia or positive blood cultures.[20]

Diagnostic criteria for non-infectious conditions

Diffuse alveolar haemorrhage was diagnosed by established criteria[5–7] and pulmonary oedema was diagnosed using clinical criteria. Bronchiolitis obliterans organising pneumonia (BOOP) was diagnosed histologically from samples obtained by transbronchial or open lung biopsies. Two patients were diagnosed with hypersensitivity pneumonitis secondary to bleomycin by clinical criteria and compatible radiological changes. In these patients the pulmonary infiltrates disappeared after bleomycin was withdrawn and corticosteroid treatment was started.

ANTIMICROBIAL PROPHYLAXIS

One hundred and thirteen patients (57%) were receiving some type of antimicrobial prophylaxis at the onset of the pulmonary infiltrates: 43% in group 1, 85% in group 2, 55% in group 3, and 33% in group 4. The most frequent prophylaxis strategy was antibacterial treatment (18%) followed by the combination of antiviral + antifungal + prophylaxis against PCP (10%), followed by a combination of antibacterial + antifungal agents (8%).

EMPIRICAL ANTIBIOTIC TREATMENT

The final decision regarding the initial antimicrobial treatment was taken by the clinician in charge. The most common combination consisted of a third generation cephalosporin plus an aminoglycoside.[21] Alternative antibiotics were ureidopenicillins and/or ciprofloxacin. If *Staphylococcus aureus* infection was suspected or proven, vancomycin was added. Amphotericin B was added if no clinical or radiographic improvement was observed after 4–5 days of antibiotic treatment. Ganciclovir plus high dose immunoglobulin G (or foscarnet as an alternative) were added if CMV infection was suspected or proven. G-CSF was occasionally given to patients with evidence of infection and prolonged neutropenia.

The initial antibiotic treatment was considered inadequate if the isolated microorganisms were not susceptible to or were not covered by the treatment administered. Changes in treatment were noted if they were based on either a positive or negative result from the non-invasive and bronchoscopic samples.

ANALYSIS OF DATA

Results are expressed as mean (SD). The median value (50th percentile) was used to evaluate the delay in establishing a specific diagnosis of the pulmonary infiltrates. All statistics were calculated using the SPSS statistical package (SPSS for Windows, version 9.0; Chicago, IL, USA). Categorical variables were compared using the χ^2 test or Fisher's exact test when appropriate, and continuous variables were analysed using the non-parametric Mann-Whitney U test. A p value of <0.05 was considered statistically significant.

Results

AETIOLOGY OF PULMONARY INFILTRATES

A specific diagnosis was obtained in 162 of the 200 cases evaluated (81%), with an infectious aetiology in 125 of the cases (77%) and a non-infectious aetiology in 37 (23%, table 2). Bacteria were the most frequent microorganisms causing pulmonary infiltrates (n=48, 24%), followed by fungi (n=33, 17%) and viruses (n=20, 10%). In 15 cases (7%) the aetiology of the pulmonary infiltrates was polymicrobial (table 3).

There were no statistically significant associations between the specific underlying immunosuppressive state and the aetiology of the pulmonary infiltrates.

NON-INVASIVE TECHNIQUES

Blood cultures were obtained from 191 cases and gave 30 positive results (16%). When only patients with an infectious aetiology were considered, the diagnostic yield of blood cultures was 23%. In 16 cases (7%) blood cultures were the only means of diagnosis (most frequently due to isolation of Gram negative bacilli and MRSA).

Detection of pp65CMV antigen in peripheral blood mononuclear cells was performed in 91 (45%) patients (47 HSCT recipients, 23 with haematological malignancies, and 21 solid organ transplant recipients) and was positive in seven of the eight cases of confirmed CMV pneumonitis. In three other cases with no evidence of CMV pneumonitis pp65CMV

Table 2 Aetiological diagnosis in relation to the underlying immunosuppressed condition

	HSCT	*Haematological malignancies*	*Solid organ transplantation*	*CIS*	*Total*
Bacterial	6 (11%)	16 (24%)	18 (35%)	8 (30%)	48 (24%)
Viral	11 (21%)	6 (9%)	1 (2%)	2 (7%)	20 (10%)
Fungal	7 (13%)	13 (19%)	6 (12%)	7 (26%)	33 (17%)
Polymicrobial	3 (6%)	1 (2%)	9 (17%)	2 (7%)	15 (7%)
Other infectious aetiologies†	1 (2%)	3 (4%)	1 (2%)	4 (15%)	9 (5%)
Pulmonary oedema	3 (6%)	3 (4%)	10 (19%)	–	16 (8%)
DAH	5 (9%)	2 (3%)	2 (4%)	–	10 (5%)
BOOP	1 (2%)	2 (3%)	–	1 (4%)	4 (2%)
Other non-infectious aetiologies‡	3 (6%)	4 (6%)	–	–	7 (3%)
Undetermined	13 (24%)	17 (25%)	5 (10%)	3 (11%)	38 (19%)

HSCT = haematopoietic stem cell transplantation; CIS = chronic immunosuppressive treatment; DAH = diffuse alveolar haemorrhage; BOOP = bronchiolitis obliterans organising pneumonia.
†Other infectious aetiologies include tuberculosis (n=5), *P carinii* pneumonia (n=4).
‡Other non-infectious entities include pulmonary involvement of Hodgkin's disease (n=3), drug toxicity due to bleomycin (n=2), alveolar proteinosis (n=1), and sarcoidosis (n=1).

Table 3 Infectious aetiologies in 125 patients diagnosed during lifetime or at necropsy

Pathogens	*No of cases*	*No of pathogens*
Bacterial		
Gram positive		
S aureus	12	17
S pneumoniae	2	5
E fecalis	2	2
E faecium	1	1
G morbillorum	1	1
S constellatum	1	1
N asteroides	2	2
Gram negative		
E coli	6	7
P aeruginosa	6	12
S marcescens	2	2
A baumanii	2	3
C freundii	1	1
M morganii	1	1
Other bacteria		
M pneumoniae	1	1
C pneumoniae	2	2
L pneumophila	2	2
Fungal		
A fumigatus	20	29
A niger	1	1
A flavus	1	1
C albicans	8	12
C kruseii	1	1
C tropicalis	1	1
S prolificans	1	2
P purpurogenum	1	1
Viral		
Cytomegalovirus	6	8
Influenza A virus	5	8
RSV	3	3
Parainfluenzae virus type 3	2	2
Virus herpes simplex (VHS)-1	1	1
Varicella zoster virus*	1	–
Enterovirus	1	1
Others		
P carinii	4	4
M tuberculosis	5	5
Mixed infections		
Aspergillus sp + MRSA	3	
Aspergillus sp + *P aeruginosa*	2	
E coli + *K pneumonia* + *S maltophila*	1	
Aspergillus sp + CMV	1	
C freundii + *P aeruginosa*	1	
MRSA + *E fecalis*	1	
A baumanii + *P aeruginosa*	1	
A flavus + *E faecium* + CMV	1	
A fumigatus + *S pneumoniae*	1	
A niger + *E coli* + MRSA	1	
A fumigatus + VHS-1	1	
C tropicalis + *A fumigatus* + *P aeruginosa*	1	

*Diagnosed by clinical criteria.

antigen was also positive. Serum *Aspergillus* spp galactomannan antigen detection was performed in 54 (27%) patients (46 HSCT recipients or with haematological malignancies) and was positive in six of seven cases with confirmed pulmonary aspergillosis and in two without evidence of pneumonia by this pathogen. Two cases of *C pneumoniae*, one case of *M pneumoniae*, and one case of *L pneumoniae* infection were diagnosed by serological tests and urinary antigen detection, respectively. One of the cases of *C pneumoniae* was also confirmed by polymerase chain reaction (PCR) detection in the pharyngeal swab.

Nasopharyngeal wash was performed in 50 (25%) patients (19 HSCT recipients, 26 with haematological malignancies, and four solid organ transplant recipients) and led to a definite diagnosis in nine (18%) cases (table 4). All cases diagnosed by NPW were also diagnosed by BAL, except one case of RSV.

Sputum analysis was performed in 96 patients but only 88 samples were accepted for microbial analysis, 27 of which (22 spontaneous, five induced) resulted in a positive culture (31%). When only the group of infectious pulmonary infiltrates was considered, this percentage increased to 46%. Sputum culture was the only technique that gave a definite diagnosis in 10 cases (8%).

Tracheobronchial aspirates (TBAS) were obtained in 55 patients on mechanical ventilation with 35 positive results (64%). All of these were in the group with an infectious aetiology (table 4). In 11 cases (9%) TBAS was the only technique that provided a definite diagnosis.

The overall diagnostic yield of non-invasive techniques was 40% and this percentage increased to 46% when we evaluated only those cases in whom the pulmonary infiltrates had an infectious aetiology.

BRONCHOSCOPIC TECHNIQUES

Fibrobronchial aspirates (FBAS) were obtained from 28 non-intubated patients and gave 16 positive results (57%), with the isolation of a single microorganism in 15 cases and two in another case (table 4).

Protected specimen brush (PSB) specimens were obtained from 125 cases and cultures yielded high colony counts ($>10^3$ cfu/ml) of different microorganisms in 30 cases (24%) (four cases with two simultaneous microorganisms) (table 4). Although the overall diagnostic yield for PSB was 24%, and increased up to 43% in those with an infectious aetiology, in only one case was PSB the only technique to provide a definite diagnosis.

Bronchoalveolar lavage (BAL) was performed in 135 cases, giving an overall diagnostic yield of 51% (68/135) which increased to 69% (56/81) in those with an infectious aetiology (table 4). Furthermore, in 12 cases in which no microorganisms were detected, the recovered BAL fluid enabled a diagnosis to be made of a non-infectious cause of the pulmonary infiltrates: diffuse alveolar haemorrhage in 10 patients, one case of eosinophilia due to pulmonary toxicity secondary to the administration of bleomycin, and one case of alveolar proteinosis. In 26 cases (20%) BAL was the only technique that provided a definite diagnosis.

Transbronchial biopsy (TBB) specimens were obtained from 11 selected cases and resulted in a specific diagnosis in six cases (55%): two cases of BOOP, two of pulmonary involvement of Hodgkin's disease, and two cases of bacterial pneumonia. Bronchial biopsy (BB) specimens were obtained in three patients and showed one case of *A fumigatus*, one of *C tropicalis*, and one of methicillin resistant *S aureus* (MRSA) pneumonia.

Videothoracoscopic lung biopsy specimens were taken in two cases, resulting in the diagnosis of one case of pulmonary tuberculosis and one case of BOOP.

*Table 4 Pathogens isolated with the different techniques employed in respiratory samples**

Pathogens	*NPW (n=50)*	*Sputum (n=88)*	*FBAS (n=28)*	*TBAS (n=55)*	*PSB (n=125)*	*BAL (n=135)*	*Total pathogens isolated*
Bacterial							
Gram positive							
S aureus		7	6	7	8	11	17
S pneumoniae		3	1	3	1		3
E fecalis			1	1	1		2
N asteroides		2					2
Gram negative							
P aeruginosa		6	2	6	2	4	11
S marcescens				1	1	2	2
A baumanii				1	1	3	3
C freundii				1			1
M morganii						1	1
E coli		2		1			1
S maltophilia					1	1	1
H influenzae		1		1		1	1
K pneumoniae				1			1
L pneumophila					1	1	2
Fungal							
A fumigatus		7	3	12	10	12	30
A flavus				1			1
A niger						1	1
C albicans		2	2	5	6	6	8
C tropicalis						1	1
C glabrata						1	1
P purpurogenum						1	2
S prolificans				1	1	1	2
Viral							
Cytomegalovirus						5	8
Influenza A virus	5					8	8
RSV	3					2	3
Parainfluenzae virus type 3						2	2
Enterovirus						1	1
VHS-1	1					1	1
Others							
P carinii						4	4
M tuberculosis		2	1			1	5

NPW = nasopharyngeal wash; FBAS = fibrobronchial aspirate; TBAS = tracheobronchial aspirate; PSB = protected specimen brush; BAL = bronchoalveolar lavage; RSV = respiratory syncytial virus; VHS-1 = virus herpes simplex-1.
*Some organisms were simultaneously isolated with more than one technique and some of these techniques (FBAS, TBAS, PSB, and BAL) allowed the identification of more than one organism.

Necroscopic examination was performed in 19 of the 81 patients. Aspergillosis was identified in five cases and CMV pneumonitis in one, none of which had been diagnosed before death. Three cases of pulmonary aspergillosis (one with associated CMV pneumonitis not diagnosed before death), two of *Pseudomonas* sp, three of CMV pneumonitis with diffuse alveolar damage, and one of *C tropicalis* were confirmed. In four cases the necropsy showed diffuse alveolar damage with negative cultures.

OPTIMISATION OF PROCEDURES PERFORMED

With the aim of optimising the application of the different procedures included in the study, we selected those 138 patients in whom a specific diagnosis was obtained using the non-invasive and bronchoscopic techniques, excluding those cases in whom the diagnosis was obtained by clinical criteria such as pulmonary oedema and those diagnosed by TBB, VTC, or necroscopic examination. In 61 of these 138 patients (44%) it was possible to achieve the diagnosis using only non-invasive procedures (including blood, serological tests, and antigen detection in blood, NPW, sputum, and TBAS cultures). The remaining 91 diagnoses (66%) required the use of different bronchoscopic procedures. No differences in clinical and radiographic characteristics were observed between the group of patients diagnosed by non-invasive procedures and those requiring bronchoscopic procedures. In the subgroup of patients diagnosed by means of bronchoscopic procedures, BAL had the highest diagnostic yield, providing 94% (65/69) of the diagnoses (including 100% of the non-infectious pulmonary infiltrates). Although PSB gave a diagnosis in 46% (29/63) of the population, all but one of the cases could also be diagnosed by BAL. The diagnostic role of FBAS cannot be properly ascertained in this population since the procedure was only performed in 19 patients, providing a diagnosis in 14 (74%); however, in none of these 14 cases did the results of PSB add diagnostic information to that already obtained by FBAS.

*Table 5 Impact of diagnostic techniques on the treatment of pulmonary infiltrates**

Diagnostic technique	*No positive/ no performed (%)*	*Modification of treatment, n (%)*
Blood cultures	30/191 (16%)	4 (5%)
CMV antigen detection	11/91 (12%)	5 (5%)
Aspergillus antigen detection	8/54 (15%)	1 (1%)
NPW	9/50 (18%)	1 (1%)
Sputum	27/88 (31%)	11 (12%)
FBAS	16/28 (57%)	4 (4%)
TBAS	35/55 (64%)	24 (26%)
PSB	30/125 (24%)	2 (2%)
BAL	68/135 (51%)	35 (38%)
TBB	6/11 (55%)	4 (4%)
VTC biopsy	2/2 (100%)	2 (2%)

NPW = nasopharyngeal wash; FBAS = fibrobronchial aspirate; TBAS = tracheobronchial aspirate; PSB = protected specimen brush; BAL = bronchoalveolar lavage; TBB = transbronchial biopsy; VTC = videothoracoscopic.
*In some patients treatment changes were due to the results obtained with several diagnostic procedures simultaneously.

COMPLICATIONS

There were only three mild complications as a result of the FOB exploration: one patient with self-limited epistaxis, one with a moderate bronchial haemorrhage following transbronchial biopsy, and hypoxaemia requiring high flow oxygen by mask for 12 hours in a third patient.

IMPACT OF DIFFERENT DIAGNOSTIC TECHNIQUES ON TREATMENT OF PULMONARY INFILTRATES

Empirical treatment was modified in 93 of the 200 cases (46%) because of the results obtained with the different techniques used. Overall, non-invasive procedures caused a change in treatment in 45 of the 93 cases (48%) whereas bronchoscopic techniques resulted in changes in 48 cases (52%). In 19 cases it was the result of both non-invasive and bronchoscopic techniques that resulted in a change in treatment (table 5). BAL and TBAS had the greatest impact with treatment modifications in 38% (35/93) and 26% (24/93), respectively. The most common reason for changing treatment was the isolation of a microorganism not covered by the empirical treatment. This occurred in 56 cases (60%) and was mostly attributable to isolation of *Aspergillus* sp, viruses, *M tuberculosis*, and MRSA. In 18 cases (20%) the reason for changing the empirical treatment was the isolation of a resistant strain. Finally, in 19 cases (20%) the empirical antibiotic therapy was discontinued after diagnosing a non-infectious cause of pulmonary infiltrates (10 cases of diffuse alveolar haemorrhage, four of BOOP, two of pulmonary lymphoma, two of pulmonary toxicity resulting from bleomycin, and one of alveolar proteinosis).

OUTCOME

Eighty one of the 200 patients (40%) included in the study died as a result of the pulmonary complication. Mortality in patients requiring mechanical ventilation was higher (73/95, 77%) than in those not requiring it (8/105, 7%; p<0.0001), being particularly high in HSCT recipients in whom the mortality rate reached 96% (26/27). Sixty four of the 125 patients with pulmonary infiltrates with an infectious aetiology died (51%) compared with only six of the 37 patients with a non-infectious aetiology (16%; p<0.0001). The mortality rate in patients in whom a specific diagnosis of the pulmonary infiltrates was established was 43% (70/162) compared with 29% (11/38) in those in whom a diagnosis could not be established during their lifetime (p=NS).

There was no relationship between the mortality rate and the specific aetiology of the pulmonary infiltrates. The mortality rates in patients with fungal, polymicrobial, viral, and bacterial pneumonia were 67%, 66%, 51%, and 42%, respectively (p=NS). There were no deaths among patients with pulmonary tuberculosis, BOOP, or diffuse alveolar haemorrhage.

A significant difference in mortality was observed between patients in whom the diagnosis was established during the first 7 days (median value for the delay in establishing the diagnosis of the overall population) and those in whom the diagnosis was established later on. In the first group the mortality rate was 34% (29/85) compared with 53% (41/77) in those in whom the delay in diagnosis was longer than 7 days (p=0.017). The observed difference in mortality depending on the delay in obtaining the diagnosis was probably related to the implications of an early change in empirical treatment. Thus, when we evaluated only patients requiring a change in empirical treatment (n=93) we observed that those in whom this change was instituted during the first 7 days had a lower mortality rate (14/42, 30%) than patients in whom the change was established later (32/51, 70%; p=0.007). This was particularly apparent in the 76 patients with pulmonary infiltrates with an infectious aetiology: mortality rate of 29% (13/34) in those in whom the pulmonary infection was diagnosed in the first 7 days compared with 71% (32/42) in patients in whom the pulmonary infection was diagnosed later (p=0.001).

Discussion

This study shows that the simultaneous use of non-invasive and bronchoscopic procedures results in a specific diagnosis being made in most cases of pulmonary infiltrates in immunocompromised patients. Furthermore, obtaining a specific diagnosis early in the course of the disease allows the empirical treatment to be changed in a high percentage of cases (46%) and may improve the survival of these patients. Using this diagnostic approach, we have diagnosed 81% of the episodes of pulmonary infiltrates, which compares favourably with 49% obtained 10 years ago by our group in a similar population.[9]

In our population the main cause of the pulmonary infiltrates was infection which constituted 77% of the episodes diagnosed. Bacterial infections and, specifically, those caused by *S aureus* (mainly MRSA) and Gram negative bacilli (mainly *P aeruginosa*) were the most frequent infectious aetiologies found. Since bacteria represent the most prevalent threat to immunocompromised patients, each institution should define the empirical antibiotic treatment based on its own data.[21–23]

Fungal species, especially *Aspergillus* spp, represented the second most frequent infectious cause of pulmonary infiltrates in the present study. Death from *Aspergillus* pneumonia in immunosuppressed patients is exceedingly high, reaching 85% in some series.[24 25] In addition to the severe immunosuppression, this poor outcome may be partially explained by the delay in establishing the diagnosis and starting specific treatment.[26] Although evidence of tissue invasion by fungi has classically been required to confirm the diagnosis, the presence of *Aspergillus* species in sputum or bronchial lavage cultures,[27] as well as the detection of galactomannan fungal antigen,[28] should be considered indicative of invasive disease until proved otherwise and warrants institution of specific therapy. In the present series *Aspergillus* pneumonia was diagnosed on 18

occasions without tissue confirmation and antifungal treatment was instituted immediately, resulting in a mortality rate of 67%. Whether this approach can improve the outcome of pulmonary aspergillosis remains to be demonstrated in well designed prospective series. The diagnosis of *Candida* pneumonia is even more controversial than *Aspergillus* pneumonia.[20] Although we cannot exclude the possibility that some of the 12 cases of pulmonary candidiasis represent airway colonisation, three had tissue confirmation and five had positive blood cultures which further supported the diagnosis.

Viral pneumonia accounted for 20 episodes of pulmonary infiltrates, CMV being the most frequent aetiological agent.[12] Evidence has been accumulating that community respiratory viruses such as respiratory syncytial virus, influenza virus, parainfluenza virus[11 13] and adenovirus also play an important role in the aetiology of respiratory infiltrates in transplant recipients and that infection with these organisms may explain some of the pneumonic episodes previously classified as idiopathic.[11] In the present series the rapid detection of parainfluenza virus in respiratory samples from two patients allowed us to initiate treatment with aerosolised ribavirin immediately, with a favourable outcome in both cases. So far, the role of any virus other than CMV or herpes simplex virus has been poorly defined. In the series published 10 years ago by our group,[9] which included 113 episodes of pulmonary infiltrates in 93 immunosuppressed patients, viral investigations were restricted to the identification by culture of CMV and the cytopathological changes produced by CMV in cells recovered from the BAL fluid. Recently, new diagnostic tools such as quantitative PCR assays and fluorescein conjugated monoclonal antibodies have been developed, permitting the rapid detection of different viruses that are frequently associated with a high rate of morbidity and mortality.[10] The systematic application of new techniques for virus identification in the present series most probably accounts for the increase in the overall diagnostic yield compared with our previous experience.[9]

Another important finding of the present study is that cultures of TBAS in intubated patients were helpful in determining the aetiology of pneumonia. Traditionally, TBAS cultures have not been considered specific enough to diagnose lower respiratory tract infections due to the presence of bacterial colonisation of both lower and upper airways. However, researchers from our institution[29] and others[30] have shown that, using quantitative cultures, the aetiology of bacterial pneumonia can be accurately ascertained in mechanically ventilated patients using TBAS cultures. In the present series we have confirmed the usefulness of TBAS cultures in immunocompromised patients, obtaining a diagnosis in 64% of intubated patients investigated. Further studies are needed to clarify the usefulness of TBAS and to compare it with other techniques such as blind BAL[31] which also does not rely on a bronchoscopist and has a good diagnostic yield in immunocompromised patients. In contrast, PSB gave few positive results (24%), many of them also achieved by BAL or FBAS. In only one case was PSB the technique that led to changes in treatment.

Bronchoalveolar lavage has been established as a reliable technique for the diagnosis of pulmonary infiltrates in the immunosuppressed host, specifically for detecting opportunistic infectious such as *P carinii* and CMV and also for bacteria.[9 32] In the present series most episodes of pneumonia caused by opportunistic pathogens were diagnosed by BAL. Although this technique has been criticised by some authors because the impact on survival is disappointingly poor,[16 17 33] BAL has a high diagnostic yield for infections and provides enough material to diagnose alternative non-infectious aetiologies. Specifically, in our population BAL provided the diagnosis in 10 cases of diffuse alveolar haemorrhage, one case of pulmonary toxicity due to bleomycin, and in one case of alveolar proteinosis.

Although TBB specimens are not routinely taken from immunocompromised patients in our centre, it has clear indications in specific cases such as those with nodular, localised or patchy infiltrates on the chest radiograph. In the present series TBB not only gave a specific diagnosis in six of the 11 patients in which it was performed, but also allowed a change in the treatment in four cases (two with pulmonary lymphoma, two with BOOP).

Compared with our previous series,[9] the current diagnostic techniques employed increased the percentage of treatment changes from 31% to 46%. However, there was no difference in mortality when patients with and without changes in treatment due to the results obtained with the different diagnostic procedures employed were compared. The lack of impact of invasive diagnostic techniques on survival has been claimed by some authors to be the major reason for not performing them in immunocompromised patients.[16] Moreover, complications and morbidity secondary to bronchoscopy have been described in this setting.[17] We suggest that the treatment of infectious pulmonary infiltrates in immunocompromised patients has a poor outcome because specific drugs are usually administered late in the evolution of the illness. In fact, we found a significant decrease in mortality in those patients with an infectious aetiology in whom an early diagnosis (7 days from the initiation of the pulmonary infiltrates) caused a change in treatment (29%) compared with those in whom this diagnosis was obtained after 7 days (71%). Although it seems logical, based on these results, to recommend the early use of diagnostic techniques to improve the clinical outcome, further specifically designed studies are needed to try to answer this critical question. Confounding factors such as the judgement of clinicians in charge to try different treatments in the face of clinical deterioration might associate changes in treatment with worse prognosis in the present study.

Based on the results obtained, our recommendation is to use simple non-invasive

procedures as a first step in the diagnosis of pulmonary infiltrates in the immunocompromised patient. These non-invasive procedures should include blood, sputum, and NPW cultures as well as TBAS in patients on mechanical ventilation. The use of non-invasive techniques provides the diagnosis in 44% of the cases and constitutes a good alternative in those patients with contraindications for a bronchoscopic exploration. Because of the importance of obtaining a diagnosis with minimum delay, BAL should always be performed when possible as this endoscopic procedure has a high diagnostic yield for both infectious and non-infectious aetiologies. The use of PSB could be avoided since it does not contribute to increasing the diagnostic yield of BAL. This rather expensive technique could probably be replaced by a more simple FBAS. Finally, provided no contraindications exist, selected patients should be submitted to transbronchial biopsies as a step before surgical procedures.

In summary, the use of both non-invasive and bronchoscopic procedures substantially increases the diagnostic yield of pulmonary infiltrates, causing changes in the empirical treatment in the majority of patients. Survival in patients who require a change in treatment seems to be higher when the specific diagnosis is established early in the course of the disease. However, further studies are needed definitively to confirm the importance of an early diagnosis on the clinical outcome in immunocompromised patients with pulmonary complications.

AR is supported by a clinical research grant from SEPAR and Hospital Clínic de Barcelona (grant 1999SGR00228 from the Comissionat per a Universitat i Recerca de la Generalitat de Catalunya). PJ is a research fellow from the Universidad Austral de Chile, Valdivia, Chile.

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JOURNAL OF CLINICAL MICROBIOLOGY, June 2002, p. 2134–2140 Vol. 40, No. 6
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.6.2134–2140.2002

Pulmonary Infiltrates in Immunosuppressed Patients: Analysis of a Diagnostic Protocol

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Received 30 April 2001/Returned for modification 20 September 2001/Accepted 6 March 2002

A diagnostic protocol was started to study the etiology of pulmonary infiltrates in immunosuppressed patients. The diagnostic yields of the different techniques were analyzed, with special emphasis on the importance of the sample quality and the role of rapid techniques in the diagnostic strategy. In total, 241 patients with newly developed pulmonary infiltrates within a period of 19 months were included. Noninvasive or invasive evaluation was performed according to the characteristics of the infiltrates. Diagnosis was achieved in 202 patients (84%); 173 patients (72%) had pneumonia, and specific etiologic agents were found in 114 (66%). Bronchoaspirate and bronchoalveolar lavage showed the highest yields, either on global analysis (23 of 35 specimens [66%] and 70 of 134 specimens [52%], respectively) or on analysis of each type of pneumonia. A tendency toward better results with optimal-quality samples was observed, and a statistically significant difference was found in sputum bacterial culture. Rapid diagnostic tests yielded results in 71 of 114 (62.2%) diagnoses of etiological pneumonia.

Both infectious and noninfectious pulmonary complications are a major cause of morbidity and mortality in the immunosuppressed host (1, 6, 29). The marked increase in the number and types of transplantations together with the advances in immunosuppressive treatment have created a subset of patients with increased risk for pulmonary complications (8). In addition, almost 70% of the individuals with human immunodeficiency virus (HIV) infection have at least one respiratory episode during the course of their disease (23). Even though a wide variety of etiologic agents are responsible for pneumonia in these patients, the type and timing of immunosuppression predispose the patient to infections by certain pathogens (1). Accordingly, in solid-organ transplant recipients, first bacterial pneumonia, mainly due to gram-negative bacilli and *Staphylococcus aureus*, and then opportunistic infections by cytomegalovirus (CMV) and *Pneumocystis carinii* are most commonly found (1). In neutropenic patients, mainly patients with hematological malignancies and bone marrow transplant recipients, bacterial and fungal pneumonia is the most important challenge (1, 9). On the other hand, in HIV-infected patients *P. carinii* remains a common respiratory pathogen together with *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* (22–24).

Prompt and accurate diagnosis of the cause of pulmonary infiltrates is essential in order to begin specific antimicrobial treatment. Various procedures have been proposed for this purpose, but the optimal management strategy remains controversial (6). Besides fiber optic bronchoscopy techniques, other new procedures have been introduced. For example, induced sputum for the detection of *P. carinii* (13) and nasopharyngeal washing (NPW) for the detection of respiratory viruses (32) are noninvasive techniques that play a role in the diagnostic strategy. In addition, recent advances in microbiological techniques such as *Legionella pneumophila* (28) and *S. pneumoniae* antigen detection in urine, *Aspergillus* sp. antigen detection in serum (20), and molecular biology methods (11, 14, 27) allow rapid diagnostic tests and therefore prompt initiation of specific therapy if pertinent.

We report the results of a prospective study on the etiology of pulmonary infiltrates in immunosuppressed patients. Several groups have been included: patients with bone marrow transplantation, solid-organ transplantation, hematologic malignancies, and HIV infection. The diagnostic yields of the different methods used were analyzed in order to optimize, if possible, the diagnostic strategy in these patients.

MATERIALS AND METHODS

Patient population. We prospectively studied immunosuppressed patients admitted to the Hospital Clínic of Barcelona with a new pulmonary infiltrate from March 1998 to October 1999. The following groups were included: patients with bone marrow transplantation, solid-organ transplantation, hematologic malignancies, and HIV infection and patients undergoing continued immunosuppressive treatment for other reasons. A total of 241 patients were considered for evaluation.

Demographic and clinical data. In all cases, the following variables were recorded at admission: age, sex, type of immunosuppression, temperature, white blood cell count, type (alveolar, interstitial, or mixed) and distribution (unilateral or bilateral) of radiographical infiltrates, and antibiotic status at the time of respiratory sampling (less or more than 48 h of antibiotic treatment).

Sampling. Noninvasive or invasive evaluation was performed according to the characteristics of the pulmonary infiltrates. Patients with a single alveolar pulmonary infiltrate underwent noninvasive evaluation consisting of two sets of blood cultures, a sputum specimen (induced sputum if no expectoration), bronchoaspiration (BAS) in intubated patients, detection of *L. pneumophila* serogroup 1 antigen in urine, NPW for detection of respiratory viruses, and serolog-

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ical tests for atypical pneumonia due to *L. pneumophila* serogroups 1 to 6 (immunoglobulin G [IgG]), *Chlamydia pneumoniae* (IgM, IgG), *Mycoplasma pneumoniae* (IgM, IgG), and *Coxiella burnetii* (IgM, IgG) and viral respiratory infections (adenovirus, respiratory syncytial virus, parainfluenza viruses 1, 2, and 3, and influenza viruses A and B).

Patients with multiple pulmonary infiltrates or interstitial infiltrate underwent invasive evaluation. Patients presenting with a single alveolar pulmonary infiltrate and remaining unresponsive after 3 days of empirical treatment also underwent invasive evaluation. This consisted of fiber optic bronchoscopy examination including a protected specimen brush (PSB) and bronchoalveolar lavage (BAL). These techniques were performed as previously reported (9, 33). Briefly, fiber optic bronchoscopy was performed transnasally or transorally after topical nasal anesthesia was instilled. Once PSB was performed, the brush was aseptically severed and introduced into 1 ml of thioglycolate broth for transport. BAL sampling was carried out by instilling three aliquots of 50 ml of sterile saline solution. The first aspirated portion of the fluid lavage was discarded, and the remainder was processed for stain and culture.

Patients without diagnosis after bronchoscopic evaluation and patients with persistent pulmonary infiltrates were subjected to transbronchial biopsy if possible.

Once the protocol had been started and due to recent advances in microbiological methods, several new diagnostic procedures were performed for selected patients: detection of *S. pneumoniae* antigen in urine, detection of *Aspergillus* sp. antigen in serum, detection by PCR enzyme immunoassay (PCR-EIA) of *C. pneumoniae* in nasopharyngeal swabs, and detection by ligase chain reaction (LCR) of *M. tuberculosis* in clinical specimens.

Laboratory processing of specimens. (i) Culture procedures. Sputum specimens were cultured for bacterial pathogens, fungi, and mycobacteria. Fiber optic bronchoscopy specimens were first homogenized by vortexing and then subjected to quantitative bacterial culture and qualitative culture for fungi and *Legionella*. For quantitative culture, 0.1 ml of PSB fluid and 0.1 ml and a 10-fold dilution of BAL fluid were plated onto each medium. BAL and BAS specimens were also cultured for mycobacteria. Media and incubation conditions were as follows. For bacterial culture, secretions were plated on sheep blood agar and chocolate agar and incubated at 37°C in a CO_2-enriched atmosphere for 2 days. For fungal culture, secretions were inoculated on Sabouraud's agar and incubated at 30°C in aerobic conditions for 4 weeks. Buffered charcoal-yeast extract agar incubated at 37°C in a CO_2-enriched atmosphere for 10 days was used for isolation of *Legionella*. For mycobacteria, Löwenstein-Jensen medium and radiometric BACTEC 12B broth culture bottles (Becton Dickinson) were incubated at 37°C in aerobic conditions for 6 weeks.

NPW and BAL specimens were examined for viruses by cell culture. After decontamination treatment, 0.3 ml of each sample was inoculated onto monolayers of HEp-2, A-549, MRC-5, and MDCK cell lines (Vircell, Granada, Spain). Cultures were maintained for 4 weeks and examined regularly for cytopathic effect. Confirmation of viral growth was done by immunofluorescence staining with monoclonal antibodies (Respiratory Panel 1 viral screening and identification kit; Light Diagnostics, Temecula, Calif.).

(ii) Direct microscopy. Sputum and BAS specimens were stained with the Gram and Ziehl-Neelsen methods. PSB and BAL smears were obtained by cytocentrifuge preparation in accordance with a modification of a described protocol (15) (0.25 and 0.5 ml of fluid for PSB and BAL, respectively, at 1,500 rpm for 15 min; Cytospin 3 centrifuge; Shandon Instruments, Cheshire, England) and subsequently Gram stained for detection of bacteria and fungi and determination of percentage of intracellular organisms. The May-Grünwald Giemsa technique was used for fungal detection and differential cell count of macrophages, squamous epithelial cells, ciliated bronchial cells, neutrophils, lymphocytes, eosinophils, and erythrocytes for assessment of specimen quality. Additional stains were used for BAL smears; these include Ziehl-Neelsen stain for detection of acid-fast organisms, Gomori methenamine silver (GMS) for *P. carinii* and fungi; periodic acid-Schiff stain, hematoxylin-eosin, and Papanicolau stain for detection of malignant cells; and Pearl's stain for detection of siderophages. Viral antigens were detected in NPW and BAL specimens by direct fluorescence employing a pool of monoclonal antibodies against a panel of respiratory viruses (influenza virus A and B, parainfluenza virus 1, 2, and 3, respiratory syncytial virus, and adenovirus). In addition, CMV detection was done by means of an immunocytochemistry method (alkaline phosphatase anti-alkaline phosphatase) using a monoclonal antibody against the pp65-CMV protein (Dako, Glostrup, Denmark).

(iii) Non-culture-dependent methods. Urinary antigen tests were performed for detection of *L. pneumophila* serogroup 1 by EIA (Biotest *Legionella* Urin Antigen EIA; Biotest) and for detection of *S. pneumoniae* by immunochromatography (Binax Now *S. pneumoniae* urinary antigen test; Binax). Detection in serum of *Aspergillus* sp. galactomannan antigen was by EIA (Platelia *Aspergillus*; Sanofi Diagnostics Pasteur). *M. tuberculosis* was determined in clinical samples by LCR (LCx *Mycobacterium tuberculosis* assay; Abbott Laboratories, Abbot Park, Ill.), and detection of *C. pneumoniae* in nasopharyngeal swabs was performed by PCR-EIA (Diasorin).

Diagnostic criteria. The diagnosis of pneumonia was based on the development of new or progressive pulmonary infiltrates together with at least two of the following: fever, cough productive of sputum, leukocytosis, or leukopenia. The diagnosis of pneumonia was definite if the above criteria were associated with one of the following: (i) histopathologic demonstration of pneumonia, with or without isolation of specific etiologic agent, (ii) positive culture of pleural fluid, (iii) positive blood cultures (two positive sets, taken within a period of 48 h), (iv) histopathologic demonstration of tissue invasion by fungal hyphae in lung biopsy specimens, (v) isolation of *L. pneumophila* or *M. tuberculosis* from respiratory samples, (vi) positive urinary antigen test for *L. pneumophila* serogroup 1, (vii) serologic diagnosis (demonstration of seroconversion), (viii) cytologic evidence of CMV inclusion bodies in cytocentrifuge smears, (ix) presence of *P. carinii* in the GMS-stained induced sputum or BAL specimens. If the criteria for definite infection were not met, a diagnosis of a probable case of pneumonia was made if one of these criteria were met: (i) bacterial pathogens yielding $\geq 10^3$ CFU/ml in cultures of PSB, $\geq 10^4$ CFU/ml in cultures of BAL, and $\geq 10^6$ CFU/ml in cultures of BAS; (ii) bacterial pathogen isolated from sputum only if there was heavy growth of the pathogen, sputum was included in group 4 or 5 of Murray and Washington's grading system (10 to 25 epithelial cells and >25 leukocytes per low-power field [group 4] and <10 epithelial cells and >25 leukocytes per low-power field [group 5]) (25), and Gram stain findings correlated with culture results; (iii) positive culture for fungal pathogen in at least two bronchoscopy specimens; (iv) positive culture for fungal pathogen in at least two sputum samples and presence of fungal hyphae in the Gram stain (pseudohyphae and yeasts were excluded); (v) positive cell culture for respiratory viruses from NPW or BAL specimens, (vi) positive antigen detection for respiratory viruses from NPW or BAL specimens.

Absence of pneumonia was indicated by failure to meet the criteria for diagnosis of pneumonia. Patients to whom this applied included those with an alternative diagnosis and those with an undetermined diagnosis.

Criteria for respiratory specimen rejection. Rejection of specimens was based on the following criteria: (i) sputum, induced sputum, and BAS specimens in group 1, 2, or 3 of Murray and Washington's grading system (>25 epithelial cells and <10 leukocytes per low-power field [group 1], >25 epithelial cells and 10 to 25 leukocytes per low-power field [group 2], and >25 epithelial cells and >25 leukocytes per low-power field [group 3]) (25); and (ii) BAL and PSB specimens with >1% squamous epithelial cells. When BAL specimens were processed for detection of *P. carinii* or respiratory viruses, these criteria were not applied.

Criteria for optimum specimen quality. Optimum conditions associated with the highest diagnostic yield were determined for each specimen as follows: (i) sputum and BAS specimens included in group 4 or 5 of Murray and Washington's grading system (25) when considering bacterial pneumonia or in group 4, 5, or 6 (<10 epithelial cells and <10 leukocytes per low-power field) when considering fungal pneumonia; (ii) BAL and PSB specimens with >25% inflammatory cells (neutrophils plus lymphocytes) for bacterial pneumonia or with >10% inflammatory cells for fungal and *P. carinii* pneumonia. For viral pneumonia these criteria were not applied.

Data analysis. Before the data were analyzed, patients were divided into those with and without pneumonia and subsequently pneumonias were divided into definite and probable and into those with an established microbial etiology and those without one. The criteria used to define these groups are the ones shown above (see "Diagnostic criteria").

(i) Diagnostic yield. The diagnostic yields of the different techniques were evaluated according to different points of view. First, a global diagnostic yield was evaluated, including yields for pneumonias with both infectious- and noninfectious-agent etiologies. For this analysis, specimens collected from all patients included in the study were considered. The diagnostic yield was calculated (a) by including all the respiratory specimens collected, irrespective of their quality, and (b) by excluding those specimens fulfilling criteria for respiratory specimen rejection.

Second, the diagnostic yield was evaluated according to the specific etiologic agent, bacterial, fungal, or viral agent. Therefore, only patients with a diagnosis of pneumonia etiology were considered. The diagnostic yield was calculated (a) by including all the specimens collected from patients with a diagnosis of bacterial pneumonia and/or fungal pneumonia and/or viral pneumonia and (b) by excluding specimens not fulfilling the criteria for optimum specimen quality.

Third, the diagnostic yields of the rapid diagnostic techniques were evaluated. The following methods were considered. (a) Staining methods included the

Gram, Ziehl-Neelsen, and GMS methods. The Gram stain was defined as positive if microorganisms were seen predominantly (>10 bacteria per high-power field) and correlated with what grew in significant concentration in culture (bacterial pathogens yielding $\geq 10^3$ CFU/ml in cultures of PSB or $\geq 10^4$ CFU/ml in cultures of BAL or $\geq 10^6$ CFU/ml in cultures of BAS; for sputum specimens, a heavy growth was considered significant). (b) Antigen detection methods included detection of *L. pneumophila* serogroup 1 antigen in urine, detection of *S. pneumoniae* antigen in urine, detection of *Aspergillus* sp. antigen in serum, and detection of respiratory virus antigen from NPW and BAL specimens. (c) Molecular biology-based methods included an LCR-based assay for detection of *M. tuberculosis* and PCR-EIA for detection of *C. pneumoniae*. Analysis of the diagnostic yield was performed by grouping the different methods according to the type of pneumonia; within each type, the rates of diagnoses obtained by rapid methods were calculated.

(ii) Cytologic patterns of BAL specimens according to type of pneumonia. To analyze whether specific cytologic patterns were associated with each type of pneumonia, three patterns were defined depending on the percentage of inflammatory cells (neutrophils plus lymphocytes): (a) >50%; (b) 25 to 50%; (c) <25%.

Statistical analysis. Epi Info software, version 6.02, (Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, Ga.) was used in conjunction with the two-tailed Fisher exact test to compare pairs of proportions. Values of $P < 0.05$ were considered significant.

RESULTS

Study population. Overall, 241 patients (160 males, 81 females; age range, 22 to 73 years, mean age, 43 years) were included in the study. Of these, 135 were HIV-infected patients, 25 had undergone bone marrow transplantation, 26 had hematological malignancies, 40 were solid-organ transplant recipients (26 kidney, 10 liver, 4 heart), and 15 had received continued immunosuppressive treatment for other reasons.

The type and distribution of the pulmonary infiltrates were as follows: 103 patients (43%) had an alveolar infiltrate, 89 (37%) had an interstitial infiltrate, and 49 (20%) had a mixed pattern. These were unilateral in 119 (49%) and bilateral in 122 (51%).

As for the number of diagnostic procedures performed, blood cultures were obtained for 208 (86%) patients, sputum was obtained from 187 (78%), induced sputum was obtained from 56 (23%), and BAS was obtained from 35 (15%). Detection of antigen for *L. pneumophila* in urine was performed for 151 patients (63%), serologic tests for atypical pneumonias and respiratory viruses were performed for 35 (15%) and 20 (8%) patients, respectively, and serologic tests of NPW were performed for 42 patients (17%). Bronchoscopy examination was performed for 146 patients (61%). Among patients with a single alveolar pulmonary infiltrate, 25% underwent bronchoscopy, whereas, among the rest, bronchoscopy was performed for 79%. On the whole, 134 BALs and 110 PSBs were performed. Ten patients underwent transbronchial biopsy.

Etiology of pulmonary infiltrates. According to the diagnostic criteria, a diagnosis of either infectious or noninfectious was achieved in 202 out of 241 patients included in the study (84%). One hundred seventy-three patients (72%) had pneumonia. Among them, 108 (63%) were classified as having a probable pneumonia and the remaining 65 (37%) were classified as definitely having pneumonia. A specific etiologic agent was found in 114 (66%). Bacterial pneumonia was the most frequent (65 cases, including 7 mixed infections), followed by fungal pneumonia (41 cases, including 7 mixed infections) and viral pneumonia (17 cases, including 6 mixed infections). The etiological agents are summarized in Table 1. HIV-infected patients had a significantly ($P < 0.0005$) higher incidence of *S. pneumoniae* and *P. carinii* infection than non-HIV-infected patients, and non-HIV-infected patients had a significantly higher incidence of *S. aureus* and *Aspergillus fumigatus* infection than HIV-infected patients.

TABLE 1. Microbial agents in 114 patients with diagnoses of pneumonia etiology

Pathogen[a]	No. (%) with indicated infection that were:	
	HIV$^+$ (67 cases)	HIV$^-$ (47 cases)
Bacterial agents	42 (63)	24 (51)
Streptococcus pneumoniae	22	1
Staphylococcus aureus		10
Haemophilus influenzae	3	
Pseudomonas aeruginosa	1	5
Acinetobacter baumannii	1	2
Serratia marcescens		1
Morganella morganii		1
Mycobacterium tuberculosis	12	4
Mycobacterium kansasii	2	
Chlamydia pneumoniae	1	
Fungal agents	23 (34)	18 (38)
Aspergillus fumigatus	1	12
Scedosporium prolificans		2
Penicillium spp.		1
Candida spp.	1	1
Pneumocystis carinii	21	2
Viral agents	6 (9)	11 (23)
IVA	3	7
PIV2	1	
PIV3		1
CMV	1	2
Enterovirus	1	
RSV		1
Polymicrobial infections	4 (6)	6 (13)
P. carinii + CMV	1	
P. carinii + *S. pneumoniae*	1	
P. carinii + *S. pneumoniae* + IVA	1	
A. fumigatus + *P. aeruginosa*		2
A. fumigatus + IVA		1
IVA + *P. aeruginosa* + *M. morganii*		1
IVA + *S. aureus*		1
PIV2 + *M. tuberculosis*	1	
RSV + *Candida* spp.		1

[a] IVA, influenza A virus; PIV2, parainfluenza 2 virus; RSV, respiratory syncytial virus.

The association of the radiographic pattern with etiology was analyzed, and a significant relationship between bacterial pneumonia and unilateral alveolar infiltrate was found (24 of 35 patients versus 23 of 88 patients; $P = 0.0002$). All patients with *P. carinii* pneumonia had a diffuse interstitial infiltrate ($P = 0.002$). Mycobacterial, fungal, and viral pneumonias mainly presented a diffuse interstitial pulmonary infiltrate, but there was no statistical significance.

The relationship between CD4 counts of HIV-infected patients and the type of pneumonia was also analyzed. Even though only 70% of patients' CD4 counts were available, the following was determined: 85% of the patients with fungal pneumonias had available CD4 counts of <200 cells/mm^3, whereas 67% of the patients with bacterial pneumonias had

TABLE 2. Diagnostic yields of the different techniques according the type of pneumonia

Specimen	Yield[a] for indicated type of:							Global diagnostic yield[d]
	Bacterial pneumonia		Fungal pneumonia		Mycobacteriosis		Viral pneumonia, AS[b]	
	AS[b]	OS[c]	AS[b]	OS[c]	AS[b]	OS[c]		
BAS								23/35 (66)
Culture	12/15 (80)	11/12 (92)	10/11 (91)[e]	10/11 (91)	1/1	1/1		
Gram stain	11/15 (73)	11/12 (92)	9/11 (82)[e]	9/11 (82)				
Ziehl-Neelsen stain					1/1	1/1		
BAL								70/134 (52)
Culture	11/18 (61)	7/7 (100)	7/12 (58)[e]	6/8 (75)	7/8 (87)	2/2 (100)	14/15 (93)	
Gram stain	8/18 (44)	5/7 (71)	2/12 (17)[e]	2/8 (25)				
Ziehl-Neelsen stain					3/8 (37.5)	2/2 (100)		
GMS stain			22/22 (100)[f]	10/10 (100)				
Viral Ag detection							9/17 (53)	
Sputum								38/187 (20)
Culture	20/35 (57)	20/21 (95)[g]	5/14 (36)[e]	5/13 (38)	13/15 (87)	13/14 (93)		
Ziehl-Neelsen stain					9/15 (60)	9/14 (64)		
PSB								14/110 (12)
Culture	8/13 (61)	4/4 (100)	6/12 (50)[e]	3/4 (75)				
Gram stain	5/13 (38)	4/4 (100)	1/12 (8)[e]	1/4 (25)				
Pleural fluid culture	0/3	0/3	0/11[e]	0/11	1/11 (9)	1/11 (9)		1/11 (9)
Blood culture	15/42 (36)	15/42 (36)	2/16 (13)[e]	2/16 (13)				17/208 (8)
NPW								3/47 (6)
Culture							3/10 (30)	
Viral Ag detection							1/10 (10)	
Bacterial serology	0/5	0/5						0/35
Viral serology							1/2 (50)	1/20 (5)
Induced-sputum GMS stain			1/8 (12)[f]	1/4 (25)				1/56 (2)
L. pneumophila Ag urine	0/28	0/28						0/151

[a] Total number of diagnoses (diagnoses of pneumonia etiology)/total number of specimens fulfilling the criteria for AS or OS (the specimens included in each classification are described in footnotes *b* and *c*, respectively. Percentages are in parentheses.
[b] All specimens (AS) from patients with the indicated diagnoses are included.
[c] Specimens not fulfilling the criteria for optimum specimen (OS) quality are excluded.
[d] Total number of diagnoses (including both infectious- and noninfectious-agent etiologies)/total number of specimens collected.
[e] Cases of filamentous fungi and yeasts.
[f] Cases of *P. carinii*.
[g] $P < 0.05$.

counts of >200 cells/mm^3 ($P < 0.05$). As for patients with mycobacteriosis and viral pneumonias, their CD4 counts were evenly distributed among both categories.

Among patients not fulfilling the criteria for pneumonia, an alternative diagnosis was found for 29 (12%) and 39 (16%) remained with an undetermined diagnosis. Noninfectious causes included pulmonary edema ($n = 8$), diffuse alveolar hemorrhage ($n = 4$), endocarditis ($n = 4$), pulmonary malignancies ($n = 4$), bronchiolitis obliterans with organizing pneumonia ($n = 3$), pulmonary chronic diseases ($n = 4$), adult respiratory distress syndrome ($n = 1$), and pulmonary drug toxicity by bleomycin ($n = 1$).

Diagnostic yield of diagnostic procedures. (i) Global diagnostic yield. On the whole, BAS and BAL proved to be the techniques with the highest diagnostic yields. Diagnosis was obtained in 23 of 35 (66%) of the BASs and in 70 of 134 (52%) of the BALs performed (Table 2).

If respiratory specimens fulfilling criteria for respiratory specimen rejection are excluded, the diagnostic yield obtained is higher but the differences are not significant (data not shown).

The diagnostic yields of the newest diagnostic procedures were not evaluated because they were performed only in selected patients. However, they contributed to the global diagnostic rate as follows: five patients had detectable *S. pneumoniae* antigen in urine, three patients had detectable serum *Aspergillus* antigen, LCR for *M. tuberculosis* was positive in two cases, and PCR-EIA for *C. pneumoniae* was positive in one case.

(ii) Diagnostic yield according to the type of pneumonia. With regard to bacterial pneumonias, the diagnostic procedure with the highest yield was BAS (12 of 15 specimens [80%]), followed by BAL (11 of 18 specimens [61%]) and PSB (8 of 13 specimens [61%]). The Gram stains of BAS, BAL, and PSB were positive in 73, 44, and 38% of the cases, respectively. A comparison of these results to those obtained if only specimens fulfilling criteria for optimum specimen quality were considered indicated significant differences for sputum culture (20 of 35 [57%] compared to 20 of 21 [95%]; $P < 0.005$). The effect of previous antibiotic administration on culture results was analyzed for each procedure, but no significant differences were observed.

As for fungal pneumonias, BAS culture and GMS-stained

TABLE 3. Diagnostic yield of rapid diagnostic techniques

Type of pneumonia (rate of diagnoses[b] [%])	Technique	Diagnostic yield[a]
Bacterial (37/48 [77])	Gram stain of sputum	20/35 (57)
	Gram stain of BAS	11/15 (73)
	Gram stain of PSB	5/13 (38)
	Gram stain of BAL	8/18 (44)
	Assay of *L. pneumophila* Ag in urine	0
	Assay of *S. pneumoniae* Ag in urine	5
	PCR-EIA of *C. pneumoniae*	1
Mycobacteriosis (12/18 [67])	Sputum Ziehl-Neelsen stain	9/15 (60)
	BAS Ziehl-Neelsen stain	1/1
	BAL Ziehl-Neelsen stain	3/8 (37.5)
	LCR for *M. tuberculosis*	2
Fungal (31/41 [76])	Gram stain of sputum	5/14 (36)
	Gram stain of BAS	9/11 (82)
	Gram stain of PSB	1/12 (8)
	Gram stain of BAL	2/12 (17)
	GMS stain of BAL	22/34 (65)
	GMS stain of induced sputum	1/56 (2)
	Assay of *Aspergillus* spp. in serum	3
Viral (10/17 [59])	BAL Ag detection	9/17 (53)
	NPW Ag detection	1/10 (10)

[a] Total number of diagnoses/total number of tests performed (percentage).
[b] Total number of diagnoses/total number of cases of pneumonia.

BAL specimens showed the highest diagnostic yield (10 of 11 specimens [91%] and 22 of 22 [100%], respectively).

The diagnostic yield of the Gram stain in sputum samples was not analyzed because a positive Gram stain was a criterion needed to consider sputum diagnostic of either bacterial or fungal pneumonia.

Mycobacteriosis were analyzed apart from bacterial pneumonias, and BAS was excluded from the analysis because it was performed for only one patient in this group. BAL and sputum cultures proved to be the techniques with the highest yields (7 of 8 specimens [87%] and 13 of 15 specimens [87%], respectively).

Finally, among viral pneumonias, cell culture of BAL and NPW yielded more diagnoses than antigen detection methods. In addition, BAL seems to be more sensitive than NPW (14 of 15 specimens [93%] and 3 of 10 specimens [30%], respectively).

(iii) Diagnostic yields of rapid diagnostic techniques. Gram stain of BAS specimens was the rapid technique with the highest yield in both bacterial and fungal pneumonias (11 of 15 specimens [73%] and 9 of 11 specimens [82%], respectively) (Table 3). As for mycobacteriosis, the best technique proved to be Ziehl-Neelsen staining of sputum (9 of 15 specimens [60%]), and antigen detection of BAL specimens was best for viral pneumonias (9 of 17 specimens [53%]). Considering all cases with a diagnosis of pneumonia, rapid methods yielded results in 71 of 114 (62.2%).

Cytologic patterns of BAL specimens according to type of pneumonia. With cases of polymicrobial infections and cases for which no cytology could be evaluated having been excluded, cytologic patterns of BAL specimens were analyzed (Table 4). Among bacterial pneumonias, six out of the seven (86%) with BAL available had >25% inflammatory cells (three of them had >50% inflammatory cells). For fungal pneumonias, the cytologic patterns for 6 BAL specimens were evenly distributed among patterns 1 to 3, while 4 out of 8 patients (50%) with viral pneumonia had <25% inflammatory cells and 16 out of 18 patients (89%) with *P. carinii* pneumonia had <25% inflammatory cells.

DISCUSSION

A diagnostic protocol was started at our hospital in order to study the etiology of pulmonary infiltrates in immunosuppressed patients and to analyze the diagnostic yields of the different techniques used. The aim of the study was to optimize the diagnostic strategy in this type of patients, both in terms of number of techniques performed and in terms of time. Almost 2 years after initiation of the protocol, the results have been analyzed.

Among the 241 episodes included, diagnosis was achieved in 202 (84%), which represents an important improvement in relation to previous studies performed at our hospital (9). The etiology was an infectious agent in 72% of the cases and a noninfectious agent in 12% of the cases. The most frequent infectious agent was bacterial, in both HIV- and non-HIV-infected patients. *S. pneumoniae* was the most frequent bacterial pathogen in HIV-infected patients (22 cases out of 28), whereas *S. aureus* was the leading pathogen in non-HIV-infected patients (10 cases out of 24). These results are in accordance with several recently published studies (8, 12). The long-known increased risk for mycobacterial infections among immunosuppressed patients, specially among individuals with HIV infection (4, 21, 30), was also confirmed in our study. Accordingly, 14 cases were diagnosed among HIV-infected patients and 4 cases were diagnosed among non-HIV-infected patients.

Fungal agents constituted the second-most-frequent etiology, followed by viral agents. Twenty-one out of 23 fungal infections in HIV-infected patients were due to *P. carinii*. The incidence of *P. carinii* pneumonia in these patients remains high although effective prophylaxis has been widely introduced (24). In the present study most cases can be explained by the fact that HIV infection status was diagnosed as a result of the *P. carinii* pneumonia and therefore there had been no prophylaxis. Interestingly, in three cases *P. carinii* coexisted with other pathogens. On the other hand, *A. fumigatus* was the most common fungal agent among non-HIV-infected patients. Over the last few years in our institution there has been an increase in the number of cases of invasive aspergillosis. Numerous reports have documented this increased incidence, and it has

TABLE 4. Cytologic patterns of BAL according to type of pneumonia

Type of pneumonia	No. of cases with cytologic pattern[a]:				Total[b]
	1	2	3	4	
Bacterial	3	3	1	3	10
Fungal	2	2	2	0	6
Viral	1	3	4	2	10
P. carinii	1	1	16	1	19

[a] Definition of cytologic patterns: 1, >50% neutrophils plus lymphocytes; 2, 25 to 50% neutrophils plus lymphocytes; 3, <25% neutrophils plus lymphocytes; 4, evaluation not possible.
[b] Polymicrobial infections have been excluded.

been related to the growing population of immunosuppressed patients, mainly those with prolonged and intense neutropenia and those undergoing high-dose corticosteroid therapy (10). In addition, outbreaks of nosocomial aspergillosis have been associated with contaminated air due to nearby construction work (10, 18, 26). In our study, the hospital renovation work that is currently taking place may have played a role in the increased number of invasive-aspergillosis cases. This speculation is based on the fact that over the last year there has been an increased isolation of fungi, among them *Aspergillus* spp., in air samples obtained within the hospital (data not shown).

Viral agents are the third-leading cause of pneumonia in both groups, with 17 cases diagnosed. Among them, the influenza A virus is the most frequent viral pathogen followed by CMV. This is specially interesting for HIV-infected patients, in whom the role of respiratory viruses in pneumonia has recently been described (16). Besides upper respiratory illness, respiratory viruses may cause pneumonia, which, due to the special characteristics of the immunosuppressed population, may have serious consequences. Accordingly, there have been several reports of moderate-to-severe pneumonia caused by these viruses (5, 31). However, their real importance in pneumonias when coinfection with bacteria or fungi occurs remains to be assessed. In our study, 6 out of 17 respiratory virus pneumonias were associated with other microbial agents.

Besides the etiology of pulmonary infiltrates, the diagnostic yields of the different procedures were analyzed. Overall, BAS and BAL proved to be the best techniques, with yields of 66 and 52%, respectively. BAS was performed in only 35 cases, 31 being for non-HIV-infected patients. This procedure was done mainly when there was a high suspicion of bacterial infection, and this may partially explain the high yield observed. In contrast, BAL was performed in patients with less-definite radiological patterns, including patients with a worse clinical response. This fact probably influenced the diagnostic yield. The agents most frequently found by BAL were viruses and *P. carinii*, mainly in HIV-infected patients, and the diagnostic yield was high in this group of patients. Seven cases caused by noninfectious agents were retrieved by BAL, all in non-HIV-infected patients. The wide range of infections and etiologies involving noninfectious agents which can be diagnosed by BAL, renders this technique very useful in immunosuppressed patients, in whom the great variety of pulmonary complications is precisely what makes their diagnosis difficult (15).

When the diagnostic yield for each type of pneumonia was considered, special emphasis was put on evaluating the importance of the quality of the samples in the final yield. Thus, although a statistically significant difference was found only in sputum culture, there was a clear tendency toward obtaining better results when the quality of the samples in all categories was optimal. This was evident in bacterial pneumonias, where the rate of diagnosis was highly influenced by the quality of the specimens; the diagnostic yield of sputum bacterial culture increased from 56 to 95% ($P < 0.05$) and the BAL and PSB culture yields increased from 61 to 100% when only optimal-quality specimens were used. Among bacterial pneumonias, all BAL and PSB specimens exhibiting inflammatory cytology (>25% inflammatory cells) showed a diagnosis of bacterial etiology. The lack of statistical significance may be explained by the small number of cases. BAS specimens were not as strongly influenced due to the fact that most fulfilled the criteria for optimum quality.

On comparison of the diagnostic yields of the various techniques used for each type of pneumonia, it is clear that again BAS and BAL were the techniques with the highest yields, regardless of the type of pneumonia. It is interesting to point out that in the diagnosis of *P. carinii* pneumonia, induced sputum had a very poor yield (12%). These results are not in accordance with previous reports (2, 19) showing that induced sputum was a reliable noninvasive means of diagnosing *P. carinii* pneumonia. In the present study, only 1 out of 23 cases was diagnosed by this technique. Moreover, in 7 cases in which BAL had diagnosed *P. carinii*, induced sputum was negative. Obtaining good-quality induced-sputum specimens requires cooperation from the patients and special training of the therapists (3). In our institution, the technique has recently been introduced, and thus more experience is needed to reassess its usefulness.

For viral pneumonia, the lower diagnostic yield of NPW viral isolation (3 of 10 specimens) compared to that of BAL (14 of 15 specimens) may be explained by a delay in sample collection. Seven out of 10 NPWs performed were collected more than 48 h after the onset of symptoms. Furthermore, among the three NPWs collected in the first 24 h, two yielded a positive result. Poor sample quality accounts for the low yield of antigen detection methods in NPW (1 of 10 specimens [10%]). It has been previously reported (7, 17) that the sensitivity of these methods can be enhanced by optimizing sample collection to obtain an adequate amount of material for testing without mucus contamination. In our study, 8 out of 42 (18%) NPWs performed did not yield an adequate number of cells for direct fluorescent-antibody assay (DFA) examination. Consequently, if poor quality samples were excluded from the analysis, the diagnostic yield of NPW DFA in viral pneumonias would be more correctly calculated as one of six specimens (17%).

The role of diagnostic techniques with rapid turnaround times was analyzed. Rapid reporting of results increases the clinical relevance of the information provided by the laboratory and is essential for instituting specific therapy for the immunosuppressed host. Over the last few years, technologic advances have provided new rapid diagnostic procedures such as antigen detection (20, 28) and molecular biology (11, 14, 27) methods. In the present study, new rapid methods have been added to conventional ones. Accordingly, among antigen detection methods, urine antigen tests for detecting *L. pneumophila* serogroup 1 and *S. pneumoniae* have been introduced as have serological tests for detecting *Aspergillus* sp. antigen. As for molecular biology methods, nucleic acid amplification methods for detecting *M. tuberculosis* and *C. pneumoniae* were performed. Except for the detection of *L. pneumophila* in urine, the remaining new procedures were performed in selected cases and therefore more studies are needed to evaluate their usefulness.

Analysis of the diagnostic yield of the rapid diagnostic procedures showed that the Gram stain of BAS and sputum specimens had a high yield in both bacterial and fungal pneumonias. Consequently, even though the information provided is not definitive, appropriate antimicrobial therapy can be selected on the basis of Gram stain results. Neither detection of *L. pneumophila* antigen in urine nor the conventional methods (serology and culture) achieved diagnosis, thereby possibly ex-

plaining the absence of *L. pneumophila* pneumonia in our study. Overall, nearly 80% of bacterial and fungal pneumonias were diagnosed by rapid methods. Regarding mycobacteriosis, the rate of rapid diagnosis was 67% and Ziehl-Neelsen sputum staining gave the best diagnostic yield. For viral pneumonias, DFA is a reliable technique for the rapid diagnosis of viral infections. In the present study, DFA allowed the diagnosis of 59% viral pneumonias.

Finally, the cytologic patterns of BAL specimens from patients with a diagnosis of pneumonia etiology were also analyzed in order to see if the type of inflammatory response could be useful in predicting the type of pneumonia. The clearest association was with *P. carinii* pneumonia: in 89% of the cases there were <25% inflammatory cells. For most of the bacterial pneumonias, even though most of the patients were immunosuppressed, an increased percentage of inflammatory cells was noted. Among viral pneumonias a cytologic pattern of <25% inflammatory cells was the most frequent, whereas in fungal pneumonias no specific pattern was found.

In conclusion, after this protocol was implemented, an increase in the number of diagnoses of the etiology of new pulmonary infiltrates in the immunosuppressed population was observed. However, in view of the analysis of the diagnostic yields of the different methods, new approaches should be considered to optimize the diagnostic strategy. Thus, in the presence of a unilateral alveolar pulmonary infiltrate, bacterial pneumonia should first be excluded by collecting a good-quality sputum sample and empirical treatment could be started immediately based on Gram stain results. For bilateral or interstitial pulmonary infiltrates unresponsive to empirical antibiotics, BAL should be performed; considering the fact that *P. carinii* pneumonia in HIV-infected patients and viral or fungal pneumonia in non-HIV-infected patients are more likely to be found, appropriate laboratory tests should be part of the diagnostic algorithm. Finally, emphasis should be placed on performing rapid diagnostic tests. The availability of results within a few hours makes rapid tests essential for the immunosuppressed population. However, more studies to evaluate the newest procedures are needed in order to improve, if possible, rapid diagnosis in these patients.

ACKNOWLEDGMENT

This study was supported in part by the grant FIS 00/0966.

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Prognostic Factors of Non-HIV Immunocompromised Patients With Pulmonary Infiltrates*

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Study objectives: **To assess the outcome and the prognostic factors in 200 non-HIV immunocompromised patients with pulmonary infiltrates (PIs).**
Design: **Prospective observational study.**
Setting: **An 800-bed university hospital.**
Patients: **Two hundred non-HIV immunocompromised patients (hematologic malignancies, 79 patients; hematopoietic stem cell transplants [HSCTs], 61 patients; and solid-organ transplants, 60 patients).**
Methods: **Investigation of prognostic factors related to mortality using a multiple logistic regression model.**
Results: **Specific diagnosis of the PI was obtained in 78% of the cases (infectious origin was determined in 74%). The overall mortality rate was 39% (78 of 200 patients). Patients with HSCT had the highest mortality rate (53%). A requirement for mechanical ventilation (odds ratio [OR], 28; 95% confidence interval [CI], 9 to 93), an APACHE (acute physiology and chronic health evaluation) II score of > 20 (OR, 5.5; 95% CI, 2 to 14.7), and a delay of > 5 days in establishing a specific diagnosis (OR, 3.4; 95% CI, 1.2 to 9.6) were the variables associated with mortality at the multivariate analysis. The subgroup analysis based on underlying disease confirmed the prognostic significance of these variables and the infectious etiology for the PI.**
Conclusions: **Mortality in immunocompromised patients is high, particularly in patients undergoing HSCT. Achieving an earlier diagnosis potentially may improve the mortality rate of these patients.** ***(CHEST 2002; 122:253–261)***

Key words: immunosuppression; lung infection; mechanical ventilation; prognosis

Abbreviations: APACHE = acute physiology and chronic health evaluation; CI = confidence interval; HM = hematologic malignancy; HSCT = hematopoietic stem cell transplant; MV = mechanical ventilation; OR = odds ratio; PI = pulmonary infiltrate; SOT = solid organ transplant

Immunocompromised patients are at high risk for developing infectious and noninfectious pulmonary complications.[1–4] The mortality rate associated with pulmonary complications is exceedingly high, reaching 85% in some series.[5] Some reports have suggested that the prognosis of these patients might be improving, especially within certain groups of immunocompromised patients.[6] New molecular diagnostic techniques, more effective prophylactic treatments, and reduced toxicity of conditioning regimens likely might be contributing to this improvement in prognosis.[7,8] There is also general agreement that the severity of pulmonary involvement (reflected by the presence of acute respiratory failure and, specifically, by the need for mechanical ventilation [MV]) is an ominous prognostic factor in hematopoietic stem cell transplant (HSCT) recipients. In this group of immunocompromised patients,

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the mortality rate for those requiring MV is > 90%, and very few survive 6 months after the onset of the pulmonary complication.[9] The impact of respiratory failure and the need for MV in other groups of immunocompromised patients has not been well-elucidated. More controversial is the significance of certain other factors as follows: identification of a specific etiologic diagnosis; the role of bronchoscopy[10]; or the inadequacy of empirical treatment in the final outcome.[11,12] This information may be decisive from a clinical point of view and for designing cost-effective diagnostic strategies. In a study involving patients with different types of immunosuppression, Poe et al[1] concluded that obtaining a specific diagnosis with the use of pulmonary biopsy did not influence outcome. Surprisingly, in the series of Poe et al,[1] patients with an etiologic diagnosis who had undergone specific treatment of the pulmonary complication seemed to have a worse prognosis than those without. By contrast, more recent studies suggest that an aggressive approach in immunocompromised patients may improve their outcomes.[13,14] Finally, it is not clear which factors are relevant for different specific groups of immunocompromised patients and which can be applied to the population in its entirety.

Recently, we have reported[7] on the clinical characteristics and diagnostic yield of different noninvasive and bronchoscopic techniques in a population of non-HIV immunocompromised patients with pulmonary infiltrates (PIs). Based mainly on this population, the present study assesses prognostic factors that are related to mortality. Specifically, we were interested in knowing whether some of the prognostic factors influencing outcome in a particular group of immunocompromised patients also could be applied to other immunocompromised groups and which of these factors might be amenable to medical intervention.

Materials and Methods

Patients

Two hundred consecutive non-HIV immunocompromised patients with a first episode of PIs were evaluated prospectively from February 1998 to January 2001. The patient group was composed of 60 patients who had received solid organ transplants (SOTs; renal, 24 patients; liver, 19 patients; cardiac, 11 patients; and renopancreatic, 6 patients), 61 HSCT recipients, and 79 patients with hematologic malignancies (HMs) treated with chemotherapy. The underlying HMs of the 140 patients in the HSCT and HM groups were acute myeloid leukemia (35 patients; 25%), Hodgkins lymphoma (18 patients; 13%), acute lymphoid leukemia (16 patients; 12%), chronic lymphoid leukemia (14 patients; 10%), multiple myeloma (13 patients; 9%), non-Hodgkins lymphoma (10 patients; 7%), and others (34 patients; 24%).

Diagnostic Procedures

Within the first 24 to 48 h after the identification of the PI, samples of blood were drawn for culture and antigen testing (*ie*, pp65 cytomegalovirus and Aspergillus spp galactomannan antigen). A sample of spontaneous or induced sputum was obtained. A Gram stain was performed to assess the quality of the sputum.[15] A sample of nasopharyngeal wash was taken for viral detection and tissue culture. Bronchoscopic methods included protected-specimen brush, bronchial aspirate, and BAL. Although the primary intention was to perform all the above-referred diagnostic procedures in all patients, the ultimate decision as to which of the procedures should be performed was always determined by the clinical state of the patient and the criteria of the physician in charge. Other diagnostic techniques such as transbronchial biopsy and open lung biopsy were carried out in selected patients.

The Ethics Committee of the Hospital Clínic approved the study protocol. Informed consent was obtained from the patients who had been referred for a bronchoscopic exploration. The details of the laboratory procedures and the diagnostic criteria for the etiology of PI have been described in a previous article.[7]

Recorded Variables

The following categoric variables were recorded: age; sex; underlying disease; prophylactic antibiotic treatment within the last month; previous admission to hospital within the last month; time between the onset of symptoms and appearance of radiographic infiltrates (> 7 days/< 7 days); presence of neutropenia; presence of graft-vs-host disease; admission to the ICU; specific etiology of the PI (infectious vs noninfectious); community vs nosocomial pneumonia; pattern of radiographic infiltrates (focal vs diffuse); inadequate empirical treatment; and requirement for MV. The continuous variables that were recorded were as follows: leukocyte and platelet counts; prothrombin rate; creatinine, serum albumin, and protein levels; Pao_2/fraction of inspired oxygen ratio; and acute physiology and chronic health evaluation (APACHE) II score.[16]

Definition

Etiology of the PI: The PI was considered to be infectious when there was clinical suspicion of a lower respiratory tract infection and a microbial agent was isolated in respiratory and/or nonrespiratory samples. Pulmonary infection was considered to be nosocomial (in-hospital) on appearance after 72 h of hospital admission. Noninfectious PIs were considered when the clinical data did not suggest an infectious etiology, no microbiological agents were isolated in any processed sample, and the clinical course and response to treatment were in accordance with an alternative noninfectious etiology.

Prophylactic Antibiotic Treatment: This was defined as the administration of prophylactic antibiotics during the last month previous to the onset of PIs.

Inadequate Empirical Treatment: This was defined as empirical treatment that was administered that does not specifically cover the particular etiology of the PI (both infectious or not infectious).

Diagnosis Delay: This was defined as the period of time between the day the presence of PIs was first demonstrated and the day that results of the diagnostic procedure were available.

Mortality: This was defined as in-hospital death.

Statistical Analysis

Differences between groups of immunocompromised patients and between survivors and nonsurvivors were assessed using the

Mann-Whitney *U* test for continuous variables, the χ^2 test for categoric variables, and the Fisher exact test in the case of small expected frequencies. The variables analyzed were those selected as potential predictors of outcome in this population according to the literature.[1,8,15,17–19] Some continuous variables were categorized. In order to optimize the threshold that would discriminate between survivors and nonsurvivors, the formula for threshold computation based on the median value (*ie*, the 50th percentile) was used. The influence of several variables on mortality was evaluated by univariate analysis using the χ^2 test (or the Fisher exact test). Thereafter, a multiple logistic regression model was applied to the variables found to be significantly associated with death. Multiple logistic regression analysis permitted an estimate of the odds ratio (OR) of death and a calculation of the 95% confidence interval (CI). A multivariate analysis of prognostic factors was performed for the whole population (n = 200) and for the following three different groups of patients studied: HSCT, 61 patients; HM, 79 patients; SOT, 60 patients. All statistics were calculated using a statistical software package (SPSS for Windows, version 10.0; SPSS; Chicago, IL). All p values reported are two-tailed, and the data are presented as the mean ± SD or as a percentage.

Results

Clinical Characteristics and Etiology of PIs

Of the 200 immunocompromised patients included in the present study, 109 (54%) were admitted to the ICU and 92 (46%) required MV. The clinical characteristics of the three groups of patients evaluated were similar (Table 1), except for gender (*ie*, more men among the SOT patients), neutropenia (*ie*, fewer cases among the SOT patients), and prophylactic antibiotic treatment (*ie*, more frequent among HSCT patients). Patients with HMs did not require MV as often as those in the other two groups. Patients who had received HSCTs had lower hematocrits and platelet counts. Creatinine levels were higher in SOT patients. Overall, a definite etiology was established in 157 cases (78%). Of the 157 patients with specific diagnoses, this information was obtained using noninvasive techniques in 44% and bronchoscopic techniques in 56%. The etiology of the PIs was infectious in 116 of 157 patients (74%), and it was noninfectious in 41 of 157 patients (26%). The different etiologies of PI are reported in Table 2. Of the infectious etiologies, bacteria were the most common microorganisms causing PI (46 of 116 patients; 23%), followed by fungi (30 patients; 15%), virus (20 patients; 10%), mixed infections (15 patients; 8%), and other infectious etiologies (5 patients; 2%). All infectious agents are summarized in Table 3. Table 4 shows the diagnostic yield of the different noninvasive and bronchoscopic techniques.

Table 1—*Clinical Characteristics of the Different Immunocompromised Groups Evaluated (n = 200)**

Variables	HSCT (n = 61)	HMs (n = 79)	SOT (n = 60)	p Value
Categoric				
Gender				
Male	38	44	47	0.02
Female	23	35	13	
Prophylactic antibiotic treatment	53 (87)	45 (57)	27 (45)	0.0001
Prior admission to hospital	19 (31)	22 (28)	12 (20)	0.36
APACHE II score > 20	25 (41)	30 (38)	23 (38)	0.93
Delay in diagnosis > 5 d	15 (34)	29 (55)	22 (58)	0.06
Acute onset of PI	36 (72)	44 (76)	49 (89)	0.07
Bilateral infiltrates in chest radiograph	37 (61)	41 (28)	28 (47)	0.3
Pao_2/Fio_2 < 250 mm Hg	37 (61)	41 (28)	28 (47)	0.3
Neutropenia†	26 (43)	34 (43)	3 (5)	< 0.0001
Infectious etiology	33 (70)	43 (75)	40 (75)	0.8
Nosocomial pneumonia	27 (63)	28 (46)	27 (66)	0.3
MV	30 (49)	25 (32)	37 (62)	0.002
Inadequate empirical treatment	26 (55)	22 (38)	18 (34)	0.08
Undiagnosed	14 (23)	22 (28)	7 (12)	0.07
Mortality	32 (53)	23 (29)	23 (38)	0.02
Continuous				
Age, yr	41 ± 12	52 ± 18	52 ± 14	< 0.0001
APACHE II score	18 ± 7	18 ± 7	20 ± 8	0.6
Hospital stay, d	29 ± 19	29 ± 17	26 ± 24	0.7
ICU stay, d	11 ± 9	13 ± 12	17 ± 14	0.08
MV, d	9 ± 9	11 ± 10	14 ± 13	0.22
Hematocrit, %	27 ± 5	29 ± 6	30 ± 4	0.014
Platelets, 10^9 cells/L	53 ± 56	94 ± 100	110 ± 81	0.001
Creatinine, mg/L	1.6 ± 1.4	1.1 ± 0.7	3.2 ± 2.3	< 0.0001
Albumin, g/L	30 ± 5	31.7 ± 5	31.8 ± 6	0.6

*Values given as No. (%) or mean ± SD, unless otherwise indicated. Fio_2 = fraction of inspired oxygen.
†Neutropenia was defined as a granulocyte count of < 1,000 cells/μL.

Table 2—*Etiologic Diagnosis in Relation to the Underlying Immunosuppressed Condition

Variables	HSCT (n = 61)	HMs (n = 79)	SOT (n = 60)	Total (n = 200)	p Value
Bacterial	7 (11)	18 (23)	21 (35)	46 (23)	0.009
Fungal	8 (13)	15 (19)	7 (12)	30 (15)	0.4
Viral	12 (20)	6 (8)	2 (3)	20 (10)	0.007
Polymicrobial	5 (8)	1 (1)	9 (15)	15 (7.5)	0.009
Other infectious etiologies†	1 (2)	3 (4)	1 (2)	5 (2.5)	0.6
Pulmonary edema	3 (5)	3 (4)	11 (18)	17 (8.5)	0.005
DAH	5 (8)	3 (4)	2 (3)	10 (5)	0.4
BOOP	2 (3)	3 (4)		5 (2.5)	0.3
Other noninfectious etiologies‡	4 (7)	5 (6)		9 (4.5)	0.13
Undetermined	14 (23)	22 (28)	7 (12)	43 (22)	0.07

*Values given as No. (%), unless otherwise indicated. DAH = diffuse alveolar hemorrhage; BOOP = bronchiolitis obliterans organizing pneumonia.

†Includes tuberculosis (three patients) and *Pneumocystis cannii* pneumonia (two patients).

‡Includes five cases of pulmonary involvement of Hodgkins disease, two cases of drug toxicity due to bleomycin, one case of alveolar proteinosis, and one case of sarcoidosis.

Mortality Rate

The crude mortality rate was 39% (78 of 200 patients). The mortality rate among patients with infectious PIs was 51% (59 of 116 patients), and the mortality rate among patients with noninfectious PIs was 17% (7 of 41 patients) [$p < 0.0001$]. No differences were observed in mortality rates between the different infectious etiologies (*ie*, bacterial, fungal, and viral). The mortality rate in undiagnosed patients was 28% and in patients with a specific diagnosis, 42% ($p = 0.1$). The mortality rate was higher in patients who had received HSCTs (53%) than in those who had received SOTs (38%) or those who had HMs (29%) [$p = 0.02$; Table 1].

Prognostic Factors

Univariate Analysis for the Whole Population: Ten variables were associated with increased mortality rate in the univariate analysis of the whole population (Table 5). Variables reflecting the severity of the disease, such as an APACHE II score of > 20 and the presence of bilateral infiltrates on chest radiographs, had a decisive influence on mortality rate. The requirement for MV had the strongest association with mortality rate. Hypoalbuminemia, an acute onset of PIs, the infectious etiology of the PI, and a nosocomial origin of infection also were associated with a higher mortality rate. Finally, three variables that are amenable to potential medical intervention, such as prophylactic antibiotic treatment, inadequacy of empirical treatment, and delay of more than 5 days (the median value for the delay in establishing the diagnosis of the whole population) in establishing the diagnosis were associated with poor outcomes.

Multivariate Analysis for the Whole Population: We used a multivariate statistical approach to identify which significant factors in the univariate analysis were independently related to mortality rate. A requirement for MV, an APACHE II score of > 20, and a delay in diagnosis of > 5 days were the variables selected in the model when the whole population was analyzed. The simultaneous presence of these three factors was associated with a mortality rate of 92% (31 of 34 patients; OR, 32; 95% CI, 9.5 to 112), whereas their absence was associated with a mortality rate of only 3% (1 of 34 patients; $p < 0.0001$). The mortality rate in intubated patients was 77% (71 of 92 patients; OR, 49; 95% CI, 20 to 121), and in nonventilated patients it was 6% (7 of 108 patients; $p < 0.0001$). The median APACHE II score in the 200 patients evaluated was 18 (range, 5 to 40). The median APACHE II score among the survivors was 14 ± 5, and it was 25 ± 6 among nonsurvivors ($p < 0.0001$). There was no difference among the three types of immunocompromised patients evaluated regarding the APACHE II score. No patient with an APACHE II score of ≥ 26 at hospital admission survived to hospital discharge. The mortality rate among patients in whom the diagnosis was established during the first 5 days was 32% (24 of 74 patients), and among patients in whom the diagnosis was established later it was 51% (42 of 83 patients; $p = 0.024$). The delay in diagnosis was also a variable related to mortality rate when only patients with an infectious etiology (n = 116) of the PI were evaluated. Thus, the mortality rate in this subgroup of immunocompromised patients was 38% (18 of 47 patients) when the diagnosis was established during the first 5 days and 60% (41/69 patients) when the diagnosis was established later ($p < 0.03$).

Different Groups of Immunocompromised Patients: Table 6 shows the variables related to mortality rate for the different groups of immunocompro-

Table 3—*Infectious Pathogens Isolated in 116 Patients (74%) With an Infectious Etiology of the PI

Pathogens	Cases, No.
Bacterial	
Gram-positive	
S aureus†	13
Streptococcus pneumoniae	3
Streptococcus mitis	1
Nocardia asteroides	1
Gram-negative	
Escherichia coli	7
Pseudomonas aeruginosa	6
Acinetobacter baumanii	3
Serratia marcescens	2
Klebsiella pneumoniae	1
Morganella morganii	1
Stenotrophomones maltophilia	1
Proteus mirabilis	1
Other bacteria	
Legionella pneumophila	3
Chlamydia pneumoniae	2
Mycoplasma pneumoniae	1
Fungal	
Aspergillus fumigatus	20
Candida albicans	6
Candida kruseii	1
Candida tropicalis	1
Scedosporium prolificans	1
Penicillium purpurogenum	1
Viral	
CMV	8
Influenza A virus	4
Respiratory syncytial virus	4
Parainfluenzae virus type 3	2
VHS-1	2
Others	
Mycobacterium tuberculosis	3
Pneumocystis carinii	2
Mixed infections	
Aspergillus sp + MRSA	3
Aspergillus sp + *P aeruginosa*	1
E coli + *K pneumoniae* + *S maltophilia* + *P aeruginosa*	1
Aspergillus sp + CMV	1
Citrobacter freundii + *P aeruginosa*	1
MRSA + *Enterococcus fecalis*	1
A fumigatus + VHS-1	1
A baumanii + *P aeruginosa*	1
Aspergillus flavus + *Enterococcus faecium* + CMV	1
A fumigatus + *S pneumoniae*	1
Aspergillus niger + *E coli* + MRSA	1
P aeruginosa + CMV	1
C tropicalis + *A fumigatus* + *P aeruginosa*	1

*MRSA = methicillin-resistant *S aureus*; CMV = cytomegalovirus; VHS = virus herpes simplex.
†Nine cases were MRSA.

Table 4—*Diagnostic Yield of the Different Procedures Performed

Diagnostic Techniques	Positive/Performed
Blood cultures	32/192 (17)
Aspergillus antigen detection	12/66 (18)
CMV antigen detection	14/98 (14)
Nasopharyngeal wash	13/60 (22)
Sputum	20/78 (26)
Bronchial aspirate	47/89 (53)
Protected specimen brush	31/129 (24)
Bronchoalveolar lavage	70/140 (50)
Transbronchial biopsy†	6/12 (50)
Open lung biopsy‡	2/2 (100)

*Values given as No./Total No. (%). See legends of Tables 2 and 3 for abbreviations not used in the text.
†Includes two cases of BOOP, two cases of pulmonary involvement of Hodgkins disease, and two cases of bacterial pneumonia.
‡Includes one case of pulmonary tuberculosis and one case of BOOP.

mised patients. Interestingly, two variables related to mortality rate when the whole population was studied (*ie*, the need for MV and an APACHE II score > 20) also had prognostic significance in each of the three different subgroups. Similarly, an infectious etiology for the PI was a variable with prognostic significance for each of the different groups. Table 7 shows the variables with prognostic significance in each group of immunocompromised patients when evaluated on a multivariate basis. The need for MV was the only variable that significantly affected mortality rate in HSCT patients. APACHE II score and an infectious etiology of the PI also had prognostic significance in patients with HMs. Finally, APACHE II score and diagnosis delay were the dominant independent variables that significantly predicted mortality in SOT patients.

Discussion

The results of the present study show that a high APACHE II score at diagnosis, the need for MV, and a delay in establishing a specific diagnosis are factors associated with mortality rate in a mixed population of immunocompromised patients. The analysis of each type of immunosuppression confirmed the prognostic relevance of the above-mentioned variables and also the significance of an infectious etiology for the infiltrates.

The in-hospital mortality rate of our population of immunocompromised patients as a whole was 39% (78 of 200 patients). The mortality rate among HSCT patients was almost twofold higher than that of patients with HMs or of those who had received SOTs. Although other studies have confirmed the high mortality rate in patients who had received HSCTs and those with HMs with pulmonary complications,[20,21] there is little information in the literature regarding the mortality rate in patients who had received SOTs and those with PIs. Torres et al[22] found a 32% mortality rate in a series of 50 patients

Table 5—*Comparison Between Survivors and Nonsurvivors for All Groups of Immunocompromised Patients Evaluated**

Variables	All Patients (n = 200)	Survivors (n = 122)	Nonsurvivors (n = 78)	p Value†	OR (95% CI) [p Value]‡
Gender					
Male	129	82	47	0.4	
Female	71	40	31		
Prophylactic antibiotic treatment	125 (63)	66 (54)	59 (76)	0.003	
Prior admission to hospital	53 (27)	28 (23)	25 (32)	0.19	
APACHE II score > 20	78 (39)	17 (14)	61 (78)	< 0.0001	5.5 (2–14.7) [0.0007]
Delay in diagnosis > 5 d	83 (53)	41 (45)	42 (64)	0.024	3.4 (1.2–9.6) [0.018]
Acute onset of PI	129 (79)	69 (72)	60 (90)	0.006	
Bilateral infiltrates in chest radiography	106 (53)	56 (46)	50 (64)	0.014	
Albumin < 29 g/L	89 (51)	47 (42)	42 (69)	0.001	
Neutropenia	63 (31)	34 (28)	29 (37)	0.21	
Infectious etiology	116 (74)	57 (63)	60 (90)	< 0.0001	
MV	92 (46)	21 (17)	71 (91)	< 0.0001	28.4 (8.7–9.3) [< 0.0001]
Nosocomial pneumonia	69 (60)	25 (43)	44 (76)	0.001	
Inadequate empirical treatment	66 (42)	32 (35)	34 (51)	0.05	
Undiagnosed	43 (22)	31 (25)	12 (16)	0.1	

*Values given as No. (%), unless otherwise indicated.
†Univariate analysis.
‡Multivariate analysis.

who had undergone orthotopic liver transplantation, and Sternberg et al[23] observed a 16% mortality rate in renal transplant recipients. In the present study, 23 of 60 SOT patients (38%) died, with a 42% mortality rate for liver transplant patients and a 29% mortality rate for renal transplant patients (p = not significant).

Two factors related to the severity of the pulmonary complications had prognostic significance in each of the three different groups of immunosuppressed patients studied and also when the whole population of immunosuppressed patients was grouped together. The usefulness of the APACHE II score as a prognostic factor in bone marrow transplant patients already has been demonstrated,[8] and the present study confirms its utility in different groups of immunosuppressed patients. The need for MV was also a predictive factor of mortality in both the univariate and the multivariate analysis of the entire population (OR, 28.4), confirming this variable as the most important determinant of mortality. The avoidance of intubation may change the dismal prognosis associated with MV, particularly in HSCT patients.[6] In this sense, two randomized studies have shown that the early implementation of noninvasive MV in both immunocompetent and immunocompromised patients with PIs decreased the requirement of intubation and the incidence of nosocomial pneumonia, and improved the prognosis of these patients.[24,25] Based on the extremely poor prognosis associated with MV and the promising results obtained in the above-mentioned studies, it seems logical to recommend the application of noninvasive MV to immunocompromised patients with PIs once significant respiratory failure has ensued. However, although the employment of this modality of ventilation may avoid intubation in these patients, it may not be appropriate for or tolerated by all of them.[26]

A delay in establishing a specific diagnosis was a prognostic factor for the whole population of evaluated patients and also for SOT patients when the different groups of immunosuppressed patients were considered separately. Diagnostic delay is a variable with important clinical implications since it is potentially modifiable by medical intervention.[27–29] Confidence in the empirical antibiotic treatment, the unavailability of specific diagnostic technologies, or, more often, the rapid development of acute respiratory failure that precludes bronchoscopy may explain the delay in diagnosis in individual patients. In the present study, the higher mortality rate among patients in whom there was a diagnostic delay of > 5 days cannot be attributed to the time spent in performing specific diagnostic procedures (*ie*, cultures), since no differences were observed in the incidence of different infectious (*ie*, bacterial, fungal, and viral) and noninfectious complications between patients who received diagnoses before or after 5 days of evolution (data not shown). Similarly, the failure to make an early diagnosis was not a marker for a patient who was too ill to undergo bronchoscopy because diagnostic delay retained its prognostic significance when only those patients undergoing bronchoscopy were selected for the analysis. We

Table 6—*Univariate Analysis for Each Group of Immunocompromised Patients Evaluated

Variables	HSCT (n = 61)			HMs (n = 79)			SOT (n = 60)		
	Survivors	Nonsurvivors	OR (95% CI) [p Value]	Survivors	Nonsurvivors	OR (95% CI) [p Value]	Survivors	Nonsurvivors	OR (95% CI) [p Value]
Prophylactic antibiotic treatment	24 (83)	29 (91)	[0.3]	30 (54)	15 (65)	[0.24]	12 (32)	15 (65)	4 (1.3–11.7) [0.013]
APACHE II score > 20	2 (7)	23 (72)	34 (6.7–176) [< 0.0001]	11 (20)	19 (83)	19.4 (5.5–68.7) [< 0.0001]	4 (11)	19 (83)	39 (8.7–175) [< 0.0001]
Delay in diagnosis > 5 d	13 (59)	18 (72)	[0.2]	19 (50)	11 (58)	[0.4]	9 (29)	13 (59)	3.5 (1.2–11.2) [0.02]
Acute onset of PI	15 (60)	21 (84)	[0.06]	27 (71)	17 (85)	[0.24]	27 (82)	22 (100)	1.8 (1.4–2.3) [0.04]
Bilateral infiltrates in chest radiograph	11 (38)	26 (81)	7 (2.2–22.7) [0.001]	30 (54)	11 (48)	[0.6]	15 (40)	13 (56)	[0.2]
Infectious etiology	12 (55)	21 (84)	4.3 (1.1–17) [0.028]	25 (66)	18 (95)	9.3 (1.1–78.2) [0.017]	20 (64)	20 (91)	5.5 (1.1–28) [0.028]
MV	1 (3)	29 (91)	270 (27–760) [< 0.0001]	6 (11)	19 (83)	39.5 (10–156) [< 0.0001]	14 (38)	23 (62)	2.6 (1.7–4) [< 0.0001]

*Values given as No. (%), unless otherwise indicated.

believe that early diagnosis using different noninvasive and bronchoscopic techniques potentially could improve the prognosis of these patients. Although there are patients with severe hypoxemia in whom it may not be safe or feasible to perform a bronchoscopy, a recent study by Hilbert et al[30] has shown that the application of a laryngeal mask in airways is a safe and effective alternative to intubation for accomplishing bronchoscopy with BAL in immunocompromised patients with suspected pneumonia and severe hypoxia. Surprisingly, the delay in establishing a specific diagnosis was not a prognostic factor for all the groups of immunosuppressed patients when they were evaluated separately, and it remained significant only in the SOT group. This does not imply that trying to get an early diagnosis for PIs in HSCT patients or in those with HMs is unhelpful. These two latter groups of patients often are treated with empirical antibiotics as a diagnostic strategy is developed. Furthermore, an intense immunosuppression may accelerate the course of the pulmonary disease in that a cutoff point of 5 days might be too late to find significant differences between survivors and nonsurvivors. The fact that patients with an acute presentation of the PI (*ie*, < 7 days) had a higher mortality rate (Tables 5 and 6) further emphasizes the importance of designing strategies aimed at obtaining an early diagnosis in immunocompromised patients. The potential benefits of a bronchoscopic evaluation performed immediately after the identification of a PI to achieve early diagnosis must be evaluated in properly designed studies.

Although it was confirmed only for the HM group in the multivariate analysis, it is interesting that the univariate analyses performed in the three groups of immunocompromised patients separately showed that patients with infectious etiologies of their PIs had worse prognoses.[31] This further supports the relevance of obtaining a specific diagnosis, not only to offer a specific treatment, but also for prognostic purposes. Another finding that further emphasizes the need for obtaining a specific diagnosis is the prognostic relevance of an inadequate empirical treatment. The prognostic significance of an inadequate empirical treatment also has been evidenced by other authors evaluating patients with nosocomial pneumonia.[11,12] In almost 42% of the patients with a specific diagnosis, the empirical treatment did not cover the concrete etiology causing the PI. This variable had prognostic significance in the univariate analysis of the whole population and was particularly worrisome for patients with an infectious etiology since it carried a mortality rate of 64%, while the mortality rate was only 21% among patients with noninfectious origins of their PIs ($p < 0.02$). The inadequacy of the empirical antibiotic treatment was

Table 7—*Prognostic Factors in Relation to the Specific Immunosuppressive Condition

Variables	HSCT	HMs	SOT
Infectious etiology		15.6 (1.1–218) [0.041]	
Delay in diagnosis > 5 d			9.8 (0.9–104) [0.057]
APACHE II score > 20		7.6 (1.3–45.2) [0.026]	35 (3.5–350) [0.002]
MV	241.5 (20.3–2861.5) [< 0.0001]	15.2 (2.7–86.7) [0.002]	

*Values given as OR (95% CI) [p value].

attributable mostly to infections by Aspergillus spp, viruses, methicillin-resistant *Staphylococcus aureus*, multiresistant Gram-negative bacilli, and mycobacterium. Finally, receiving prophylactic antibiotic treatment prior to the appearance of the PI had prognostic implications in SOT patients. This variable is a well-known factor predisposing patients to lung infections by multiresistant microorganisms, and it underlines the importance of establishing a judicious antibiotic policy.[19]

The present study has limitations that have to be considered for the interpretation of the results. This was a noncontrolled observational study that evaluated different groups of immunocompromised patients. Although the total number of patients evaluated was rather high, the number of patients in any of the three groups might be insufficient to identify certain variables as being relevant for outcome.

In summary, we have described the mortality rate and have analyzed the prognostic factors of a large series of immunocompromised patients with PIs. Of these factors, MV requirement, a high APACHE II score at the onset of the pulmonary complication, and a diagnostic delay of > 5 days are associated with a high mortality rate when the population is studied as a whole. The use of methods aimed at achieving the early diagnosis of PIs is recommended to try to decrease the high mortality rate observed in this population.

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RESULTADOS Y DISCUSIÓN

En esta serie sobre infiltrados pulmonares en pacientes con infección por el VIH, la tasa de incidencia durante el primer año del estudio fue de 18 episodios por 100 ingresos hospitalarios/año (intervalo de confianza 95%: 15-21).[78] Al finalizar los dos años de estudio, la incidencia acumulada era del 25% (249 episodios sobre 1016 ingresos hospitalarios consecutivos).[79] Esto supone que, de cada cuatro pacientes VIH que ingresan en el hospital, uno de ellos presenta a su ingreso, o durante el mismo, una complicación respiratoria que se manifiesta mediante la presencia de infiltrados pulmonares radiológicos. La neumonía bacteriana fue el diagnóstico más frecuente, seguido de la NPC y las micobacteriosis, por este orden.

En los primeros estudios sobre complicaciones pulmonares en pacientes con infección por VIH, la NPC constituía la primera causa, produciendo hasta el 85% de los casos[11,80]. No obstante, estos estudios iniciales incluían sólo pacientes que cumplían las antiguas definiciones de SIDA, casi todos con avanzada inmunosupresión. Entre el 70-90% de estos pacientes en Norteamérica y el Oeste de Europa desarrollaban algún episodio de NPC en algún momento de su enfermedad.[34] La epidemiología de la NPC ha cambiado profundamente tras la generalización de la profilaxis primaria frente a *P. carinii*. Así, mientras en Europa en la década de los ochenta la NPC era la enfermedad definitoria de SIDA en el 30-50% de los pacientes, sólo lo fue en el 10-20% de los sujetos incluidos en 1994 en la cohorte del estudio EuroSIDA.[81,82] Estos datos son similares a los de otros estudios observacionales realizados en Norteamérica y Australia tras la introducción de la profilaxis primaria.[83,84,85,86] Además la incidencia de NPC disminuyó en aproximadamente un tercio en

pacientes con similares grados de inmunosupresión, comparada con los pacientes con SIDA en los años 80.[81] Posteriormente, la introducción del TARGA también ha supuesto un claro descenso en la incidencia de NPC, como en la de otras infecciones oportunistas.[44,46,87,88] Pero aunque la infección por *P. carinii* se ha vuelto menos prevalente en los últimos años, continúa siendo la principal causa de enfermedad definitoria de SIDA y la infección oportunista más frecuente en los pacientes VIH en Norteamérica y Europa.[28,83,89,90,91,92] En el estudio actual, la NPC constituye la segunda causa de infiltrados pulmonares, con una incidencia acumulada del 17% que se mantuvo tanto en el primer año, como al cabo de los dos años de estudio.[78,79] La tasa de incidencia al cabo de los dos años de estudio fue de 4 episodios de NPC por 100 ingresos hospitalarios-año (IC 95% 3-5), que se mantuvo prácticamente constante durante cada uno de los años del estudio. Ninguno de los pacientes que presentó la neumonía realizaba profilaxis frente a *P. carinii* de forma regular, y el desarrollo de la NPC se produjo antes del diagnóstico de la infección por el VIH en el 40% de los casos. Hubo una diferencia significativa en la incidencia de NPC entre los pacientes que recibían TARGA y los que no.[78] Todos estos datos muestran cómo ha cambiado la epidemiología de la infección por *P. carinii*. En la actualidad, ésta se produce en los pacientes que no realizan la profilaxis (primaria o secundaria), o lo hacen de forma irregular, lo que en la mayoría de los casos coincide con pacientes que no reciben o no realizan adecuadamente el tratamiento antirretroviral, y otro importante porcentaje (que puede llegar al 50%)[93] en aquellos en los que el diagnóstico de infección por el VIH se hace coincidiendo con el desarrollo de la NPC, como han confirmado otros estudios.[94,95,96,97] Esta situación enfatiza la importancia de

realizar un diagnóstico precoz de la infección por VIH, particularmente en los pacientes de riesgo.[93]

La media de linfocitos CD4 en los pacientes con NPC de este estudio era de 20 células/mm^3 y todos tenían un número inferior a 200/mm^3, de forma similar a lo descrito previamente[98,99]. Apenas hay información en la literatura sobre la relación entre la carga viral plasmática del VIH y las diferentes infecciones o tumores que se producen con mayor frecuencia en las personas infectadas por el mismo. Un estudio reciente basado en una cohorte previa a la introducción de la profilaxis frente a *P. carinii*, ha puesto de manifiesto la existencia de un notable incremento del riesgo de desarrollar NPC entre los pacientes con mayores niveles basales de carga viral (al diagnóstico de la infección por el VIH), en las tres categorías de CD4 consideradas (≥ 350, 200-349 y <200/mm^3). En nuestro estudio, la mediana de carga viral en los casos de NPC fue significativamente superior a la de los otros grupos diagnósticos (neumonía bacteriana y micobacteriosis) (Figura 1, referencia 78). La carga viral era superior a 50000 copias/ml en todos los pacientes, y no hubo ningún caso de NPC en pacientes con menos de 200 copias/ml.

En las investigaciones realizadas en las últimas décadas se ha hecho evidente que hay otras enfermedades pulmonares importantes en las personas infectadas por el VIH, no sólo en las que tienen criterios de SIDA. En nuestra serie se confirma, como en otras recientes, que la neumonía bacteriana constituye la primera causa de enfermedad pulmonar en pacientes con infección por el VIH, y que se produce con mucha mayor frecuencia que la NPC.[42,100] También se ha constatado que es mucho más frecuente en estos pacientes que en la población general (la neumonía bacteriana recurrente de

hecho constituye en la actualidad un criterio de SIDA), incluyéndose entre los principales factores de riesgo el descenso del número de linfocitos CD4/mm^3 y el uso de drogas por vía parenteral. Además, la neumonía bacteriana constituye en la era del TARGA la causa más frecuente de ingreso hospitalario en esta población.[101,102] En nuestra serie, casi la mitad de los infiltrados pulmonares se debieron a neumonías bacterianas, al cabo de los dos años de estudio (47%)[79]; un porcentaje superior al observado en los estudios realizados en los últimos años ochenta (20-45%),[103,104] y sobre todo al de las primeras series, que sólo incluían pacientes con criterios de SIDA (2-10%).[10,11,105] Sin embargo, la incidencia de neumonías bacterianas en la serie actual (12 episodios por 100 ingresos hospitalarios-año [IC 95% 10-13]) fue similar a la encontrada en otras dos investigaciones realizadas en la década de los noventa, previas a la introducción del TARGA: 12,5 casos por 100 ingresos y año.[106,107] En nuestra serie, no hubo diferencia en cuanto al porcentaje de neumonías bacterianas entre los pacientes que recibían o no TARGA. Pocos trabajos -todos ellos retrospectivos- han evaluado el impacto de la introducción del TARGA sobre la incidencia de las neumonías bacterianas en la población de pacientes VIH.[108,109,110] Uno de ellos se realizó sobre pacientes con menos de 200 linfocitos CD4/mm^3 a su entrada en el estudio, encontrándose un descenso significativo de 22,7 episodios/100 personas y año durante la primera mitad de 1993 a 12,3 episodios/100 personas y año durante la primera mitad de 1996 y finalmente 9,1 episodios/100 persona y año en la segunda mitad de 1997.[110] Sin embargo, las características inmunitarias basales de estos pacientes no los hacen comparables con los de otros estudios. La introducción del TARGA en este grupo probablemente favoreció una clara mejoría

inmunológica, disminuyendo por lo tanto el riesgo de desarrollo de neumonías bacterianas. Los otros dos estudios arrojan resultados contradictorios. En uno de ellos la incidencia de neumonía bacteriana aumentó en la era del TARGA (de 29% a 47% de los pacientes VIH con complicaciones pulmonares que ingresaron en un hospital de referencia).[108] El otro estudio mostró un descenso significativo de 13,1 a 8,5 por 100 ingresos/año; el decremento fue mayor en el caso de las neumonías nosocomiales (2,4 a 0,8 episodios/100 ingresos/año) que en las neumonías bacterianas adquiridas en la comunidad (10,7 a 7,7 episodios por 100 ingresos y año).[109] Por lo tanto, y aunque los resultados no son concluyentes, parece que la introducción del TARGA no ha supuesto como mínimo un importante descenso en la incidencia de neumonías bacterianas adquiridas en la comunidad, aunque quizás el grupo de pacientes más inmunodeprimidos (con menos de 200 CD4/mm^3) han resultado más beneficiados por el empleo generalizado de tratamientos antirretrovirales combinados de mayor eficacia. El mayor descenso de neumonías bacterianas nosocomiales probablemente está en relación con el decremento de infecciones oportunistas graves, que ha conllevado una menor duración del tiempo de hospitalización de estos pacientes.[109]

En los pacientes VIH las neumonías bacterianas se producen con mayor frecuencia que en la población general en todos los estadios inmunitarios, en cuanto a recuento de linfocitos CD4/mm^3, aunque el riesgo se incrementa a medida que disminuyen los CD4.[25,42] Así, el 80% de los casos de neumonía bacteriana ocurren con CD4 inferiores a 400/mm^3 y, cuando las neumonías son recurrentes, con menos de 300 CD4[111]. En el presente estudio, la mediana de linfocitos CD4 en los casos de neumonía bacteriana era de 200 células/mm^3,

significativamente superior al de los casos de TBC y NPC (Figura 2, referencia 78). En nuestro estudio se pudo comprobar cómo el incremento en el riesgo de neumonía es ya evidente en estadios iniciales de alteración de la inmunidad celular (Figura 2, referencia 78). Puesto que el TARGA retrasa el deterioro inmunológico causado por el VIH, podría incrementarse el periodo de susceptibilidad aumentada a las neumonías bacterianas. La mediana de la carga viral fue también menor en los casos de neumonía bacteriana que en las de las otras etiologías (Figura 1, referencia 78).

Las bacterias identificadas con mayor frecuencia fueron *Streptococcus pneumoniae* y *Haemophylus influenzae*, de forma semejante a lo descrito en otras series.[103,106,109,112,113,114] Diversos estudios han descrito la elevada incidencia de neumonía neumocócica en este grupo de pacientes comparado con la población general y han evidenciado sus características epidemiológicas y clínicas.[115,116,117,118] Múltiples estudios han evaluado la posible eficacia de la vacuna antineumocócica en los pacientes VIH, con diversos resultados.[17,119,120,121,122,123,124,125] Los estudios realizados en USA demuestran una eficacia moderada en los pacientes con más de 200 linfocitos CD4/mm^3, mientras que un estudio realizado en África –el único ensayo clínico controlado- no demostró eficacia de la vacuna.[120] No obstante, los CDC y las guías de la Sociedad Americana de Enfermedades Infecciosas recomiendan vacunar a los pacientes VIH con más de 200 CD4/mm^3 tan pronto como sea posible tras el diagnóstico de la infección por el VIH.[126] Recientemente se ha caracterizado la epidemiología, clínica y pronóstico de la neumonía por *H. influenzae* en estos pacientes.[127] *Sthaphylococcus aureus* fue la tercera causa más frecuente en la serie actual, coincidiendo con lo observado por otros autores.[38,112,128] Por el

contrario, a diferencia de la elevada frecuencia con que se ha encontrado *Pseudomonas aeruginosa* como agente causal de neumonía bacteriana en algunos estudios,[42,106,114129] en el presente trabajo no encontramos episodios causados por este microrganismo. Los mencionados estudios fueron realizados en la era pre-TARGA, en la que –como se ha comentado anteriormente- había una mayor incidencia de neumonías nosocomiales en los pacientes VIH, en las que *P. aeruginosa* constituía la causa más frecuente.[130] Por otra parte también se han descrito infecciones broncopulmonares por *P. aeruginosa* en pacientes con muy avanzado estado de inmunodepresión (mediana de linfocitos CD4 de 11/mm^3), situación en la que cada vez permanecen menos tiempo los pacientes tras la introducción del TARGA.[131] En nuestro trabajo encontramos cinco casos de neumonía por *Legionella pneumophila*. Aunque se han descrito pocos casos de neumonía por *Legionella* en pacientes VIH, hay estudios que sugieren que el riesgo es superior en las personas con SIDA que en la población general.[132,133] Algunos autores han referido un peor pronóstico en estos pacientes, con mayor número de complicaciones, mientras que otros mostraban una evolución similar a la de la infección en pacientes inmunocompetentes.[132,134,135,136] Nuestro cinco pacientes con neumonía por *Legionella* evolucionaron satisfactoriamente con el tratamiento habitual y no presentaron complicaciones.

En Estados Unidos se observó una disminución de la incidencia de TBC a partir de 1992, a pesar del aumento en el número de casos de SIDA.[137] En una cohorte de pacientes HIV reclutados entre 1988 y 1990, y seguidos durante una mediana de 53 meses, la incidencia de TBC fue de 0,7 casos por 100 personas/año.[138] De forma similar, en Europa, la incidencia de micobacteriosis

en pacientes con infección por el VIH entre 1994 y 1999 era de 0,8 casos por 100 personas y año de seguimiento,[139] lo que suponía una clara disminución respecto a la incidencia en los años ochenta (3 casos por 100 personas y año).[140] Este marcado descenso se ha relacionado con la introducción del TARGA.[139] Sin embargo, se observan importantes diferencias entre las distintas áreas geográficas. Así, la incidencia de tuberculosis en el sudoeste europeo es de 3,1 casos por 100 personas/año, esto es, entre cuatro y siete veces superior a la incidencia en otras regiones.[139] De forma similar, en nuestro estudio se observó una incidencia de 3 episodios por 100 ingresos/año (IC 95% 2-4), que no ha cambiado con respecto a la era pre-TARGA.[141] Además, las micobacteriosis constituyeron la tercera causa más frecuente de infiltrados pulmonares en los pacientes VIH estudiados en nuestro medio (12%, IC 95% 8-16), una zona endémica en TBC. Esta elevada incidencia, junto con la frecuente asociación de micobacteriosis con otras infecciones pulmonares en esta serie, apoya la realización sistemática de cultivos para micobacterias en el diagnóstico de los infiltrados pulmonares en pacientes VIH en nuestra área geográfica. Además, los casos de micobacteriosis por otras especies distintas de *M. tuberculosis* (*Mycobacerium kansasii, Mycobacterium avium* complex, *Mycobacterium fortuitum* y *Mycobacterium xenopi)* que requieren tratamientos específicos, refuerzan este planteamiento diagnóstico. Además, algunas de estas especies –como *Mycobacterium xenopi*- constituyen patógenos emergentes en los pacientes con infección por VIH.[142]

Al cabo de los dos años de estudio, se confirmó una frecuencia de etiología polimicrobiana del 9% (IC 95% 5-14) de los episodios en los que se llegó a un diagnóstico etiológico. Otros trabajos han llamado la atención sobre

la importancia de las infecciones pulmonares producidas por más de un microrganismo en los pacientes VIH, en las que, con frecuencia, alguno de ellos no se sospechó en el diagnóstico inicial.[143,144]

Llama la atención en la presente serie, compuesta fundamentalmente de pacientes jóvenes (edad media 39 años), el diagnóstico de tres casos de carcinoma de pulmón. Se ha sugerido que la incidencia de cáncer de pulmón podría estar aumentada en las personas con infección por el VIH, pero los estudios disponibles son limitados y los resultados dispares.[145,146]

Es difícil contrastar nuestros resultados sobre la evolución y los factores asociados con mortalidad con los de la literatura, ya que apenas hay estudios sobre factores pronósticos en series globales de infiltrados pulmonares en pacientes VIH. En una serie previa de pacientes con infiltrados pulmonares que requirieron ingreso en una unidad de cuidados intensivos, los factores asociados con mayor mortalidad fueron la NPC y el requerimiento de ventilación mecánica.[147] La mayoría de las investigaciones se han centrado en la NPC, con una mortalidad media que ha oscilado en la mayoría de las series entre un 10-30% (17% en el estudio actual).[93,148,149,150,151] En varios estudios de pacientes con NPC, la insuficiencia respiratoria y la necesidad de ventilación mecánica se han asociado de forma consistente con un peor pronóstico.[148,149,150] Otros factores asociados con una mayor mortalidad han sido: una edad superior a los 45 años, unos niveles de LDH superiores a 800 IU/L, una elevada neutrofilia en el lavado bronquioalveolar (LBA), una situación nutricional deficitaria o un número de linfocitos CD4 inferior a 50/mm^3. [149150,152] Un estudio reciente sugiere que el uso de TARGA ha conllevado una disminución de la mortalidad asociada a la NPC grave (que requiere ingreso en

la unidad de cuidados intensivos).[153] Los factores predictores de muerte por neumonía bacteriana que se han descrito en los pacientes VIH han sido la presencia de neutropenia, una pO2 arterial inferior a 70 mm Hg y un índice de Karnofsky menor o igual a 50[154]. La tasa de mortalidad asociada a la neumonía bacteriana ha sido muy variable en estudios anteriores (5-30%), aunque el promedio oscila entre 10-15%.[17,42,105,106] Estas tasas de mortalidad, aunque no son desdeñables, no son superiores a las de la neumonía bacteriana en los pacientes no infectados por el VIH.[17] No obstante, estas comparaciones son difíciles de realizar, puesto que la neumonía –en ausencia de infección por VIH- se produce más frecuentemente en personas mayores que los infectados por el VIH. Sin embargo, la mortalidad por bacteriemia neumocócica en adultos emparejados por edad es similar,[155] aunque podría estar aumentada en los pacientes VIH de más edad y en pacientes con SIDA.[156,157] La mortalidad atribuible a neumonía bacteriana en nuestra serie es del 3,4% (4 de 118 pacientes). Esta baja tasa podría estar relacionada con la baja prevalecía de neumonías causadas por enterobacterias y *P. aeruginosa*, que son las que conllevan un peor pronóstico en estudios previos. Los factores independientes asociados con mayor mortalidad en nuestro estudio de pacientes VIH con infiltrados pulmonares fueron: la edad superior a los 50 años, la necesidad de ventilación mecánica y la ausencia de un diagnóstico etiológico. Este último factor reviste un especial interés, puesto que enfatiza la importancia de optimizar la sistemática y las técnicas diagnósticas empleadas en estos pacientes.

No hay un claro consenso sobre cuál es el mejor algoritmo diagnóstico en los pacientes con infección por el VIH que presentan infiltrados pulmonares.

Algunos investigadores han recomendado el inicio de un tratamiento empírico, teniendo en cuenta las características clínicas y la epidemiología local. La puesta en marcha de técnicas diagnósticas se llevaría a cabo en aquellos pacientes que evolucionasen desfavorablemente con el tratamiento empírico.[26,158] Por el contrario otros autores recomiendan intentar establecer un diagnóstico etiológico en todos los casos, inicialmente mediante pruebas no invasivas, seguido por otras invasivas si las primeras no son diagnósticas[159]. Nuestro estudio apoyaría este último planteamiento, puesto que la ausencia de un diagnóstico etiológico se asoció con una mayor mortalidad. Además, la notable prevalencia de infecciones polimicrobianas (9%), en las que, con frecuencia, uno de los diagnósticos no se ha sospechado de entrada, avala la importancia de este abordaje. En el estudio actual destaca la elevada rentabilidad de una técnica no invasiva como el estudio microbiológico del esputo (51%), que alcanza el 57% en el caso de las neumonías bacterianas. Esta rentabilidad es superior a la obtenida en un estudio reciente (35%) que, no obstante, subraya la utilidad del cultivo de esputo en el diagnóstico de las neumonías bacterianas en los pacientes VIH, debido tanto a su disponibilidad y facilidad de obtención, como a su buena correlación con el cultivo de muestras estériles.[160] Aunque no lo hemos evaluado en nuestra serie, una técnica no invasiva que probablemente pueda tener un importante papel en el diagnóstico de las neumonías bacterianas en estos pacientes es la detección del antígeno neumocócico en orina, dado que el neumococo es la causa más frecuente. No hay estudios específicos realizados en pacientes VIH, pero los buenos resultados obtenidos en la población general, sugieren su probable utilidad en este grupo de pacientes.[161] El rendimiento diagnóstico del estudio de las

muestras obtenidas mediante broncoscopia -realizada de acuerdo con el protocolo establecido- fue elevado, dando lugar al diagnóstico en el 56% de los casos. Estos resultados son concordantes con trabajos realizados previamente, que demostraron una rentabilidad similar, así como la importancia diagnóstica de la broncoscopia en este grupo de pacientes.[104,162,163] Por el contrario, la ausencia de diagnósticos de neumonías "atípicas" (distintas de *Legionella*), como en otras series, sugiere que la realización de rutina de pruebas serológicas para diagnosticar los agentes etiológicos no es necesaria en la mayoría de los casos.[114,164]

Mediante nuestro protocolo de estudio se llegó a un diagnóstico etiológico en el 66% de los episodios, al cabo de los dos años de estudio, lo que puede considerarse una elevada rentabilidad diagnóstica. No obstante, son necesarios más estudios que establezcan cuál es el algoritmo diagnóstico más adecuado en los pacientes VIH con infiltrados pulmonares, que debe tener en cuenta las características epidemiológicas de cada área geográfica.

Los hallazgos más significativos del segundo estudio son: 1) los pacientes VIH con neumonía bacteriana tienen niveles plasmáticos de PCR e IL-8 al ingreso superiores a los pacientes con NPC y micobacteriosis; 2) el valor plasmático de TNF-α es mayor en los pacientes VIH con micobacteriosis que en los pacientes con neumonía bacteriana y NPC; 3) la IL-8 plasmática es un factor independiente asociado con mortalidad en los pacientes VIH con complicaciones pulmonares.

En los últimos años se ha estudiado la respuesta inflamatoria local y sistémica en la neumonía bacteriana de la población inmunocompetente, con especial énfasis en los patrones de citocinas y los niveles de PCR.[165,166,167,168]

Así, se ha descrito un sustancial incremento de las citocinas proinflamatorias, como la IL-1β, IL-6 y TNF-α, y las proteínas de fase aguda como la PCR.[168] Además estas citocinas parecen estar asociadas con la gravedad de la neumonía.[167] La IL-8 es otra citocina asociada con la inflamación. Se trata de una quimiocina con capacidad de reclutar y activar los neutrófilos,[169,170] que aumenta en el pulmón neumónico, pero no en el que no está afectado por la neumonía.[171,172] La IL-10 es una citocina antiinflamatoria que inhibe la producción de citocinas proinflamatorias.[171] En estudios recientes se ha demostrado una asociación entre los niveles de IL-10 y la gravedad de la neumonía bacteriana.[172] No se ha estudiado la respuesta inflamatoria en la neumonía bacteriana en los pacientes con infección por el VIH.

En nuestro estudio, los pacientes VIH con neumonía bacteriana tenían niveles elevados de las citocinas proinflamatorias IL-6, TNF-α e IL-8 (Tabla 3, referencia 79). Además, los niveles plasmáticos de PCR estaban elevados. Globalmente estos resultados son similares a los encontrados en la población general con neumonía bacteriana, aunque no encontramos niveles séricos elevados de IL-1β, ni de IL-10. Es interesante señalar que aunque la IL-8 está habitualmente confinada en el pulmón afectado y raramente se encuentra en el plasma,[173] en nuestro estudio hubo niveles plasmáticos detectables de IL-8 en 75% de los pacientes VIH con neumonías bacterianas. En cambio en un estudio previo de neumonías en la población general, la IL-8 sérica era sólo detectable en el 25% de los pacientes.[174] En relación con las citocinas proinflamatorias IL-1β, IL-6 y TNF-α, Dehoux y colaboradores encontraron que sólo los niveles de IL-6 estaban ligeramente aumentados en el suero de los pacientes con neumonía bacteriana en la población general, mientras que los

niveles elevados de las otras citocinas estaban circunscritos al pulmón afectado.[175] Otros estudios han documentado la presencia de respuesta inflamatoria sistémica en pacientes con neumonía bacteriana más grave, así como una correlación entre los niveles plasmáticos de IL-6 y TNF-α y la gravedad y mortalidad de la neumonía.[165,167,176,177] Así, en un estudio de pacientes consecutivos con neumonía bacteriana, los niveles séricos de IL-6 y TNF-α estaban presentes sólo en el 23% y 41% de los pacientes respectivamente. Sin embargo, en otro estudio de pacientes con neumonía grave que requirió ventilación mecánica, la IL-6 y el TNF-α se detectaron en todos los pacientes,[177] y la IL1-β era detectable en el 45% de los pacientes con neumonía bacteriana grave.[177] En el estudio actual, encontramos en los pacientes VIH con neumonías bacterianas un patrón de citocinas similar al observado en los pacientes inmunocompetentes con neumonía grave: 99 y 100% de los pacientes tenían niveles plasmáticos detectables de IL-6 y TNF-α, respectivamente, y 55% en el caso de la IL1-β. Sin embargo, nuestros pacientes con neumonía bacteriana no cumplían criterios clínicos de gravedad: solo 5 de 118 (4%) requirieron ingreso en la UCI y 4 (3%) requirieron ventilación mecánica, con una mortalidad del 3%. Estos hallazgos parecen demostrar una respuesta inflamatoria sistémica más pronunciada en los pacientes VIH con neumonía bacteriana, lo que podría estar relacionado con algún tipo de incapacidad de contener la respuesta inflamatoria en el sitio de infección. En cualquier caso, el perfil sérico de PCR y citocinas encontrado en los pacientes infectados por VIH con neumonía bacteriana frente a los otros diagnósticos principales –NPC y micobacteriosis- es bastante característico y

permite distinguir la neumonía bacteriana de los otros procesos con buenos niveles de sensibilidad, especificidad y valores predictivos.

En este estudio, el nivel de TNF-α estaba elevado en los pacientes con neumonía bacteriana, NPC y micobacteriosis. Sin embargo, fue significativamente mayor en las micobacteriosis que en los otros grupos diagnósticos (Tabla 3, referencia 79). De hecho, un valor de TNF-α $\geq$ 60 ρg/ml tuvo un elevado VPN para el diagnóstico de micobacteriosis (97%). Se sabe que el TNF-α contribuye en los mecanismos de defensa del huésped en la infección por micobacterias.[178,179,180] Estudios realizados en ratones demuestran que el TNF-α es importante en la formación de granulomas y en el control de la extensión de las micobacteriosis.[178,179] En humanos, el uso de anticuerpos monoclonales anti-TNF-α, como infliximab, se ha asociado con una tasa aumentada de reactivación de TBC.[181] En pacientes con TBC no inmunodeprimidos, la producción de TNF-α está presente en el sitio afectado, pero rara vez se encuentra en la circulación.[181,182] No obstante, según publicaciones recientes, los niveles plasmáticos de TNF-α pueden correlacionarse con la actividad y gravedad de la tuberculosis.[183] También se ha demostrado que la fagocitosis de *M. tuberculosis* induce mayor producción de TNF-α en los macrófagos de los pacientes infectados por el VIH que en las células no infectadas.[184] En un estudio de pacientes VIH con TBC activa, las mediciones seriadas de los niveles plasmáticos de TNF-α se correlacionaron con la respuesta al tratamiento tuberculostático.[185] Sin embargo, no había sido previamente señalado el posible papel de los niveles de TNF-α en el diagnóstico de las micobacteriosis en los pacientes VIH. La elevada incidencia de micobacteriosis, la frecuente asociación de éstas con otras infecciones

pulmonares en los pacientes VIH, así como la importancia epidemiológica de este proceso, apoyan la importancia de excluir este diagnóstico al ingreso.[78] Sin embargo, el diagnóstico microbiológico directo no es siempre posible al ingreso y las tinciones de esputo no siempre son positivas en los pacientes con micobacteriosis. Puesto que el TNF-α tiene un muy elevado VPN, podría ser usado como herramienta para excluir el diagnóstico de micobacteriosis en estos pacientes.

En nuestra serie, se encontraron niveles elevados de las citocinas proinflamatorias IL-6 y TNF-α en la NPC (Tabla 3, referencia 79). *P. carinii* es capaz de estimular la producción de TNF-α por los macrófagos alveolares, y, estudios in vitro sugieren que esta citocina es capaz de matar al microorganismo.[186] En ratones, el TNF-α es importante en el aclaración de *P. carinii*, mientras la IL-6 regula la respuesta inflamatoria.[187,188] Las citocinas proinflamatorias parecen estar más prominentemente presentes en el lavado broncoalveolar que en el plasma en los pacientes VIH con NPC.[189] Así, se ha descrito que los niveles de IL-8 en el lavado broncoalveolar están elevados en la NPC y, además, se correlacionan con la gravedad clínica de la neumonía.[190,191] Sin embargo, en el presente estudio no encontramos niveles elevados de IL-8 en el plasma de estos pacientes. Otro hallazgo de interés es que los niveles de PCR eran bajos en los pacientes con NPC, y esto podría ser usado como una herramienta diagnóstica: un nivel bajo de PCR en plasma (< 10 mg/dL), junto con niveles elevados de LDH (> 900 U.I), tuvieron un elevado valor predictivo positivo y valor predictivo negativo (73% y 87% respectivamente) para el diagnóstico de NPC.

En el segundo artículo que forma parte de esta tesis doctoral se discute con detalle sobre las posibilidades de que estas diferencias de patrones de respuesta inflamatoria se deban realmente a las distintas etiologías de los infiltrados pulmonares o a los diferentes estadios de la infección por VIH.[79] Razonablemente puede concluirse que las diferencias reseñadas son hallazgos independientes del estado de la infección por VIH.

No hubo suficiente número de fallecimientos en cada grupo diagnóstico para analizar los factores pronósticos en cada uno de ellos. En el grupo global de pacientes VIH con infecciones pulmonares, unos niveles elevados de IL-8 (superiores a 61 ρg/ml) se asociaron de forma independiente con una mayor mortalidad. Aunque el diagnóstico etiológico no parece ser un factor relacionado con esta asociación, esto no puede ser completamente asegurado. En los pacientes que fallecieron, todas las citocinas séricas estudiadas estaban elevadas y eran mayores que en los supervivientes (Tabla 5, referencia 79). Dada la profunda alteración de las citocinas que se asocia con la infección por VIH, algunos de estos hallazgos pudieron realmente ser debidos a un estado de infección avanzada por el propio VIH. Sin embargo, cuando se consideró también el estatus virológico e inmunológico, la IL-8 se asoció independientemente con una mayor mortalidad. Este hallazgo subraya la importancia de la respuesta inflamatoria sistémica del huésped en el pronóstico de las infecciones pulmonares en este grupo de pacientes.

Por lo tanto, los pacientes infectados por el VIH con infecciones pulmonares parecen tener una respuesta inflamatoria sistémica importante contra estas infecciones, especialmente en el caso de las neumonías bacterianas y las micobacteriosis. Las diferencias encontradas en el patrón de

respuesta de los grupos etiológicos estudiados pueden permitir una aproximación precoz y no invasiva al diagnóstico en estos pacientes. Sin embargo, una excesiva respuesta inflamatoria sistémica puede tener efectos perjudiciales y estar asociada con una mayor mortalidad.

CONCLUSIONES

1. Con respecto al OBJETIVO 1:
    A. Los infiltrados pulmonares constituyen una causa frecuente de ingreso hospitalario en los pacientes con infección por el VIH (25 episodios/100 ingresos hospitalarios y año) en la era del tratamiento antirretroviral de gran eficacia (TARGA).
    B. Las causas más frecuentes de infiltrados pulmonares en pacientes con infección por el VIH en la actualidad son la neumonía bacteriana (*S. pneumoniae, H. influenzae*), la neumonía por *P. carinii* y las micobacteriosis (fundamentalmente tuberculosis), por este orden.
        a. No hubo diferencias con respecto a la incidencia de neumonías bacterianas o por micobacterias en los pacientes que recibían o no TARGA previamente. La incidencia de neumonía por *P. carinii* fue inferior en los pacientes que recibían TARGA.
        b. El recuento de linfocitos CD4/mm3 es significativamente superior en las neumonías bacterianas con respecto a las neumonías por *P. carinii* y las micobacteriosis. La carga viral plasmática del VIH fue significativamente superior en la neumonía por *P. carinii* que en las neumonías bacterianas y las micobacteriosis pulmonares.
    C. La mortalidad global debida a estas complicaciones es del 10%. Los factores independientes asociados con una mayor mortalidad en estos pacientes son: la edad superior a 50 años, el requerimiento de ventilación mecánica y la no obtención de un diagnóstico etiológico.
2. Con respecto al OBJETIVO 2:

A. Con el protocolo empleado en este estudio se obtuvo un diagnóstico etiológico en el 66% de los casos.
B. Los procedimientos diagnósticos más rentables globalmente en el estudio de los infiltrados pulmonares en los pacientes con infección por el VIH son el cultivo del esputo (51%) y de las muestras obtenidas mediante broncoscopia (56%).

3. Con respecto al OBJETIVO 3:
   A. Al ingreso, la proteína C reactiva (PCR) y la interleucina 8 (IL-8) plasmáticas están significativamente más elevadas en los pacientes infectados por VIH con neumonía bacteriana que en los pacientes con otros diagnósticos (micobacteriosis y neumonía por *P. carinii*). El valor predictivo positivo y el valor predictivo negativo de unos valores de PCR superiores o iguales a 10 mg/dl y de IL-8 mayor o igual a 20 ρg/ml para el diagnóstico de neumonía bacteriana (versus micobacteriosis y neumonía por P. carinii) son de 71% y 83% respectivamente.
   B. EL TNF-α plasmático al ingreso es significativamente superior en los pacientes con micobacteriosis que en los pacientes con otros diagnósticos (neumonía bacteriana y neumonía por P. carinii). El valor predictivo negativo de un valor de TNF-α superior o igual a 60 ρg/ml para el diagnóstico de micobacteriosis versus neumonía bacteriana y neumonía por P. carinii es del 97%.
   C. Un valor plasmático elevado de IL-8 al ingreso (superior a 61 ρg/ml) constituye un factor independiente asociado con mayor mortalidad en los pacientes VIH con infiltrados pulmonares.

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Tabla 1. Características de las citocinas estudiadas en este trabajo

Citocina	Características
IL-1	Polipéptido de unos 15-20 kDA. La principal *fuente fisiológica* de IL-1 son los macrófagos activados. Entre sus *funciones* destaca su capacidad proinflamatoria. Existen dos *formas*: IL-1α e IL-1β, con una homología del 26%. Se derivan de una proteína precursora (pro-IL-1α y pro-IL-1β). Ambas actúan sobre un mismo receptor. El antagonista del receptor de la IL-1 (IL-1-RA) compite por este mismo receptor, impidiendo la actuación de la IL-1. • La IL-1α actúa principalmente en el ámbito intracelular; no se encuentra en la circulación general excepto en los casos de patología grave. • La IL-1β es la forma predominante en el espacio extracelular.
IL-6	Glicoproteína de entre 22 y 29 kDa. *Producida por* múltiples tipos celulares, entre los que destacan: macrófagos activados, monocitos, fibroblastos y células endoteliales. Interviene en las reacciones de fase aguda, en la regulación de la respuesta inmunológica y la hematopoyesis. Ejerce su actividad biológica a través de un receptor de membrana compuesto por dos subunidades denominadas sR-IL-6 y gp 130. Ambos receptores se solubilizan una vez se han unido a la IL-6,

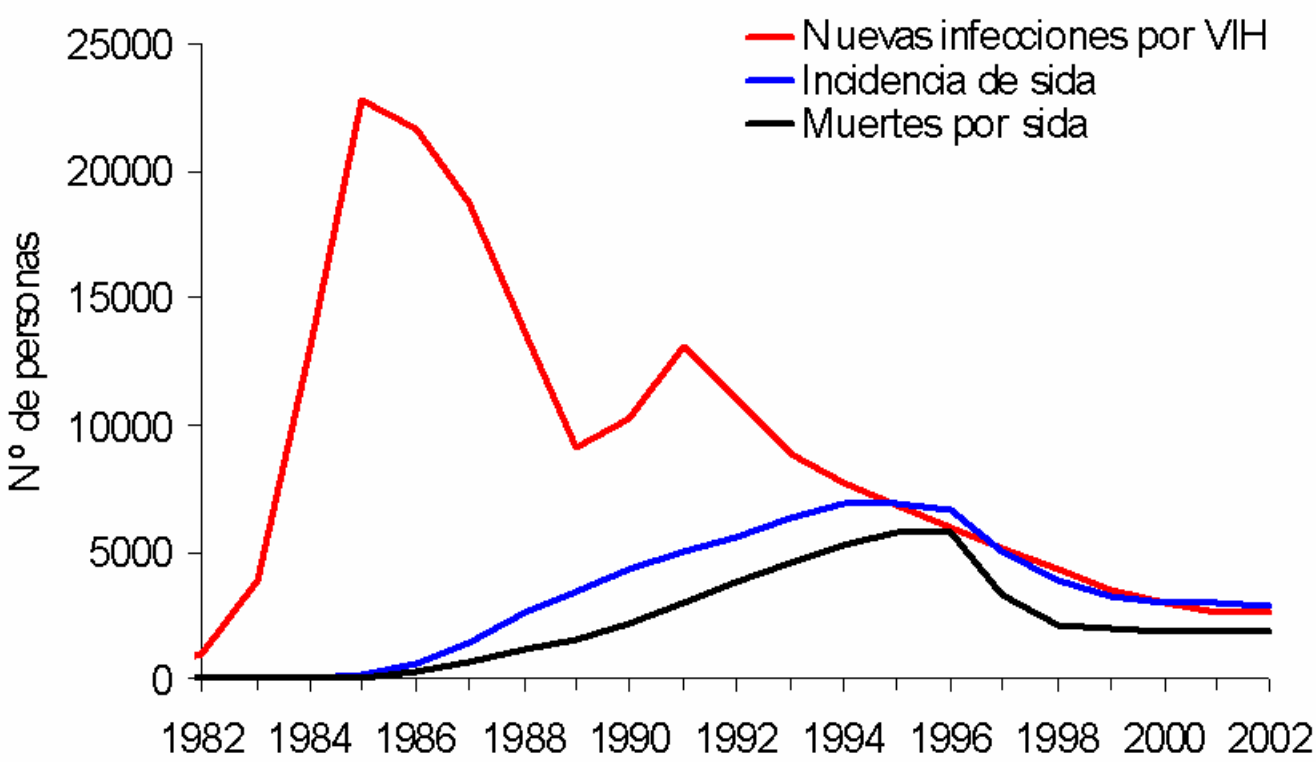
	pero mientras que el sR-IL-6 es un agonista de la IL-6, el gp130 soluble antagoniza la acción de la IL-6. Tiene propiedades proinflamatorias, como la IL-1 y el TNF-α, pero también antiinflamatorias, como muestran recientes evidencias de que las citocinas que se unen al receptor gp130 tienen actividad antiinflamatoria.
IL 8	Es una proteína no glicosilada de 8 kDa que es sintetizada como un precursor de 99 aminoácidos. El *origen* principal de la IL-8 son los monocitos, si bien también puede ser producida por células epiteliales, hepatocitos, fibroblastos y células endoteliales. Se trata de una quimiocina que *actúa* como factor quimiotáctico para leucocitos, fundamentalmente neutrófilos. También actúa favoreciendo su degranulación y estimulando la fagocitosis.
IL-10	*Producida por* los linfocitos T de tipo Th2 y con capacidad de inhibir la síntesis de IFN-χ y de IL-2 por parte de los linfocitos T. A la vez, es una citocina con capacidad antiinflamatoria, pudiendo inhibir la síntesis de IL-1, IL-6 y TNF-α por parte de los macrófagos. También interviene como coestimulador en el crecimiento de varias células hemopoyéticas, incluyendo los linfocitos B y T.
TNF-α	Es una proteína de unos 45-51 kDa. *Producida* fundamentalmente por monocitos, macrófagos y linfocitos.

	El TNF-α ejerce su función a través de dos receptores: el TNF-R-I (de 55 kDa) y el TNF-R-II (de 75 kDa). La diferente actividad del TNF-α en varios tipos celulares depende probablemente de una distinta expresión o regulación de sus receptores. El TNF-α: - ejerce un efecto antitumoral a través de un doble mecanismo que incluye la inhibición de la angiogénesis y el aumento de la respuesta inmunitaria antihumoral - tiene actividad proinflamatoria y actúa como mediador en el desarrollo del shock séptico - interviene como mediador en la caquexia, por lo que también se le ha denominado “caquectina”

Figura 1. Número estimado de personas que viven con VIH/SIDA en el mundo.*

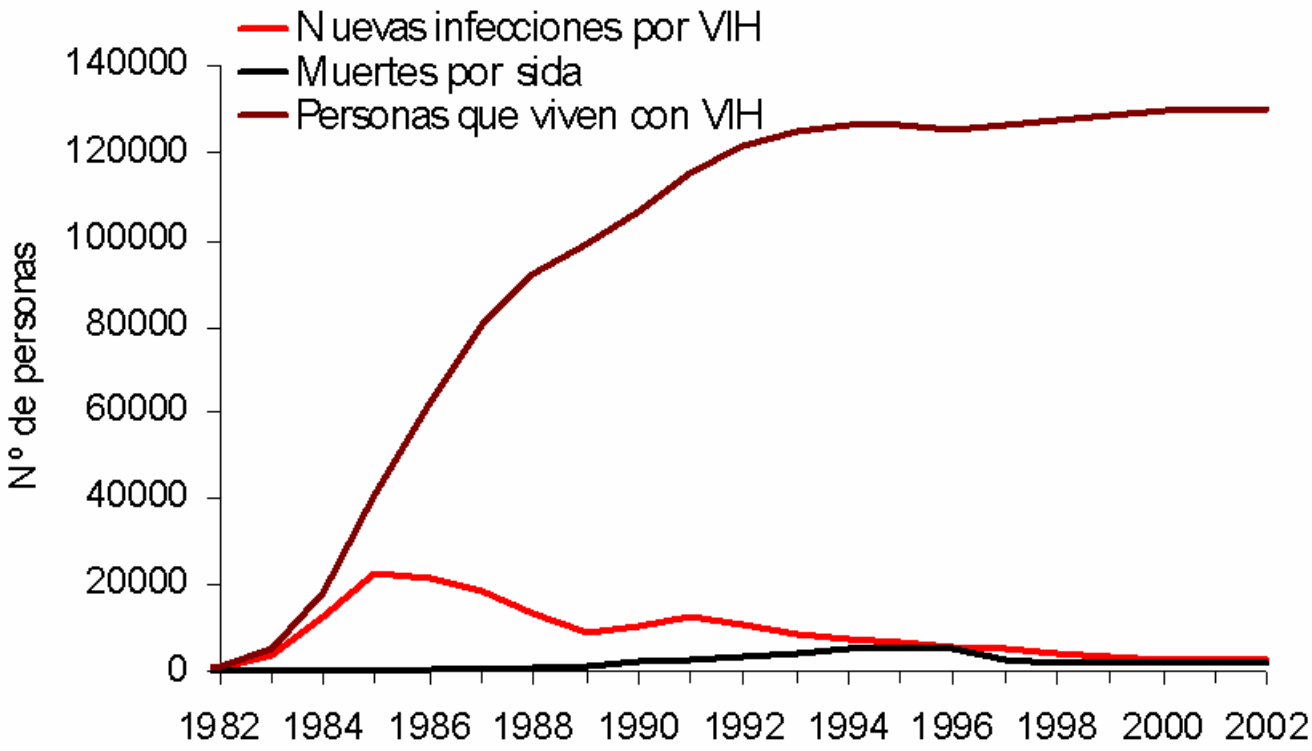
* ONUSIDA/OMS – 2003. Programa Conjunto de las Naciones Unidas sobre el VIH/SIDA (ONUSIDA). Organización Mundial de la Salud (OMS). ONUSIDA/03.39S (versión española, diciembre de 2003). Versión original inglesa, UNAIDS/03.39E, diciembre de 2003: AIDS epidemic update: December 2003. Traducción – ONUSIDA

Figura 2. Evolución de la epidemia de VIH y SIDA en España*



* Instituto de Salud Carlos III. Centro Nacional de Epidemiología - Ministerio de Sanidad y Consumo. Dirección General de Salud Pública. Secretaría del Plan Nacional sobre el SIDA. Vigilancia epidemiológica del SIDA en España. Registro nacional de casos del SIDA. Actualización a 21 de Diciembre de 2003. Informe semestral nº 2, Año 2003. http://cne.isciii.es/sida/informe.pdf

Figura 3. Evolución de la epidemia de VIH y SIDA en España



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