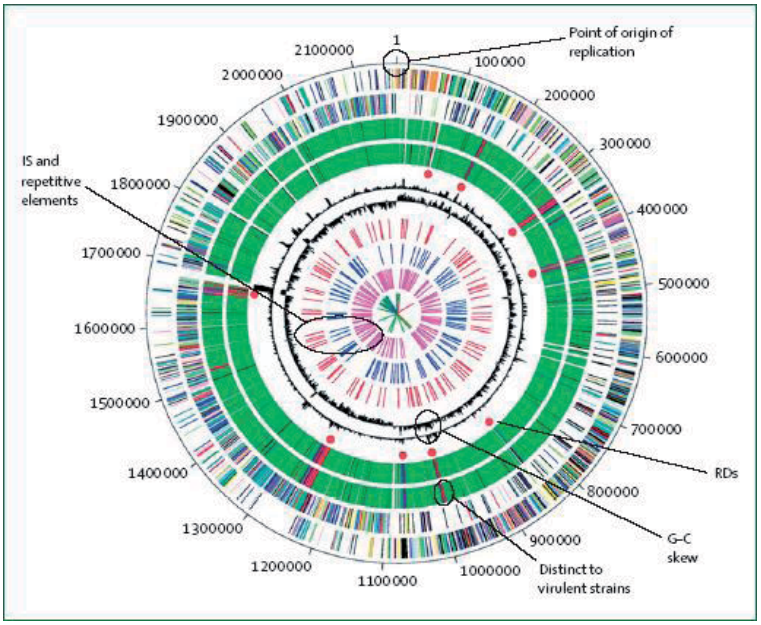
Universitat de les Illes Balears

MECANISMOS DE DEFENSA Y PREVENCIÓN DE LA ENFERMEDAD NEUMOCÓCICA EN PACIENTES CON INFECCIÓN POR VIH



Maria Peñaranda Vera
Universitat de les Illes Balears
Palma de Mallorca, 2010

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MECANISMOS DE DEFENSA Y PREVENCIÓN DE LA ENFERMEDAD NEUMOCÓCICA EN PACIENTES CON INFECCIÓN POR VIH

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Maria Peñaranda Vera

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Con el beneplácito del Director y de la Ponente

Dra. Maria del Pilar Roca Salom
Catedrática del Departamento de Bioquímica y Biología Molecular
de la Universitat de les Illes Balears

Dr.Antoni Payeras Cifre
Especialista Medicina Interna en el Hospital Son LLatzer
Doctor en Medicina por la Universitat de les Illes Balears

El interesado

Maria Peñaranda Vera

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Sólo si nos detenemos a pensar en las pequeñas cosas llegaremos a comprender las grandes.

Jose Saramago

A Catalina y Miguel

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ABREVIATURAS

ADVP: adicción a drogas vía parenteral
Ag: antígeno
Ac: anticuerpo
BAL: lavado bronco alveolar
BAS: aspirado bronco alveolar
CD4: linfocitos T Helper CD4
CPV: vacuna antineumocócica conjugada heptavalente
CV: carga viral del VIH
DE: desviación estardar
EN: enfermedad neumocócica
EIN: enfermedad invasiva por neumococo
IC95%: intervalo de confianza del 95%
Ig: inmunoglobulina
IL: interleucina
LCR: líquido cefalorraquídeo
LB: linfocito de clase B
LT: linfocito de clase T
MASP: proteína sérica asociada a la MBL
MBL: lectina unidora de manosa (mannose-binding lectin)
NAC: neumonía adquirida en la comunidad
NN: neumonía neumocócica
OR: odds ratio
p: significación estadística
PCR: proteína C reactiva
PCR: reacción en cadena de la polimerasa
PGN: peptidoglicano
PPV: vacuna antineumocócica polisacárida 23-valente
SI: sistema inmune
SIDA: síndrome de inmunodeficiencia adquirida
TAR: tratamiento antiretroviral
TLRs: toll like receptors
TNF: factor de necrosis tumoral
NLRs: nod like receptors
VHB: virus de hepatitis B
VHC: virus de hepatitis C
VIH: virus de inmunodeficiencia humana

RESUMEN

Esta tesis está basada en cuatro artículos sobre enfermedad neumocócica. Incluimos en el apartado de Apéndice una revisión de los mecanismos de defensa conocidos hasta la actualidad frente a neumococo.

Inicialmente realizamos un estudio retrospectivo con el objetivo de determinar el papel de la MBL y de la PCR como reactantes de fase aguda y marcadores de gravedad en pacientes ingresados por neumonía neumocócica, evidenciando que no se comportaban como marcadores de gravedad, aunque los pacientes con bacteriemia asociada tenían mayores concentraciones de ambas.

Posteriormente analizamos en un estudio de casos y controles las variables que se asociaban a enfermedad neumocócica en pacientes con infección por VIH y la eficacia de la vacuna polisacárida (PPV) en la prevención de dicha enfermedad objetivando que se asociaban a mayor riesgo de enfermedad neumocócica el enolismo, la enfermedad pulmonar obstructiva crónica (EPOC) y la cirrosis mientras que se asociaban a menor riesgo el tomar tratamiento retroviral (TAR) y la vacunación previa con PPV, que protegía a los que tenían CD4 mayores a 200 céls/μL y también a los que tenían CD4 menores de 200 céls/μL, manteniéndose el efecto protector más allá de los 5 años de la vacunación.

De los pacientes con infección por VIH ingresados por enfermedad invasiva por neumococo (EIN) se estudiaron de forma retrospectiva las variables pronósticas y se compararon los pacientes previamente vacunados y los no vacunados, observando que los vacunados tenían menor estancia hospitalaria, menos complicaciones y menor mortalidad que los no vacunados.

Finalmente realizamos un ensayo clínico para evaluar si la vacunación antineumocócica con dos vacunas, conjugada 7-valente (CPV) seguida de polisacárida 23-valente (PPV) a las 4 semanas, inducía mayor concentración de anticuerpos y con mejor capacidad funcional, medida por avidez, que una sola dosis de PPV. Evidenciamos que las dos estrategias vacunales producían respuestas similares en cuanto a concentración de anticuerpos y que la avidez de dichos anticuerpos no aumentaba tras la vacunación en ninguna de las estrategias vacunales. El hecho de tener CD4 nadir mayores a 200 céls/μL y no haber padecido neumonía previa parecía asociarse a mejor respuesta vacunal.

LISTADO DE PUBLICACIONES

- Mannose-Binding Lectin does not act as an acute-phase reactant in adults with community-acquired pneumococcal pneumonia. M. Perez-Castellano, M. Peñaranda, A. Payeras, J. Milà, M. Riera, J. Vidal, F. Pujalte, A. pareja, C. Villalonga and N. Matamoros. Clin Exp Immunol. 2006; 145: 228-234.

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- Impact of prior pneumococcal vaccination on clinical outcomes in HIV-infected adult patients hospitalized with invasive pneumococcal disease. A. Imaz, V. Falcó, M. Peñaranda, Q. Jordano, X. Martínez, C. Nadal, A. Curran, AM. Planes, D. Dalmau, E. Ribera, M. Riera, E. Ruiz de Gopegui and A. Pahissa. HIV Medicine. 2009; 10: 356-363.

- Conjugate and Polysaccharide Pneumococcal Vaccines do not improve initial response of the Polysaccharide Vaccine in HIV infected adults. Maria Peñaranda, Antoni Payeras, Ana Cambra, Joan Mila, Melcior Riera, and the Majorcan Pneumococcal Study Group. AIDS. 2010; 24: 1226-1228.

APÉNDICE

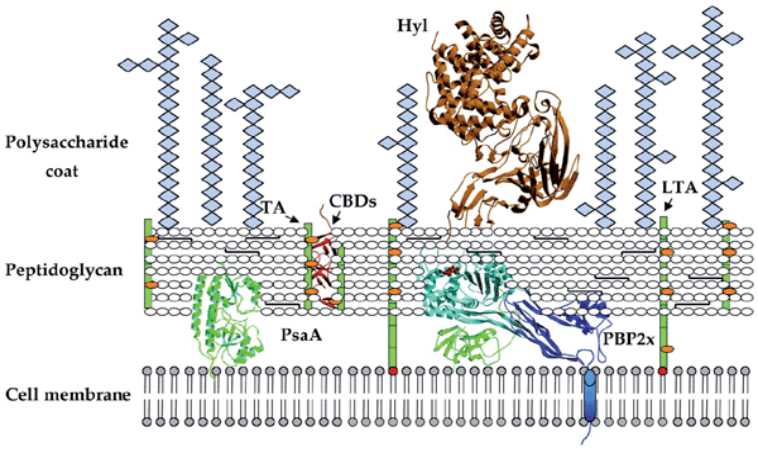
- Innate Immunity In Pneumococcal Pneumonia: New and Old Players: Antoni Payeras, Joan Milà, Melchor Riera, Maria Peñaranda and Jaime Pons. Current Research in Immunology 1, 2007 (59-89).

INTRODUCCIÓN

1. INTRODUCCIÓN

1. 1. NEUMOCOCO. DESCRIPCIÓN

El *Streptococcus pneumoniae* sigue siendo la causa más frecuente de neumonía, meningitis y otitis media, a pesar de la vacunación a menores de 2 años y a la población de riesgo.



Es un coco grampositivo, catalasa negativo, α-hemolítico, sensible a la optoquina y soluble en sales biliares.

Envolviendo a la membrana celular tiene una pared celular constituida por el polisacárido C, compuesto por el peptidoglicano y ácido teicoico. El PGN está formado por cadenas alternadas de N-acetilglucosamina y N-acetilmurámico. La pared celular, a su vez, está envuelta por una cápsula de polisacáridos unidos covalentemente al PGN lo que explica la dificultad para separar la cápsula de la pared celular. Basado en las diferencias antigénicas de los polisacáridos capsulares se han identificado 91 serotipos [1] .

La mayoría de colonizados tiene un sólo serotipo aunque es posible tener varios [2], pero es la adquisición reciente de un serotipo invasivo el factor de riesgo más importante para infección neumocócica [3].

El neumococo, aunque no es altamente contagioso, está bien adaptado al medio humano, coloniza las vías aéreas superiores del 20-40% de niños y del 5-10% de adultos sanos, lo que permite una transmisión baja pero duradera. La colonización aumenta con la edad llegando a un pico a los dos años y luego disminuye progresivamente. Este estado de colonización aparentemente inocuo es un proceso dinámico entre los factores de virulencia que intentan anclarse al huésped, proliferar e invadir las vías respiratorias bajas y los mecanismos de defensa del huésped que intentan evitarlo [4].

El genoma del neumococo tiene un DNA de estructura circular acompañado de pequeños plásmidos, en el que hay entre 1 y 2 millones de pares de bases dependiendo de la virulencia de la cepa, con un core de 1553 genes esenciales para su viabilidad y 154 genes adicionales que contribuyen a la virulencia (viruloma). Existen cepas no invasoras que no contienen todos los elementos del viruloma y dentro de un mismo serotipo pueden existir cepas invasoras y cepas colonizadoras [5].

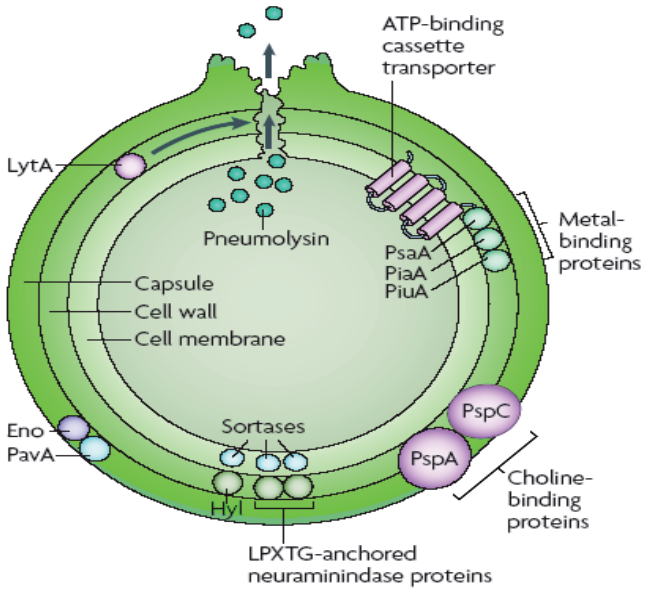
El genoma tiene gran cantidad de genes para los componentes de la cápsula, su principal factor de virulencia, y regula la cantidad de material capsular según el estadio.
El neumococo tiene capacidad de "quorum sensing" y capacidad de internalizar DNA de otros neumococos y de otras bacterias de la nasofaringe y con ellos intercambiar los polisacáridos capsulares. Tiene dos sistemas "sensor kinasas" que reconocen el entorno y alteran sus programas genéticos en respuesta, estos sistemas dirigen la síntesis de bactericidas, la recombinación génica, la formación del biofilm y la expresión de factores de virulencia [4].

1. 2. COMPETENCIA

Existen más de 700 microorganismos en la faringe humana, por lo que la colonización implica la competición entre ellos y entre las distintas cepas de neumococo. Hay cepas más resistentes a la opsonofagocitosis que otras que se benefician de la respuesta inmune del huésped (sobre todo frente a *Haemophilus*).
La competencia implica a más de 100 genes y está controlada por una feromona de "quórum sensing". Las neumocinas son pequeños péptidos antimicrobianos expresados en el locus "blp" y bajo control de una feromona de "quórum sensing" (BlpC) codificada por el mismo locus, que es una región muy variable lo que indica que es producto de una intensa presión selectiva [6].
Dependiendo de la fase en que se encuentra el neumococo y del medio regula sus factores de virulencia, así en el torrente circulatorio aumenta la expresión del "ply" (gen que codifica la neumolisina) y del "pspA" (gen que codifica la proteína de superficie A), mientras que en el pulmón y en el cerebro aumenta la expresión de los genes que codifican las neuraminidasas (nan A y nan B) y de los "genes de competencia" comA, comB y comX.

La gran población de la nasofaringe favorece que haya DNA exógeno y permite la incorporación de DNA de otros neumococos y de estreptococos cercanos, lo que puede mejorar la "fitness" del neumococo. La incorporación de DNA podría ser un medio para compensar la alta tasa de mutaciones producidas durante el metabolismo oxidativo del neumococo, las cuales podrían ser deletéreas para el propio neumococo, por este mecanismo es como se adquieren los genes que codifican las proteínas unidoras de penicilina alteradas que confieren resistencia a los β-lactámicos.
El neumococo puede producir la misma cantidad de peróxido de hidrógeno (H_2O_2), por daño tisular o por lisis de otros microorganismos, que los propios neutrófilos y un ambiente rico en H_2O_2 conduce a mutaciones del DNA como las mutaciones en la girasa y la topoisomerasa que conllevan la resistencia a quinolonas [6].

1. 3. FACTORES DE VIRULENCIA DEL NEUMOCOCO



1. 3. 1. Cápsula neumocócica:

Es el principal factor de virulencia, crucial durante la colonización, invasión y diseminación extrapulmonar, aunque existen cepas acapsulares que se han aislado en infecciones superficiales como conjuntivitis.
Los polisacáridos capsulares están cargados negativamente lo que les repele del ácido siálico del que son ricos los mucopolisacáridos del moco, favoreciendo el acceso del neumococo a la superficie de las células epiteliales de los espacios nasales. Esta carga negativa también le repele de la unión al factor del complemento C3b y de la unión a las inmunoglobulinas.

La cápsula tiene lo que se llama "variación de fase" que le permite la expresión de ciertas moléculas que hacen la cápsula más fina, llamadas variantes transparentes, que prevalecen en la colonización lo que dificulta el reconocimiento por el sistema inmune y favorece su unión al huésped, en la invasión se expresan ciertas moléculas que hacen la cápsula más gruesa, llamadas variantes opacas, que los hacen más resistentes a la fagocitosis y favorecen el paso al torrente circulatorio [7,8].
La reducción del tamaño capsular es necesaria para exponer las adhesinas neumocócicas pero aumenta su vulnerabilidad a la opsonofagocitosis lo que el neumococo minimiza produciendo un biofilm en el que participan las neuraminidasas, el biofilm es una matriz de polímeros que reduce la actividad antimicrobiana, previene la deshidratación del neumococo y aumenta su adherencia al epitelio.

1. 3. 2. Moléculas de adhesión del neumococo:

La **Fosforilcolina (ChopP)** es una adhesina que forma parte tanto de los ácidos teicoicos asociados a la pared celular como de los ácidos lipoteicoicos unidos a la membrana celular. Muchos microorganismos que colonizan las vías respiratorias superiores contienen dicha proteína (*Haemophilus influenzae*, *Neisserias*) por lo que ésta debe ser particularmente importante para la colonización [5]. En el neumococo, la ChopP se adhiere al receptor del factor activador de plaquetas, ampliamente distribuido en las células de la nasofaringe humana (el neumococo puede simular ser el PAF para unirse a su receptor).

La **Proteína A unidora de colina** (CbpA, también PspC o SpsA).

Algunos serotipos de neumococo poseen **estructuras similares a pili** que promueven la adhesión enganchándose a receptores todavía no identificados.

El neumococo produce tres **exoglicosidasas**: **neuraminidasa** (NanA), **β-galactosidasa** (βBgaA) y la **β-N-acetilglucosaminidasa** (StrH) que actúan de forma secuencial para eliminar los glúcidos terminales que se encuentran en muchos glicoconjugados humanos inhibiendo su función de aclaramiento o proveyendo nutrientes que son las moléculas glicosiladas y también pueden revelar receptores de adherencia.

La **Hialuronidasa de unión a superficie** puede facilitar la diseminación bacteriana a través de los polisacáridos del tejido conectivo que contienen hialuronano.

La **enolasa** y la **adhesina de virulencia neumocócica A** son dos adhesinas de superficie que se unen al plasminógeno y a la fibronectina respectivamente, que son componentes de la matriz extracelular y su ausencia se relaciona con menores cargas bacterianas y mejor supervivencia en estudios experimentales [6].

La **proteasa IgA1** escinde la unión del neumococo a la IgA1 (que constituye más del 90% de la IgA de las vías respiratorias) por lo que el aclararamiento mediado por Ac sólo se puede producir tras la producción de grandes cantidades de otras clases y subclases de Ac.

La **neuraminidasa** también promueve la adhesión exponiendo lugares de adhesión en las células epiteliales.

La neumolisina es una citolisina (sintetizadas por la mayoría de grampositivos), potente factor de virulencia que se encuentra en todos los neumococos aunque su importancia puede variar de cepa a cepa.
Es una proteína soluble en forma de monómero que se oligomeriza en la membrana de las células epiteliales hasta formar un poro transmembrana en forma de gran anillo y produce la lisis celular, inhibe el batir ciliar del epitelio respiratorio y del epéndimo cerebral, inhibe los fagocitos del sistema respiratorio, induce la síntesis de citocinas inflamatorias y estimula los TLR4 que activan los CD4, e inhibe el complemento por la vía clásica.
Fundamental para la invasión, ya que la lisis del endotelio pulmonar y epitelio alveolar facilita la penetración al torrente circulatorio.

En estudios experimentales en que se sobreexpresa la neumolisina, se encuentran gran número de bacterias en la sangre e importantes signos inflamatorios y en su ausencia se toleran grandes cantidades de bacterias en la sangre sin que haya respuesta inflamatoria, desarrollándose una bacteriemia crónica. Su actividad citotóxica está favorecida por la hialuronidasa y el H_2O_2 [9,10].
La neumolisina también puede proteger de la infección neumocócica, si hay poca cantidad de neumococos y poca neumolisina con formación subletal de poros, se estimula la entrada de calcio lo que activa la cascada inflamatoria intracelular estimulándose la trascripción de genes que inducen la producción de citocinas, especialmente de IL-8 y de ciclooxigenasa II por el epitelio alveolar, que estimulan el reclutamiento de neutrófilos que pueden controlar la colonización inicial [11] y al degradar la bacteria exponen sus Ag al tejido linfoide nasofaríngeo activando la inmunidad en las mucosas [12].

1. 3. 3. Proteínas de Superficie Celular:

Fosforilcolina (ChopP), descrita anteriormente.

Proteínas unidoras de colina. Existen 15 proteínas unidoras de colina incluidas las PspA, PspC y LytA.

La Proteína de superficie A (PspA) es una proteína que protuye en la superficie celular, su carga negativa repele el C3 del complemento y por tanto la opsonización. También se une a la proteína unidora de lactoferrina y por ello protege al neumococo de la actividad bactericida de la apolactoferrina (forma depleccionada de hierro de la lactoferrina). Altamente variable.
La Proteína de superficie C (PspC) también conocida como proteína unidora de colina A (CbpA) ya que se une a las colinas de las células epiteliales y del ácido siálico.

También llamada proteína secretora de superficie A (SpsA) ya que también se une al componente secretor humano, glicoproteína del epitelio necesaria para el transporte de inmunoglobulinas poliméricas y sobre todo IgA a través de las superficies de las mucosas.
También se une al factor H (componente de la vía alternativa del complemento que interviene su regulación), con lo que previene la formación del C3b de la vía alternativa del complemento y por tanto previene la opsonización. También es capaz de unirse al componente C3 del complemento.

Autolisina A (LytA) escinde la unión de N-acetil-muramil-L-alanina con el PGN del neumococo, lo que provoca la lisis del neumococo. Participa en el crecimiento y turn-over del neumococo y es capaz de liberar neumolisina, PGN y ácido teicoico de las células lisadas aumentando la respuesta inflamatoria.

1. 3. 4. Lipoproteínas unidoras de metales:

Antígeno lipoproteico de superficie unidor de metales (PsaA): lipoproteína de la pared celular que puede unirse al zinc o al manganeso con mayor especificidad por el último. La unión al manganeso parece esencial en la resistencia del neumococo al estrés oxidativo.

Proteína de adquisición de hierro A (PiaA) y **Proteína unidora de hierro A** (Piu A): Parece que hay una redundancia en los sistemas unidores de hierro de manera que sólo los mutantes dobles de PiaA y PiuA tienen menor crecimiento en medios deficientes de hierro.

1. 3. 5. Neuraminidasas:

También conocidas como sialidasas al escindir ácidos siálicos terminales de las glicoproteínas, glicolípidos y oligosacáridos de la superficie celular como lactoferrina, IgA2 y componente secretor. El neumococo codifica tres neuraminidasas en los genes nanA, nanB y nanC. Se ha visto que las nanA y nanB son necesarias para la supervivencia del neumococo en el tracto respiratorio y en la sangre, y que los ratones mutantes de nanA eran aclarados rápidamente de las vías respiratorias y que los mutantes nanB persistían pero sin capacidad para aumentar. Las nan C eran más frecuentes en aislados cerebrales que en respiratorios.

1. 4. MECANISMOS DE RECONOCIMIENTO DEL HUESPED

Los receptores de reconocimiento son componentes fundamentales para el SI innato y contribuyen al inicio de la respuesta inmune adaptativa.

- La PCR es una proteína de fase aguda que también funciona como molécula de reconocimiento del neumococo, se une a la fosforilcolina de la pared celular del neumococo y activa el complemento.

- El receptor para el factor activador de plaquetas: el neumococo puede utilizar el rPAF (mediante la Chop) para pasar del pulmón a la sangre [4].

- El receptor de macrófagos con estructura de colágeno (MARCO) está en los macrófagos alveolares y puede unir e internalizar el neumococo "in vitro" [13].

- Los receptores de manosa de los macrófagos se unen a los polisacáridos de la cápsula del neumococo.

- El CD14 es un receptor del epitelio alveolar que el neumococo utiliza para diseminarse, así ratones que no tienen CD14 son resistentes a la infección diseminada por neumococo [14].

- El SIGNR1 es una lectina C (proteína unidora de carbohidratos) que se expresa en los macrófagos de la zona marginal del bazo implicada en la captura de polisacáridos capsulares por macrófagos de la zona marginal del bazo. El SIGNR1 también media en la presentación de los polisacáridos capsulares a los linfocitos B que sintetizan IgM por lo que el déficit de SIGNR1 disminuye la capacidad de montar una respuesta inmune. Los ratones deficientes en SIGNR1 tienen más la bacteriemia [15].
- PRRs: receptores de reconocimiento de patógenos (Pathogen Recognition Receptors), que a su vez se dividen en **Toll Like Receptors** (TLRs) que son receptores transmembrana y **Nod Like Receptors** (NLRs) que son receptores del citosol. La otra cara de la moneda son los ligandos del neumococo reconocidos por los PRRs: las PAMPs (patrones moleculares asociados a patógenos), componentes del neumococo no susceptibles de grandes variaciones.

Los receptores Toll Like (TLRs) pueden detectar Ag de patógenos tanto en su superficie como en endosomas o en lisosomas [16]. Se encuentran tanto en linfocitos CD4 naïve como en linfocitos CD4 memoria y en linfocitos citotóxicos.

Se han descrito 12 TLRs que son centinelas del SI innato, aunque también intervienen en el SI adaptativo activando la secreción de citocinas que estimulan la producción de Ac. A su vez, los adyuvantes de las vacunas (aluminio) actúan activando dichos TLR.

El TLR1 y el TLR2 reconocen de forma sinérgica el neumococo (ácidos lipoteicoicos) lo que activa la vía MyD88 y TRAF6 en el epitelio alveolar que estimula la liberación de IL-8 y TNFα [17], la inhibición del TLR1 inhibe la producción de TNFα por los macrófagos de la sangre periférica [18].
El TLR2 reconoce el PGN, los ácidos teicoicos y lipopéptidos bacterianos, aunque algunos autores han visto que los ratones sin TLR2 tienen una modesta disminución del aclaramiento de los neumococos por lo que su papel podría ser más limitado de lo que se creía y cooperativo con el TLR1 [17,19].
El TLR4 reconoce la neumolisina [17,20] y activa una cascada de señales que conducen a la producción de IL-8, IL-1B, IL-6, TNFα, citosina activadora de macrófagos o interferón gamma por linfocitos T naïve, lo que estimula el reclutamiento de neutrófilos y de macrófagos favoreciendo el control del neumococo y la inmunidad mucosa por células T sin intervención de los Ac [20-22]. Los ratones defectuosos en TLR4 tienen más riesgo de enfermedad a menores dosis infectivas de neumococo [23].
El TLR9 reconoce DNA bacteriano. Actúa en las fases tempranas de la infección antes de la intervención de las células inflamatorias de la circulación. Activa la vía MyD88 que estimulan los macrófagos alveolares locales que aclaran el neumococo del tracto respiratorio. Los ratones defectuosos en TLR9 son más susceptibles a la infección neumocócica [24].
El neumococo también puede modular la expresión de los TLR aumentando la expresión de TLR1 y TLR2 en las células epiteliales bronquiales pero sin efecto sobre los TLR4 o TLR6 [17].

Los NLRs pueden reconocer los neumococos mal opsonizados y eliminados de forma más lenta en los lisosomas y que invaden el citosol.
El NOD1 (dominio oligomerizado unidor de nucleótidos 1) es ubicuo, en cambio el NOD2 se encuentra en las células epiteliales y en las células presentadoras de Ag. El NOD2 reconoce el muramil dipéptido y los "inflamosomas" que activan la caspasa-1, que estimula la secreción de IL-1, IL-18 y IL-33. Las implicaciones en la infección neumocócica de estos dos receptores aún están poco claras.
A su vez el neumococo regula la expresión de NOD1 y NOD2 en las células epiteliales [25].

1. 5. FACTORES DE DEFENSA FRENTE AL NEUMOCOCO. INMUNIDAD INNATA Y ADAPTATIVA

1. 5. 1. Inmunidad Innata:

Dentro de la defensa frente al neumococo se encuentran: mucosa, moco, cilioperistalsis, microflora saprofita, secreciones locales, epitelio pulmonar, macrófagos e histiocitos alveolares, complemento...

Las secreciones locales contienen inmunoglobulinas, IgG y sobre todo IgA secretora que aglutinan los microorganismos y bloquean su unión a las células del huésped, la IgA también es capaz de unirse a patógenos intracelulares o a sus productos. Las secreciones bronquiales contienen lisozima, N-acetil Muramil L-alanino amidasa (NAMLAA), β-defensinas, lectinas pulmonares y colectinas del surfactante [1].

Posteriormente actúan los receptores de reconocimiento que se unen a señales comunes en bacterias, virus y hongos y activan una serie de citocinas inflamatorias que forman parte del SI innato, que estimulan los fagocitos y las células citotolíticas y también activan el SI adaptativo.

Los macrófagos alveolares son la primera defensa en los pulmones y pueden eliminar y fagocitar un número reducido de neumococos, cuando la cantidad de bacterias es mayor los neutrófilos se convierten en las principales células fagocíticas, entonces los macrófagos son relegados al aclaramiento de neutrófilos apoptóticos [26].

El sistema de coagulación colabora en la contención de la infección precoz. La infección neumocócica activa la coagulación tanto sistémica como la intrapulmonar iniciadas por el factor tisular, así en animales de experimentación la infusión con inhibidor del factor tisular disminuye la coagulopatía y la infusión de proteína C activada recombinante disminuye la mortalidad a 28 días en pacientes con sepsis por neumococo [27].

El objetivo de estos factores es el control de la infección pero puede producirse una hiper activación de estos mecanismos en infecciones descontroladas, lo que causa la disrupción del epitelio bronco alveolar por las proteasas de los fagocitos y la diseminación extra pulmonar, potenciado por la neumolisina que destruye el epitelio bronco alveolar, activa el complemento y estimula los fagocitos.

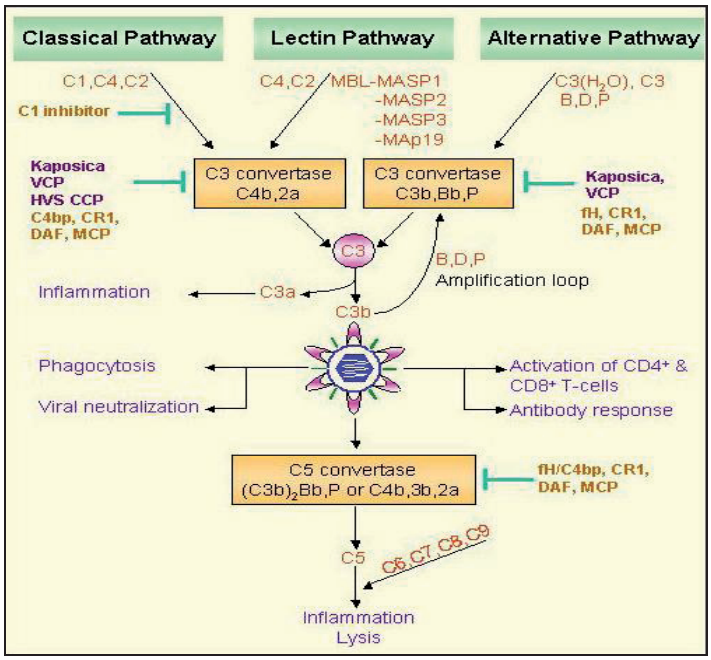
Complemento:

Sistema de de proteínas sintetizadas en el hepatocito, aunque pueden sintetizarlas monocitos, macrófagos, adipocitos, microglía, astrocitos, fibroblastos y células endoteliales.

El C3 es la molécula fundamental del complemento, su escisión puede producirse por la vía clásica, por la vía alternativa o por la vía de las lectinas. Las tres estimulan la C3 convertasa, que escinde el C3 en C3a y C3b.
El C3b es una potente opsonina y puede escindir el C5 en C5a (péptido inflamatorio y quimiotáctico de neutrófilos) y C5b, que interacciona con el complejo C6-C7-C8-C9 que es complejo de ataque de membrana (MAC) el cual induce poros celulares y la subsiguiente lisis celular.

La vía clásica es activada por los complejos Ag-Ac, la unión del Ag produce un cambio conformacional en el Ac que permite su unión al C1q que estimula C2-C4 que genera C3 convertasa.
En la vía de las lectinas la MBL es funcional y estructuralmente igual al C1 que estimula las MASP1, MASP2, MASP3 y MAp19, la MASP2 es fundamental para escindir C4 y C2 y generar C3 convertasa.
La vía alternativa es activada por grandes polisacáridos como lipopolisacárido, y ácido teicoico que activan el factor B y D formándose un producto que activa el C3. La vía alternativa no necesita Ac aunque éste puede facilitar la activación.

El C3b como producto de la escisión del C3 de la vía clásica o alternativa puede iniciar la rueda de la vía alternativa.



El complemento no es un sistema fundamental para la defensa frente al neumococo en las fases precoces, pero adquiere relevancia en la bacteriemia [28]. La vía más importante frente a neumococo es la vía clásica mediada parcialmente por IgM naturales [29,30].

A su vez el neumococo puede inhibir el complemento mediante:
- Cápsula: repele el complemento [31].
- PhpA: tiene actividad degradadora de C3 [32].
- Neumolisisna: inhibe el complemento por la vía clásica [33].
- PspA: repele el complemento.
- PspC: se unen al factor H (regulador del complemento) [34,35].

1. 5. 2. Inmunidad Adaptativa:

En este sistema intervienen los linfocitos B y los linfocitos T estimulados por los Ag. Los LB son los encargados de producir Ac; en neumococo intervienen las IgM, IgG e IgA.

- IgM: tiene baja afinidad* pero sus 10 lugares de unión al Ag le confieren una alta avidez. Es la que más eficazmente activa el complemento por la vía clásica.

- IgG: la más cuantiosa en plasma y la única que pasa la placenta. Hay 4 subclases, la 1 y la 3 fijan el complemento, la 2 con menor eficacia y la 4 no lo fija. Los Ac frente a proteínas son sobre todo IgG1 e IgG3 y los Ac frente a polisacáridos suelen ser IgG2.

- IgA: la mayoría está en las secreciones (producida por los LB de la submucosa). Bloquea los patógenos y evita su unión a los receptores celulares.

Reaccionan con el PGN y pueden activar el complemento por las vías clásica y alternativa [36]. Las IgA poliméricas y secretoras son las más eficaces en la activación del complemento frente a *Streptpcoccus pneumoniae* [37].

**- Afinidad es la fuerza de unión al Ag que tiene el Ac en su lugar de unión al Ag, influida por fuerzas electrostáticas, uniones de hidrógeno, uniones de van der Walls e interacciones hidrofóbicas.*

- Avidez es la medida de la unión entre un Ac con un Ag y esta influida por la afinidad del lugar de unión y el efecto aditivo de múltiples lugares de unión al Ag. Las IgG, IgE e IgD tienen 2 lugares de unión, la IgA se puede dimerizar y tener 4 lugares de unión y la IgM es un pentámero con 10 lugares de unión, por lo que IgM de baja afinidad pueden tener alta avidez para un Ag multivalente ya que sería difícil que se desunieran todas las uniones simultáneamente.

Los LB, tras el primer contacto con el Ag, producen inicialmente IgM, sólo tras recibir señales de los LT pueden cambiar y producir IgG, IgA e IgD. El LB necesita dos señales para producir Ac de manera eficaz, la primera del Ag y la segunda del LT, algunos Ag pueden proveer la segunda señal sin necesidad de los LT (Hib, virus,..).

Bajo la influencia de los LT algunos LB se transforman en LB memoria, los cuales ante repetidas exposiciones del Ag se dividen rápidamente y producen grandes cantidades de Ac en 1 ó 2 días y con mayor afinidad que tras la primera respuesta y producen IgG, IgA e IgE.

Los polisacáridos producen vacunas pobres porque no pueden estimular la producción de IgG específicas, los LB que producen anticuerpos frente a polisacáridos no pueden hacer el cambio de IgM a IgG y no pueden generar LB memoria al no haber LT que produzcan las segundas señales.

Los LT están programados para reconocer péptidos y no polisacáridos. Las vacunas conjugadas unen los polisacáridos a una proteína para la que el organismo produzca LT específicos. Los LB internalizan el conjunto (proteína-polisacárido) mediante su IgM polisacárido específico de membrana, se degrada y los péptidos se muestran en las moléculas de HLA clase II.

1. 5. 3. Papel de los Anticuerpos capsulares en la colonización y en la EIN:

Los Ac frente a los polisacáridos capsulares del neumococo contribuyen a la resistencia progresiva a la colonización.
La gran respuesta a la vacuna CPV ha llevado a pensar que los Ac anticapsulares son los principales mediadores en la resistencia a la colonización del neumococo. Pero no se sabe si es precisamente este mecanismo el que induce la resistencia natural a la colonización a partir de los dos años, ya que la reducción de la colonización parece que es anterior a la adquisición de Ac anticapsulares en niños no vacunados, por lo que algunos autores proponen que son los Ac frente a Ag no capsulares los que confieren resistencia a la colonización [38], que además no es seroespecífica [39].

Los Ac frente a la cápsula son suficientes para proteger frente a la EIN, aunque queda menos claro si constituyen la resistencia natural a la infección neumocócica.
La EIN aumenta hasta un pico a los 9-15 meses de edad, luego disminuye para todos los serotipos y a los 24 meses es la mitad del pico. Se vio que en niños de 3 años no vacunados, los Ac frente a los serotipos 6 y 14 no alcanzaban los niveles protectores de 0,35µg/mL mientras que la EIN por esos serotipos era 10 veces menor que a los 12 meses [40], lo que apoya la teoría de una resistencia común para todos los serotipos o inmunidad frente a Ag no capsulares.

Existen Ag no capsulares como la proteína de superficie A (PspA), la adhesina A (PsaA), la neumolisina, el polisacárido de pared celular (CWPS) y el ácido lipoteicoico de la membrana (LTA), que serían inmunogénicos en edades tempranas por lo que los Ac frente a dichas proteínas podrían proteger frente a la colonización, frente a la EIN y frente a la otitis media [38].

1. 5. 4. Papel de los linfocitos CD4 frente a la colonización:

Durante el estudio de vacunas se vio que existe una segunda inmunidad frente a la colonización en la que intervienen los CD4. En modelos animales la inmunización intranasal con vacuna de célula completa (WCV) protegía de manera excelente frente a la colonización y a la sepsis por neumococo y esa protección era independiente de los Ac y dependía de los CD4 de la línea IL-17A [38], ya que la infección con neumococos a los ratones inmunizados, protegía aquellos con déficit de Ac pero no a ratones con déficit de CD4.

Se ha visto que en los pulmones de ratones infectados intranasalmente con neumococo hay un rápido aumento de CD4 en el lugar de la invasión en el que es fundamental la interacción neumolisina-TLR4 que estimula el reclutamiento de CD4 [38]. Las funciones dependientes de CD4 que eliminan la colonización todavía no están aclaradas [41].

La secreción de IL-17A de estas células podría reclutar fagocitos al lugar de la colonización y disminuir su duración. Así Malley y Weiser [6,38] proponían que en la inmunidad adquirida frente a neumococo intervenían los Ac (capsulares y no capsulares) que protegían frente a la invasión y probablemente frente a la colonización y los CD4 IL-17A que prevenían frente a la colonización y probablemente frente a la enfermedad mucosa. Por lo que en la prevención deberían incluirse ambas vías.

1. 6. FACTORES DE RIESGO PARA INFECCIÓN NEUMOCÓCICA

Se han descrito numerosos factores que facilitan la infección neumocócica como son las edades extremas (menores de 2 años y mayores de 65 años), la obesidad [39,42-44], la malnutrición [45,46] y el estatus socioeconómico bajo [45].

Los hábitos tóxicos también son factores relacionados con mayor riesgo de enfermedad neumocócica, sobre todo la ADVP [45,47], el alcoholismo [48-50] y el tabaquismo [51-53].

Entre los cambios que provoca el tabaco en las vías aéreas están la disminución de varias Ig entre ellas la IgA secretora, la disminución de polimorfonucleares, macrófagos y linfocitos locales así como la inhibición de su función. También disminuye el batir ciliar, aumenta la producción de moco aunque disminuye la calidad de dicho moco [54]. También se ha visto en fumadores un aumento de la adherencia del neumococo a las células epiteliales bucales [55].

También se han asociado a mayor riesgo de enfermedad neumocócica la raza negra [45-47,56] y el sexo femenino [45].

Existen comorbilidades que se han relacionado con mayor riesgo de infección neumocócica como la enfermedad pulmonar obstructiva crónica (EPOC) [50], la cirrosis hepática [50,57], tratamientos antibióticos previos, gripe previa u hospitalización previa [47] y las inmunodeficiencias primarias y las inmunodeficiencias adquiridas, tumores hematológicos [50,56], esplenectomía [50], tratamientos inmunosupresores o la infección por el VIH [45-47,50,56-60].

1. 7. INFECCIÓN POR VIH Y POR NEUMOCOCO

La enfermedad por *Streptococcus pneumoniae* es un problema frecuente y con elevada morbimortalidad en la población general y principalmente en pacientes con infección por VIH, quienes tienen un riesgo de neumonía bacteriana entre 60 y 100 veces mayor que la población general [45,50,58,60-63], sobre todo neumonía neumocócica [64,65].

El neumococo causa el 20% de todas las neumonías bacterianas en pacientes con infección por VIH, en ellos el riesgo aumenta proporcionalmente al descenso de linfocitos CD4, siendo mayor en aquellos con recuento de CD4 inferior a 200 céls/µL [66] y es especialmente importante en aquellos lugares sin acceso al TAR. Así la carga de infección neumocócica en niños africanos con VIH es 42 veces mayor que en seronegativos [69].

La infección por VIH avanzada (estadío C del CDC, CD4 menores a 200 céls/µL y CV elevada) y la asociación de otras comorbilidades (EPOC, cirrosis, asma, hipoalbuminemia) aparecen como los factores de mayor riesgo de EIN en estos pacientes [45-47,56,67,68], pero incluso en aquellos con CD4 mayores a 200 céls/mL la incidencia de EIN es mayor que en sanos [50].

Desde el uso expandido del TAR muchos autores evidenciaron una disminución de la tasa de EIN en pacientes con infección por VIH [46,47,57,60,66,70,71], otros trabajos no observaron cambio alguno [45,50]. Aún así sigue siendo mucho mayor que en seronegativos, entre 10 y 35 veces más [47,50,53,57,60,72].

En un estudio español se vio que en la era TAR la infección neumocócica había disminuido de 2410 a 820 episodios por 100000 pacientes-año, había menos recurrencias pero más mortalidad debida a una mayor comorbilidad de los pacientes, y con alta tasa de resistencia a penicilina en ambos periodos [57]. En otro estudio español se evidenció que los pacientes con infección por VIH tenían más riesgo de infecciónes por neumococos resistentes a penicilina que los seronegativos [73].

Otras publicaciones objetivaron que la profilaxis con cotrimoxazol [53] protegía frente a infección neumocócica, aunque en algunos estudios la profilaxis con dicho antibiótico se asociaba a infecciones por neumococo resistente al cotrimoxazol [47,50,57].

1. 7. 1. Patogenia de la infección neumocócica en pacientes con infección por VIH

Todavía se desconocen las bases moleculares de la susceptibilidad a la infección neumocócica en pacientes con infección por VIH.

Un estudio en madres de Zambia demostró que la infección por VIH se asociaba a mayor colonización nasofaríngea y menor tiempo a una nueva colonización [74].

Kadioglu objetivó un menor un menor reclutamiento de CD4 en el lugar de la infección y menor aclaramiento del neumococo en pacientes con infección por VIH [41]). Malley sugería que era la disminución de CD4 secretores de IL-17A lo que determinaba la disminución de la inmunidad innata frente al neumococo [38].

En pacientes con infección por VIH se vió que la activación de los TLR2 y TLR4 aumentaba la expresión de los receptores CCR5 en los CD4 naïve y memoria, lo que aumentaba su susceptibilidad a ser infectadas [75]. Schleicher encontró en pacientes VIH naïve una disminución de CD4 y de linfocitos totales en la fase aguda de la infección neumocócica con recuperación de los mismos al mes de la infección [76].

Armbruster evidenció que no había diferencias en el recuento de células inflamatorias en el BAL de pacientes con y sin infección por VIH, pero en los pacientes con VIH la expresión de los receptores de neutrófilos de las IgG estaba alterada lo que podía aumentar la susceptibilidad a las infecciones [77].

Takahashi encontró una menor actividad opsonofagocítica frente a neumococo a pesar de una mayor concentración en suero de IgG frente a los serotipos 3 y 9 en pacientes con infección por VIH [78].

Benito no encontró diferencias en IL-1, IL-6, o IL-10, en pacientes con VIH, entre aquellos con neumonía bacteriana, PCP o tuberculosis, pero los que tenían neumonía bacteriana tenían PCR e IL-8 más altas y que la concentración de IL-8 era un predictor de mortalidad [79].

En cambio Gordon encontró que los pacientes con infección por VIH tenían más IL-1, más IL-6 y menos IL-8 en BAL que los seronegativos tras infección neumocócica y que la menor concentración de IL-8 provocaba un menor reclutamiento de neutrófilos y peor pronóstico [80].

1. 8. VACUNAS FRENTE A NEUMOCOCO EN PACIENTES CON INFECCIÓN POR VIH

En la actualidad se recomiendan dos vacunas frente a neumococo, la polisacárida 23-valente (Pneumo 23, Aventis-Pasteur, que contiene 25 ug de polisacáridos de 23 serotipos) y la vacuna conjugada heptavalente (Prevenar, Wyeth-Lederle, que contiene 2 ug de polisacáridos de los serotipos 4, 14, 19F, 23F, 18C y 9V y 4 ug de serotipo 6B conjugados a la proteína toxoide diftérica).

La vacuna polisacárida (PPV) ha demostrado ser inmunógena en pacientes con infección por VIH [81-87] y especialmente en aquellos con CD4 mayores a 200 céls/µL [85,87-89] y aquellos con TAR [88-90].

Es la vacuna recomendada en pacientes con infección por VIH, [91,92] con mayor énfasis en fases precoces cuando los CD4 están por encima de 200 céls/µL [46,93,94] y aunque se trata de una vacuna con bajo coste y bien tolerada, los estudios en cuanto a su eficacia en pacientes con infección por VIH son controvertidos. Quizás por ello no todos los profesionales siguen dichas recomendaciones, como reflejaron las bajas coberturas vacunales en varios estudios en pacientes con infección por VIH [47,50,95].

Algunos estudios de cohortes y casos controles evidenciaron una disminución de la incidencia de EIN especialmente en pacientes en TAR y con CD4 elevados pero también en pacientes con CD4 por debajo de 200 céls/µL [46,47, 57,93,95-98].

Otros estudios no pudieron demostrar dicho beneficio [45,99] incluyendo el único ensayo clínico randomizado, doble ciego controlado con placebo realizado en pacientes con VIH ugandeses sin TAR en el que no se evidenció disminución de la enfermedad neumocócica en vacunados ni menor mortalidad, incluso los vacunados tenían más neumonías por cualquier causa [100,101]. Tampoco se evidenció beneficio de la vacuna en pacientes inmunodeprimidos en un metanálisis que incluía estudios con PPV y placebo [102].

Varios estudios en pacientes con NAC observaron que los que habían sido vacunados previamente con PPV tenían menos complicaciones, una resolución de los síntomas más rápida y menor estancia hospitalaria que los no vacunados [103-105]. Se desconoce si la vacunación previa con PPV aporta dichos beneficios adicionales en pacientes con infección por VIH que desarrollan infección neumocócica.

Actualmente se recomienda en niños la vacunación frente a neumococo con la vacuna conjugada heptavalente (CPV). Muchos estudios demostraron que tras la vacunación de lactantes con CPV disminuyó la incidencia de EIN no sólo en niños [106-116], sino también en inmunodeprimidos [108-110], ancianos [109,110,117,118] y en pacientes con infección por VIH [59,119-121].

La inmunogenicidad de la CPV ha sido ampliamente demostrada en lactantes y niños [122-131] pero no ha demostrado ventajas sobre la PPV ni en ancianos [132-134] ni en inmunodeprimidos [135,136].

En España, la CPV se introdujo en 2001 como vacuna voluntaria y privada, en 2002 la Sociedad Española de Pediatría recomendó su uso con tres dosis a los 2, 4 y 6 meses y una dosis de recuerdo (booster) a los 24 meses. Desde esa fecha ha disminuido la enfermedad invasiva por neumococo debido a un descenso de la infección por serotipos incluidos en la vacuna conjugada con un pequeño aumento de los serotipos no incluidos en la vacuna como el serotipo 5 y el 19A [115].

En estudios prospectivos se vió que la CPV era menos inmunógena en niños con infección por VIH que en niños sanos e inducía Ac con menor actividad opsonofagocítica [131,137-140]. Los estudios en adultos también evidenciaron una menor respuesta inmune en los infectados por VIH [141,142], especialmente en los que tenían CD4 menores a 200 céls/µL [134].

La comparación de ambas vacunas, CPV y PPV, en pacientes con infección por VIH ha arrojado resultados contradictorios, así en algunas publicaciones se encontró una mejor respuesta con la CPV con mayor concentración de Ac [59,121,142,144] o mejor opsonofagocitosis [59,142] mientras que en otras no se evidenciaron diferencias [141,143].

Un estudio reciente, en pacientes con infección por VIH de Malawi en el que se comparaba la CPV con placebo, la CPV se asociaba a menor riesgo de enfermedad neumocócica por serotipos incluidos en la vacuna, pero no disminuía el riesgo de enfermedad neumocócica global por cualquier serotipo, ni la mortalidad [145].

Los trabajos que compararon la actividad funcional de los Ac inducidos por las dos vacunas en pacientes con infección por VIH, medida por actividad opsonofagocítica [59,90,142] o por avidez, se encontró mejor respuesta con la CPV.

1. 8. 1. Nuevas estrategias vacunales:

La PPV induce inmunidad contra los polisacáridos capsulares por lo que confiere una protección seroespecífica y aunque protegería frente al 90% de los serotipos que causan infección, los polisacáridos generan una inmunidad independiente de los linfocitos LT y son poco inmunógenos en menores de 2 años y en pacientes inmunodeprimidos (los más susceptibles a la infección).

Esta pobre inmunogenicidad se ha resuelto parcialmente mediante la conjugación de los polisacáridos a proteínas, las cuales pueden inducir una respuesta inmune con intervención de los LT, aunque la protección sigue siendo seroespecífica y debido a su alto coste el número de serotipos incluidos en las vacunas es limitado (siete, nueve, once o trece). Su precio, la necesidad de múltiples dosis y de conservación en nevera las hace poco adecuadas para países subdesarrollados.

Su uso en la mayoría de países desarrollados ha disminuido la EIN y la colonización nasofaríngea [122], aunque en algunos estudios se ha visto un aumento de la colonización nasofaríngea [145] y de la EIN [109,113,119] por serotipos no vacunales, por lo que se ha generado la inquietud de si las vacunas que sólo incluyen una parte de los serotipos no disminuirían la EIN sino que provocarían un cambio en el nicho ecológico con el reemplazamiento de los serotipos vacunales por serotipos no incluidos en las vacunas [5,145].

Por ello se está investigando en vacunas basadas en proteínas no seroespecíficas (derivados no tóxicos de la neumolisina, PsaA, PspA, NanA, lipoproteínas unidoras de metales, el polisacárido de pared celular, el ácido lipoteicoico de la membrana) que contribuyan a la virulencia, induzcan inmunidad y memoria inmunológica incluso en menores de 2 años y sean comunes a todos los serotipos.
Investigando Ac de pacientes expuestos al neumococo, se ha identificado un conjunto de proteínas que se ha llamado ANTIGENOMA, dos de las cuales, la serin-treonin proteinkinasa junto a PsaA y la proteína necesaria para la separación de la pared celular junto a PsaA, están en fase I como vacunas inyectables [38].

En general los Ag de especie parecen ser menos potentes que los polisacáridos capsulares para crear vacunas, pero se desconoce si la combinación de Ac frente a polisacáridos y Ac frente a Ag no capsulares sería sinérgica [38].

1. 9. MANNOSE-BINDING LECTIN

1. 9. 1 Descripción:

La MBL es una proteína plasmática de la familia de las lectinas, calcio dependiente, sintetizada por el hígado durante la fase aguda de las infecciones (la IL-6 la estimula y la IL-1 la inhibe, también regulada por las proteínas de fase aguda, PCR y amiloide sérico A) que se une a los polisacáridos del PGN, el cual está en la superficie de las bacterias pero no en las células humanas.
Tiene alta afinidad por la N-acetilglicosamina pero también a la N-acetilmanosamina, manosa, L-fucosa, y glucosa.

1. 9. 2. Funciones:

La MBL al unirse a los carbohidratos activa el complemento por la vía de las lectinas, que incluye varias proteasas séricas sobretodo la MASP (Proteasa Sérica Asociada a la Manosa): MASP1, MASP2, MASP3, y la MAP19 o sMAP (proteína asociada a la manosa sin actividad proteasa). La MASP2 es la principal iniciadora del complemento por la vía de las lectinas, la MASP1 actúa como amplificadora de la activación del complemento, se desconoce el papel de la MAPS3 y de la sMAP.
La MBL interactúa con la inmunidad innata al iniciar la cascada del complemento por la vía de las lectinas y con la inmunidad adaptativa estimulando la opsonofagocitosis, la MBL puede opsonizar bacterias sin que intervenga el complemento.

Se une al PGN con gran afinidad y su unión inhibe la estimulación de los macrófagos por parte del PGN, y por tanto se inhibe la liberación de citocinas pro-inflamatorias por parte de los macrófagos.

La MBL reconoce estructuras de las células apoptóticas necrosadas y estimula los fagocitos.

Se ha descubierto un nuevo papel de la MBL como co-receptor de los TLRs por lo que no sólo aumenta la degradación de los microorganismos sino también coordina, amplifica y sincroniza los mecanismos de inmunidad innata [147]. La MBL se ha encontrado en el BAL de pacientes con neumonía pero no en sanos indicando que podría filtrarse de la circulación sanguínea a los lugares de inflamación pulmonar para contenerla [148].

1. 9. 3. Gen MBL y polimorfismos:

La MBL está codificada por el gen MBL2 que está en el cromosoma 10.

Existen tres polimorfismos frecuentes en el gen MBL2 que consisten en un cambio de nucleótidos en el exón 1, las mutaciones simples se producen en los codones 52, 54 o 57 y se conocen como alelos D, B y C respectivamente. En estudios epidemiológicos estas variantes alélicas se unen en el alelo O (que aglutina estas tres variantes para facilitar el análisis) frente al alelo normal que se denomina alelo A.

Estas variantes producen concentraciones menores de MBL (las MBL variantes son más inestables, sufren una degradación enzimática más rápida y tienen menor vida media, menor afinidad a sus ligandos y menor capacidad para activar el complemento) [149].

Existen también mutaciones puntuales en el promotor del gen MBL2 y hay polimorfismos en tres regiones que se asocian a menores concentraciones de la MBL, en la región -50 son variantes H/L, en la región -221 son variantes X/Y y en la región -70 variantes C/T y la mutación en el extremo 5' variantes P/Q, se han descrito hasta 87 polimorfismos aunque la relevancia fenotípica en cuanto a los niveles plasmáticos es desconocida.

Los haplotipos de promotor también afectan las concentraciones plasmáticas de MBL siendo el haplotipo HY el que se correlaciona con mayores concentraciones, LY con concentraciones intermedias y LX con las concentraciones más bajas, similares a las de la alteración estructural B.
El 33% de la población es heterocigota para estas variantes y el 5% homocigota, en los heterocigotos las concentraciones de MBL son un 20% de las normales y en los homocigotos menores al 2%.

Los individuos con alelos XA en un cromosoma y O en el otro (XA/O) tienen niveles muy bajos MBL de manera que los genotipos XA/O y los O/O se consideran genotipos insuficientes de MBL. Aunque la deficiencia de MBL no se considera una inmunodeficiencia en sentido estricto ya que su penetrancia clínica es muy baja.
Hay una gran variacion en los niveles plasmáticos de MBL en la población (0.1 a 10000 ng/L), la deficiencia de MBL definida como menos de 100 ng/mL aparece en el 5-10% de la población, aunque se han utilizado diferentes cut-offs que puedan indicar deficiencia de MBL sin gran justificación (<100, <500 o <1000 ng/mL).

1. 9. 4. Relación con las infecciones:

En los últimos años ha crecido el interés en la MBL, debido a su papel como molécula de reconocimiento del complemento pero también en la relación entre las variantes genéticas y las concentraciones plasmáticas y la susceptibilidad a infecciones, tumores y enfermedades auto inflamatorias.

Algunos trabajos concluyeron que podría comportarse como reactante de fase aguda [150,151] pero otros no consiguieron demostrarlo [152,153] y algunos sólo evidenciaron dicho comportamiento en las sepsis por gramnegativos [154].

Varios estudios evaluaron la relación entre las mutaciones del gen de la MBL o el déficit de MBL y la susceptibilidad a infecciones con resultados contradictorios, así en estudios en niños Koch, Neth y Wiertsema [155-157] objetivaron mayor susceptibilidad a infecciones en los homocigotos y en los que tenían entre 6 y 17 meses.

Garred evidenció en niños con LES y niños con fibrosis quística [158,159] que los homocigotos tenían mayor susceptibilidad a infecciones, trató un paciente con fibrosis quística e infecciones por *P. aeruginosa* con MBL recombinante con estabilización de la función pulmonar [160], tratamiento que proponen otros autores para disminuir las infecciones en la población con déficit de MBL [161].

Según García-Laorden aunque las variantes del MBL2 no predisponían a NAC sí se asociaban con más complicaciones y mayor mortalidad una vez desarrollada [162].

Jorgensen encontró en pacientes con cáncer colorrectal que concentraciones bajas de MBL se asociaban a mayor riesgo de infecciones y por tanto menor supervivencia [163].

Otros estudios intentaron relacionar el déficit de MBL con enfermedad neumocócica con resultados contradictorios, así Roy [164] vio que los homocigotos para variantes del gen MBL tenían mayor riesgo de EIN, en cambio Kromborg, Dean, Tax y Hundt no evidenciaron tal riesgo [153,165-167].
La vía de la manosa tiene un papel menor en la activación del complemento frente a neumococo en comparación con las vías clásica y alternativa, de manera que los polimorfismos genéticos predispondrían sólo débilmente a infecciones por neumococo [29].

1. 10. PROTEÍNA C REACTIVA EN NEUMONÍA NEUMOCÓCICA

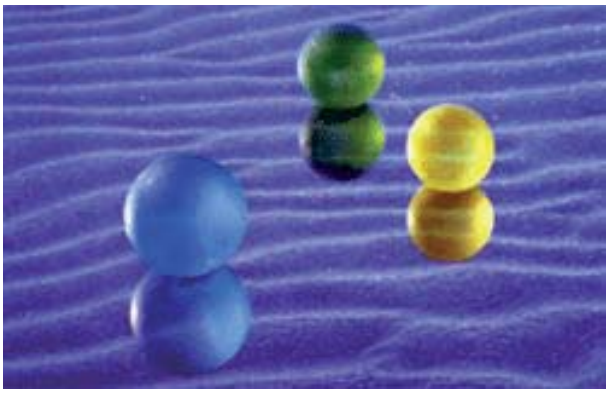
La PCR es una proteína sintetizada en el hígado como respuesta al estímulo de la IL-6, que juega un papel significativo en la respuesta inmune frente a bacterias.

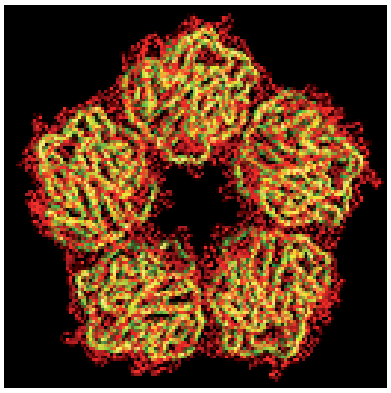
Se une al Polisacárido C de la pared celular de las bacterias grampositivas, entre ellas el neumococo (se une específicamente a la ChopP) e interactúa con el Cq1 activando la vía clásica del complemento y favoreciendo la opsonofagocitosis.

También bloquea la adherencia del neumococo a través del receptor del factor activador de plaquetas [5].

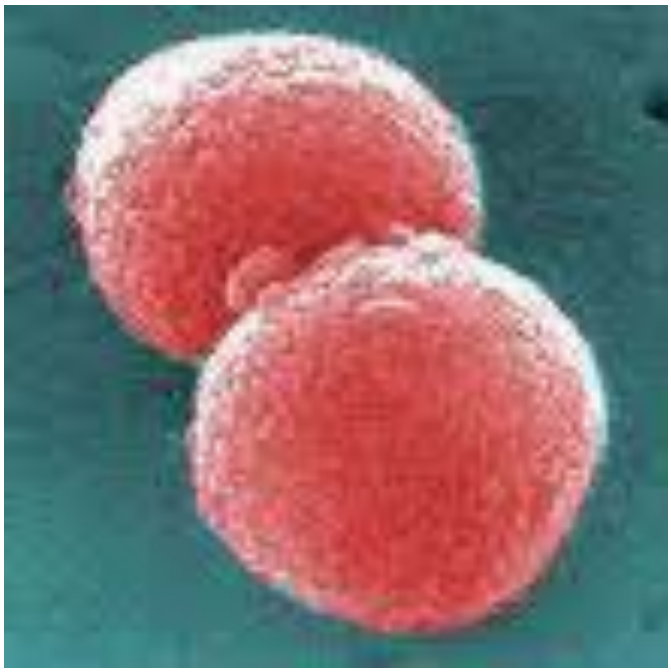
Los estudios recientes sobre PCR se centran en su utilidad como reactante de fase aguda, como marcador de riesgo de enfermedad coronaria y como marcador de actividad en enfermedades auto inflamatorias.

En la neumonía adquirida en la comunidad algunos estudios concluyeron que una PCR elevada era indicativa de etiología bacteriana y se relacionaba con la gravedad [168-171], aunque otros estudios concluyeron que la PCR no era útil para diferenciar etiología bacteriana en una NAC [172].





OBJETIVOS Y PLANTEAMIENTO EXPERIMENTAL



2. OBJETIVOS Y PLANTEAMIENTO EXPERIMENTAL

2. 1. HIPÓTESIS

2. 1. 1. La Mannose Binding Lectin y la Proteína C Reactiva se comportan como reactantes de fase aguda en la neumonía neumocócica.

2. 1. 2. El déficit de MBL, por determinación genética o por menores concentraciones plasmáticas se asocia a peor pronóstico en las neumonías neumocócicas.

2. 1. 3. Los pacientes con infección por el VIH tienen más riesgo de enfermedad neumocócica y se asocian a ese mayor riesgo factores inmunológicos, virológicos, diversos hábitos tóxicos, comorbilidades, y podrían asociarse a un menor riesgo el tratamiento del VIH o determinadas estrategias de prevención (cotrimoxazol, macrólidos o vacunación frente a neumococo).

2. 1. 4. La PPV de 23 serotipos recomendada en pacientes con infección por el VIH es eficaz en la prevención de enfermedad neumocócica, incluso en pacientes con infección avanzada.

2. 1. 5. La PPV de 23 serotipos, en pacientes con infección por el VIH, puede aportar beneficios adicionales a la prevención de la enfermedad neumocócica, de manera que los pacientes VIH vacunados que adquieren infección neumocócica, padecerían una enfermedad más leve con menor mortalidad que los no vacunados.

2. 1. 6. En pacientes con infección por el VIH e inmunodepresión moderada la vacunación secuencial con CPV seguida de PPV produce mayor concentración de anticuerpos y con mayor avidez por sus polisacáridos que la vacunación sola con PPV, siendo ambas estrategias vacunales seguras.

2. 2. OBJETIVOS:

Realizamos una serie de tres estudios retrospectivos y un ensayo clínico cuyos objetivos principales eran:

2.2.1. Evaluar el papel de la MBL y de la PCR en la neumonía neumocócica, su valor como reactantes de fase aguda y la correlación entre niveles de MBL y gravedad de la neumonía neumocócica.

2.2.2. Determinar los factores de riesgo de desarrollar enfermedad neumocócica en pacientes con infección por VIH.

2.2.3. Definir el papel de la vacuna antineumocócica PPV en la prevención frente a la neumonía u otras infecciones invasivas por neumococo en pacientes con infección por VIH.

2.2.4. Establecer los posibles efectos adicionales de la PPV en la evolución y pronóstico de pacientes con infección por VIH ingresados por enfermedad invasiva por neumococo, comparando los previamente vacunados con los no vacunados.

2.2.5. Determinar si la estrategia de vacunación secuencial con PPV seguida de CPV produce mayor concentración de anticuerpos específicos frente a los serotipos incluidos en la vacuna conjugada y con mayor avidez por sus polisacáridos, en comparación con la PPV sola, en pacientes con infección por VIH e inmunodepresión moderada.

2.2.6. Comparar la respuesta de anticuerpos a las 4 semanas tras cada una de las vacunas, evaluar el efecto "priming" de la CPV administrada 4 semanas antes de la PPV en pacientes con infección por VIH.

2.2.7. Valorar la seguridad de ambas vacunas.

2.2.8. Estudiar los factores que se asocian a respuesta vacunal a las dos vacunas, CPV y PPV, en pacientes con infección por VIH.

2. 3. PLANTEAMIENTO EXPERIMENTAL

2. 3. 1. Papel de la MBL y de la PCR en la neumonía neumocócica

Para evaluar el papel de la MBL y de la PCR en la neumonía neumocócica (NN), su valor como reactante de fase aguda y la correlación entre niveles de MBL y gravedad de la NN se realizó un estudio retrospectivo de adultos ingresados con NN entre junio de 2003 y junio de 2005, incluyendo de forma consecutiva aquellos pacientes en los que se aisló *Streptococcus pneumoniae* en hemocultivos, o en esputo junto a Ag urinario positivo, o en BAL o BAS.

Sólo se consideró el primer episodio si hubo más de uno y se excluyeron inmunodeficiencias primarias, infecciones nosocomiales, aquellos que rechazaron el consentimiento informado o aquellos en los que no se pudo realizar el seguimiento.

Se recogió una muestra de suero de cada paciente en las primeras 48 h del ingreso, en las que se determinaron los niveles de PCR por nefelometría (BNAII, Dade Behring, Marburg, Alemania) y de MBL por ELISA (AntibodyShop, Gentofte, Dinamarca) y se secuenció el gen de la MBL (PCRSSP, Stefensen) [173].

Se recogieron muestras de suero en la fase de convalecencia en las que se determinaron los niveles de PCR y de MBL.

Se compararon los genotipos de MBL y los niveles de MBL y de PCR entre los pacientes con neumonías de bajo riesgo y los pacientes con neumonías de alto riesgo, según la escala de Fine [174].

También se compararon los niveles en la fase aguda y de convalecencia y entre los pacientes con o sin bacteriemia o comorbilidad asociadas.

Finalemente se correlacionaron los niveles de MBL y de PCR en la fase aguda y en la convalcencia.

2. 3. 2. Factores de riesgo de enfermedad neumocócica y eficacia de la vacuna polisacárida en pacientes con infección por VIH

Para determinar los factores de riesgo de enfermedad neumocócica en pacientes VIH y la eficacia de la PPV, se realizó un estudio retrospectivo de casos y controles, entre enero de 1995 y diciembre de 2005, en 4 hospitales españoles: Hospital Son Dureta, Hospital Vall d'Hebrón, Hospital Son Llatzer y Mútua de Terrasa.

Los casos se seleccionaron de las bases de datos de pacientes con infección por VIH de cada hospital, los criterios de inclusión eran: infección VIH, mayores de 18 años, que hubieran presentado neumonía o enfermedad invasiva por *S. pneumoniae* entre 1995 y 2005. Se excluyeron los pacientes en los que no se disponía de una determinación de CD4 en los tres meses previos al episodio de infección neumocócica.

Se seleccionaron tres controles para cada caso, de las mismas bases de datos, pacientes sin infección neumocócica ni neumonía previa apareados con los casos por: sexo, edad (edad del caso en el momento del diagnóstico ± 5 años), CD4 (CD4 del caso en el momento del diagnóstico ± 50 céls/μL), mecanismo de transmisión del VIH (ADVP y otros mecanismos).

Se estudiaron en casos y controles las siguientes variables: edad, sexo, administración de la PPV (previa al episodio de infección en los casos y en cualquier momento en los controles) y fecha de la vacuna, mecanismo de transmisión del VIH (ADVP y otras transmisiones), ADVP, tabaquismo activo o enolismo activo (más de 80 gr de alcohol diarios), recuento de CD4, CV, estadío de la infección por VIH (estadío C y otros), profilaxis con cotrimoxazol o con macrólidos, estar en tratamiento retroviral (TAR), coinfección con virus de hepatitis B (VHB) o virus de hepatitis C (VHC) y comorbilidad (EPOC o cirrosis).

Para evaluar las variables asociadas a riesgo de enfermedad neumocócica se realizó un análisis bivariado y multivariado mediante un modelo de regresión logística.

Se evaluó el beneficio de la vacuna comparando casos y controles en aquellos con CD4 iguales o mayores a 200 céls/μL y en aquellos con CD4 menores a 200 céls/μL y también se compararon los vacunados hacía 5 años o menos y los vacunados hacía más de 5 años.

2. 3. 3. Beneficios adicionales de la vacuna polisacárida en pacientes con infección por VIH con enfermedad neumocócica

Para establecer los posibles efectos adicionales de la PPV en el pronóstico de la enfermedad invasiva por neumococo en pacientes con infección por VIH, realizamos un estudio observacional en el que se recogieron de forma consecutiva todos los episodios de EIN en adultos con infección por VIH ingresados entre enero de 1996 y octubre de 2007 en tres hospitales españoles: Hospital Son Dureta, Hospital Vall d'Hebrón y Mútua de Terrasa.

Se revisaron las historias clínicas de los pacientes de forma retrospectiva de 1996 a 1999 y desde el año 2000 de forma prospectiva.

Se recogieron las siguientes variables: edad, sexo, ADVP activa o previa, consumo de tabaco, abuso de alcohol, vacunación previa con PPV, comorbilidades (enfermedad hepática crónica, EPOC, neoplasia sólida o hematológica y esplenectomía), mecanismo de transmisión del VIH, recuento de CD4, CV, TAR, profilaxis con cotrimoxazol, estadío CDC de la infección por VIH, síndrome clínico por el que ingresó (neumonía, meningitis, peritonitis o bacteriemia primaria), gravedad de la neumonía (escala de Fine), serotipo (realizado en el Instituto de Salud Carlos III), sensibilidad de la cepa de neumococo (por Etest) y finalmente variables pronósticas como mortalidad hospitalaria, necesidad de intubación oro traqueal (IOT) o necesidad de ingreso en la unidad de cuidados intensivos (UCI).

Se compararon las variables en vacunados y no vacunados.

2. 3. 4. Evaluación de la respuesta inmune de una estrategia de vacunación secuencial con vacunas conjugada y polisacárida frente a la vacuna polisacárida en pacientes con infección por VIH

Para determinar si la vacunación doble con PPV y CPV producía mayor concentración de anticuerpos específicos frente a los serotipos incluidos en la CPV y con mayor avidez que la PPV sola, en pacientes con infección por VIH e inmunodepresión moderada, se llevó a cabo un ensayo clínico randomizado y abierto en los hospitales de Son Dureta y de Son Llatzer de Mallorca entre diciembre de 2007 y abril de 2008.

Eran elegibles los pacientes que acudían a consultas con CD4 entre 200 y 500 céls/µL y CV menor a 5 log copias/mL no vacunados frente a neumococo.

Era criterio de exclusión: embarazo, ADVP en los 6 meses anteriores, tratamiento antibiótico en las 4 semanas previas, tratamiento radio o quimioterápico, enfermedad renal o hepática avanzadas, uso de otros fármacos en investigación, inmunoglobulinas u otras vacunas en las 8 semanas previas e inmunodeficiencias primarias o secundarias diferentes al VIH.

Se recogieron las siguientes variables: edad, sexo, tabaquismo, enolismo, consumo de cannabis o cocaína, recuento de CD4 y CV en el momento de la inclusión, CD4 nadir y CV cénit, estadío CDC de la infección por VIH, mecanismo de transmisión del VIH, TAR, profilaxis con cotrimoxazol, neumonía previa y número de episodios, asma, EPOC, cirrosis hepática, coinfección por VHB o VHC o diabetes mellitus.

El tamaño muestral se calculó para asegurar un 80% de poder para detectar diferencias del 15% en el porcentaje de respondedores en los dos grupos para una muestra de 2500 pacientes (error tipo I: 5%). Se necesitaba una muestra de 200 pacientes y asumiendo una pérdida de un 10% de pacientes, se seleccionaron 220 pacientes.

Los pacientes que aceptaron y firmaron el consentimiento informado, se randomizaron 1:1 (sobres sellados con la estrategia vacunal) a una de las dos estrategias.
Los asignados al grupo 1 recibieron una dosis de CPV y una dosis de PPV 4 semanas después.
Los pacientes asignados al grupo 2 recibieron una sola dosis de PPV.
Los efectos secundarios se recogieron mediante entrevista telefónica a los 3 días de la vacunación.

Se recogieron las muestras de suero antes de la CPV (basal), antes de la PPV (4 semanas) y a las 8 semanas en el grupo 1, y en el grupo 2 antes de la PPV (basal) y a las 4 semanas.

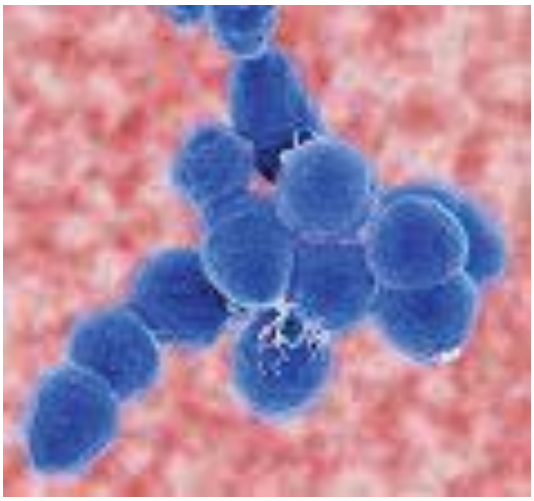
Se determinaron las IgG específicas frente a los polisacáridos capsulares 4, 14, 23F, 19F, 6B, 18C y 9V en todas las muestras, mediante la técnica de ELISA descrita por Wernette et al. [175], los sueros fueron absorbidos con polisacárido de pared celular y con polisacárido 22F.

La avidez de cada IgG por sus polisacáridos se determinó en todas las muestras utilizando la metodología descrita por Romero-Steiner et al. [176] que utiliza un ELISA con una dilución única de agente careotrópico (tiocianato sódico a 0,45M) y diferentes diluciones del suero muestra. Los resultados se expresaron como índice de avidez que indica el porcentaje de Ac que permanecen unidos al Ag en presencia del agente careotrópico.

Se compararon las variables en los dos grupos vacunales (T Student no pareada para variables cuantitativas y Chi cuadrado para variables cualitativas).
Se compararon las concentraciones medias geométricas de las IgG (GMC) en los 2 grupos vacunales (T Student para muestras no pareadas) basalmente, a las 4 y a las 8 semanas.
Se comparó en los dos grupos el porcentaje de pacientes respondedores para cada serotipo a las 8 semanas (4 semanas tras la PPV en ambos grupos) utilizando dos criterios:
- primer criterio de respuesta vacunal: aumento dos veces el valor inicial de IgG tras la vacunación.
- segundo criterio de respuesta vacunal: aumento dos veces el valor inicial de IgG y alcanzar como mínimo el valor de 1 µg/mL tras la vacunación.

Se realizó un análisis bivariado y multivariado mediante un modelo de regresión logística para determinar los factores asociados a respuesta a un mínimo de 3, 4 o 5 serotipos.
Se compararon los índices de avidez antes y tras la vacunación (T Student para muestras apareadas) y entre las dos estrategias vacunales (T Student para muestras independientes) y se correlacionaron con las concentraciones de dichos Ac. Se estudió la relación entre avidez y neumonía previa, EPOC, tabaquismo, enolismo, estadío de la infección VIH, CV detectable o indetectable, CD4 por encima o debajo de 350 céls/µL, CD4 nadir mayores o menores a 200 céls/µL, TAR o coinfección por VHB o VHC.

RESULTADOS Y DISCUSIÓN



RESUMEN DE LAS PUBLICACIONES: RESULTADOS Y DISCUSIÓN

3. 1. LA MANNOSE-BINDING LECTIN NO ACTÚA COMO REACTANTE DE FASE AGUDA EN ADULTOS CON NEUMONÍA ADQUIRIDA EN LA COMUNIDAD

La PCR, la MBL y el gen MBL se estudiaron en 100 pacientes consecutivos con NN (68 hombres y 32 mujeres), 33 tenían EPOC, 15 VIH, 9 diabetes, 9 insuficiencia cardiaca, 7 enfermedad hepática y 4 llevaban corticoides. En 43 se obtuvieron muestras en la convalecencia.
De ellos 43 tuvieron neumonía de bajo riesgo y 67 de alto riesgo.
Cincuenta tuvieron el genotipo wild type (AA), 43 heterocigotos (AO) y 4 homocigotos (OO). Los pacientes AA tenían niveles de MBL mayores que los genotipos mutados AO y OO tanto en la fase aguda (mediana de 4225 ng/mlvs. 700 ng/ml, p<0.001), como en la convalecencia (4000 ng/ml vs. 650 ng/ml, p<0.001).
Aquellos con genotipo AA tenían más riesgo de bacteriemia que los AO y OO (OR 2,74, CI95% 1,01-7,52, p: 0,02), pero sin diferencias en cuanto a la severidad de la NN.

Los niveles de MBL eran mayores en aquellos con bacteriemia en la fase aguda (3038,49 ng/ml vs. 2532,17 ng/ml) y en la de convalecencia (2751,94 ng/ml vs. 1923,12 ng/ml) pero sin diferencias significativas. Los niveles de PCR eran mayores en aquellos con bacteriemia en la fase aguda (79,53 mg/l vs. 36,68 mg/l, p: 0,003) aunque estas diferencias desaparecían en la fase de convalecencia (6,03 mg/l vs. 12,78 mg/l). Los niveles de PCR en la fase de convalecencia eran mayores en aquellos con comorbilidad (31,90 mg/l vs. 5,77 mg/l, p: 0,01) en cambio los de MBL eran similares.

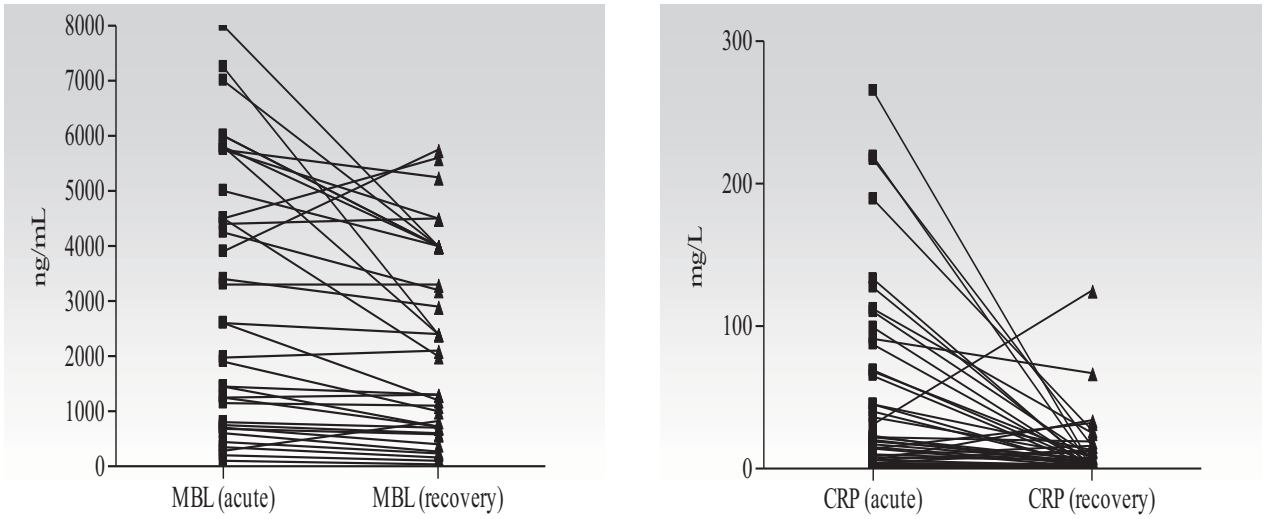
Los niveles MBL eran ligeramente mayores en los grupos de alto riesgo pero sin diferencias significativas, tanto en fase aguda como en convalecencia, tampoco hubo diferencias al separar por genotipos. Los niveles de PCR eran mayores en la fase de convalecencia para los grupos de alto riesgo. Tabla 1.

No hubo correlación entre los niveles de MBL y PCR ni en la fase aguda (p: 0,87) ni en la de convalecencia (p: 0,53) así como tampoco había relación entre esos niveles y el índice de gravedad de Fine. Fig. 1.
Cinco pacientes murieron, sin diferencias en las concentraciones de MBL o de PCR con los que sobrevivieron.

Tabla 1. Concentraciones de MBL y PCR en la fase aguda y en la fase de convalecencia dependiendo del grupo de riesgo de la neumonía.

	RIESGO BAJO (Fine I, II y II)	RIESGO ALTO (Fine IV y V)	P
Toda la población			
MBL en la fase aguda (ng/ml) Mediana (rango)	1450 (35-7250)	2600 (90-10000)	ns
MBL fase convalecencia (ng/ml) Mediana (rango)	1850 (100-5250)	2400 (35-5750)	ns
PCR fase aguda (mg/l) Mediana (rango)	22 (1-578)	31 (1-294)	ns
PCR fase convalecencia (mg/l) Mediana (rango)	2 (0-25)	6 (1-125)	0,005
Sólo genotipos AA			
MBL fase aguda (ng/ml) Mediana (rango)	4500 (1400-7250)	3750 (1150-10000)	ns
MBL fase convalecencia (ng/ml) Mediana (rango)	2400 (1700-5250)	4000 (1200/5750)	ns
Sólo genotipos AO/OO			
MBL fase aguda (ng/ml) Mediana (rango)	675 (35-2500)	750 (90-2200)	ns
MBL fase convalecencia (ng/ml) Mediana (rango)	720 (100-2100)	580 (35-1300)	ns

Figura 1. Concentraciones de MBL y de PCR durante la fase aguda y durante la fase de convalecencia.



DISCUSIÓN

3. 1. 1. PAPEL DE LA PCR EN EL DIANÓSTICO DE LA ENFERMEDAD NEUMOCÓCICA

En nuestro trabajo, en pacientes con neumonía neumocócica, la PCR era mayor en la fase aguda que en la de convalecencia y mayor en aquellos con bacteriemia asociada.
En la fase de convalecencia se mantenía más alta en los pacientes con comorbilidad. Pero no se correlacionaba con la gravedad de la neumonía, como tampoco se correlacionaba en el estudio de Hedllund en pacientes hopitalizados por NAC [169].
En cambio, Ortqvist en pacientes ingresados por NAC, encontró que los pacientes con niveles más altos de PCR tenían mayor duración de la fiebre y mayor estancia hospitalaria [168], también encontró una asociación entre niveles más altos de PCR y causa neumocócica y niveles más bajos y causa vírica.

Algunos autores propusieron la cuantificación de la PCR para determinar la gravedad de las neumonías y para diferenciar causa bacteriana de causa viral.
Así, García-Vázquez encontró que las NAC por *Legionella* tenían PCR más altas que las NAC por otras etiologías y que el punto de corte de 25 mg/l diferenciaba *Legionella* de otras etiologías con una sensibilidad del 60% y una especificidad del 83% [170].
Almirall también vio que las NAC por neumococo y por *Legionella* tenían PCR más altas que las NAC por virus o por *Coxiella* o de causa no filiada y que los pacientes hospitalizados tenían niveles mayores que los tratados ambulatoriamente y que el punto de corte de 10,6 en hombres y 11 mg/l en mujeres diferenciaba los pacientes ingresados de los ambulatorizados con una sensibilidad y una especificidad del 80%, nivel que proponían para decidir el ingreso en pacientes con NAC [177].

Por el contrario, un meta-análisis realizado por Van der Meer que incluía estudios con niveles de PCR para discernir la etiología de las NAC, no encontró evidencia de que la PCR fuera útil para confirmar o descartar ninguna etiología [172].

No encontramos correlación entre los niveles de MBL y los de PCR, en concordancia con otros trabajos [178].
Las muestras se recogían en las 48h del diagnóstico de neumonía lo que podía haber alterado los niveles de PCR ya que la vida media es de 19 horas, pero no los de la MBL cuya vida media es 5-7 días.

3. 1. 2. PAPEL DE LA MBL EN EL DIAGNÓSTICO DE LA ENFERMEDAD NEUMOCÓCICA

En este estudio la MBL, a diferencia de la PCR, no se comportaba como reactante de fase aguda tal como describieron otros autores [150,153,156,178-180]. Tampoco sus concentraciones se relacionaban con la gravedad de la neumonía, aunque había una tendencia a mayor bacteriemia en aquellos con mayores concentraciones.

Múltiples trabajos habían encontrado asociación entre déficit de MBL y mayor susceptibilidad a infecciones [155-158,164,167,181], en cambio otros tantos no evidenciaron asociación alguna [153,165, 166,182,183].

Endeman en pacientes con NAC no encontró relación entre genotipos deficientes de MBL y susceptibilidad a NAC ni a mayor gravedad de las NAC [184]. García Laorden tampoco encontró relación entre genotipos deficientes y riesgo de NAC, dentro de las NAC los genotipos deficientes se asociaban a mayor necesidad de UCI, distress respiratorio, fracaso multiorgánico y mortalidad a 90 días, pero no se asociaban ni con el índice de gravedad de Fine ni con mayor bacteriemia [162].

Como en otros estudios [149,185,186] en nuestro estudio los genotipos mutados del gen MBL eran los que tenían menores concentraciones plasmáticas de MBL.
Curiosamente los pacientes con genotipo wild-type presentaban una tendencia a mayor bacteriemia, quizás como adelantó Nadesalingan la unión MBL a la N-acetilglicosamina inhibe la inducción por el PGN de citocinas pro-inflamatorias y estimula el reclutamiento de fagocitos favoreciendo el buen anclaje a la superficie de estas células y permite el paso al torrente circulatorio [187].

Eisen, uniendo varios estudios de MBL y sepsis, determinó que el punto de corte de 500 ng/ml era el de mayor sensibilidad (82%) y especificidad (82%) para predecir genotipos de MBL deficientes y con dicho nivel se diagnosticaban más pacientes con déficit de MBL que utilizando los genotipos deficientes. También concluyeron que en pacientes con sepsis el déficit de MBL se asociaba a mayor mortalidad de forma no significativa y en pacientes con NN el déficit de MBL se asociaba a mayor mortalidad de forma significativa [188].
En nuestro trabajo no se encontró más déficit de MBL entre los que murieron (sólo 5 pacientes).

3. 2. FACTORES DE RIESGO DE ENFERMEDAD NEUMOCÓCICA EN PACIENTES CON INFECCIÓN POR VIH Y EL PAPEL DE LA VACUNA ANTINEUMOCÓCICA: ESTUDIO DE CASOS Y CONTROLES

Se incluyeron 736 pacientes, 73% hombres, con una edad media de 38 años. Solamente habían recibido vacunación antineumocócica 151 pacientes (20%), 20 casos (11%) y 131 controles (24%). Tabla1.

En el análisis bivariante se relacionaron con enfermedad neumocócica: ADVP (OR 3,33; CI95% 2–5,55), alcoholismo activo (OR 3,03; CI95% 1,86–4,91), EPOC (OR 2,58; CI95% 1,3–5,1), cirrosis hepática (OR 6,05; CI95% 3,2–11,4), TAR (OR 0,23; CI95% 0,16–0,32), profilaxis con cotrimoxazol (OR 0,66; CI95% 0,45–0,97), CV menor a 5000 copias/mL (OR 0,38; CI95% 0,26–0,54) y la vacunación previa con PPV (OR 0,39; CI95% 0,24-0,65), no se encontró relación entre infección neumocócica y sexo, tabaquismo, estadío de la infección VIH, recuento de CD4 o coinfección con VHB o VHC. Tabla 2.

En el análisis multivariante los factores de riesgo asociados a enfermedad neumocócica fueron cirrosis hepática (OR 5,64; CI95% 2,53-12,53), EPOC (OR 2,90; CI95% 1,21-6,94) y enolismo activo (OR 2,15; CI 95% 1,11-4,19) y los factores que se asociaban a protección frente a enfermedad neumocócica fueron estar tomando TAR (OR 0,23; CI95% 0,14–0,36) y haber sido vacunados frente a neumococo con PPV. Tabla 2.

Tras estratificar la muestra entre aquellos con CD4 iguales o mayores de 200 céls/µL (mediana 325) y aquellos con CD4 por debajo de 200 céls/µL (mediana 89) la vacunación con PPV seguía siendo un factor protector en ambos grupos con una mayor protección en el grupo con CD4 menores a 200 céls/µL (OR 0,15; CI 95% 0,05-0,50) frente al grupo con CD4 iguales o mayores de 200 céls/µL (OR 0,55; CI 95% 0,31-0,99).

Tras estratificar los pacientes entre los que habían sido vacunados hacía 5 años o menos y los vacunados hacía más de 5 años, el efecto protector se mantenía tanto en los vacunados en los 5 años previos (OR 0,36; CI 95% 0,17-0,77) como en los vacunados hacía más de 5 años (OR 0,55; CI 95% 0,34-0,98).

Tabla 1. Características basales en casos y controles.

Variables	184 Casos N(%)	552 Controles N(%)	p
Edad (mediana)	38	38	1
Sexo masculino	134 (73%)	402 (73%)	1
Tabaquismo	137 (75%)	235 (72%)	0,43
Alcoholismo	36 (20%)	41 (7%)	<0,001
ADVP activa	37 (26%)	41 (10%)	0,03
CD4 (mediana)	204	210	0,60
CD4≥200 céls/µL	95 (52%)	291 (53%)	0,79
CV (mediana log)	4	3	0,04
CV<5000copias/mL	60 (38%)	309 (61%)	<0,001
Estadio C	70 (38%)	190 (34%)	0,37
Cotrimoxazol	43 (23%)	174 (31%)	0,04
Macrólidos	0 (0%)	4 (1%)	0,24
TAR	79 (43%)	422 (77%)	<0,001
VHB	15 (8%)	33 (6%)	1
VHC	130 (71%)	390 (71%)	1
EPOC	16 (9%)	19 (4%)	0,005
Cirrosis	29 (16%)	16 (3%)	<0,001
PPV 23-valente	20 (11%)	131 (24%)	<0,001

Tabla 3. Factores asociados a enfermedad neumocócica en adultos VIH

Variables	Análisis bivariado OR (CI95%)	P	Análisis multivariado OR (CI95%)	P
Sexo	1 (0,68-1,45)	1		
Tabaquismo	0,84 (0,56-1,28)	0,43		
Alcoholismo	3,03 (1,86–4,91)	<0,001	2,15 (1,11-4,19)	0,02
ADVP activa	3,33 (2–5,55)	0,03		0,46
CD4≥200 céls/µL	1,04 (0,75-1,46)	0,79		
CV<5000cop/mL	0,38 (0,26–0,54)	<0,001		0,24
Estadío C	0,85 (0,60-1,20)	0,37		
Cotrimoxazol	0,66 (0,45-0,97)	0,04		0,80
TAR	0,23 (0,16–0,32)	<0,001	0,23 (0,14–0,36)	<0,001
VHB	0,71 (0,38-1,35)	0,30		
VHC	1 (0,69-1,44)	1		
Cirrosis	6,05 (3,2–11,4)	<0,001	5,64 (2,53-12,53)	<0,001
EPOC	2,58 (1,3–5,1)	<0,001	2,90 (1,21-6,94)	0,02
PPV 23-valente	0,39 (0,24-0,65)	<0,001	0,44 (0,22-0,88)	0,02

DISCUSIÓN

3.2.1. FACTORES DE RIESGO DE ENFERMEDAD NEUMOCÓCICA EN PACIENTES CON INFECCIÓN POR VIH

Publicaciones previas determinaron algunos factores asociados a enfermedad neumocócica en pacientes con infección por VIH como la raza negra [45,47,56], el tabaquismo [50,53], alcoholismo [47,57], ADVP [45,47], CD4 menores a 200 céls/μL [45,46,50], neumonía previa u hospitalización previa [45,46,57], profilaxis con cotrimoxazol [53] y enfermedades de base como linfoma [50,56], cirrosis [45,50,57], EPOC [50] o hipoalbuminemia [45].

En nuestro estudio el enolismo activo y sobre todo las comorbilidades como la cirrosis y la EPOC se revelaron como factores de riesgo para la enfermedad neumocócica.
Ni la ADVP ni el uso de cotrimoxazol aparecieron como factores de riesgo en el análisis multivariado aunque eran más frecuentes en los casos que en los controles.
Tan sólo Kohli et al. [53] concluyeron que el cotrimoxazol se asociaba a menor riesgo de neumonía bacteriana en una cohorte prospectiva de mujeres VIH, al contrario que Jordano et al. [50] quienes no sólo no encontraron relación alguna entre el uso de cotrimoxazol y el riesgo de EIN sino que dicho uso se asociaba a mayor resistencia a cotrimoxazol y a penicilinas.

Una de las limitaciones del estudio era su diseño retrospectivo, por lo que algunos datos epidemiológicos como el tabaquismo o el enolismo, podrían no haberse recogido de forma exacta en las historias clínicas y ello podría explicar el hecho de que el tabaquismo no se asociara a mayor riesgo de enfermedad neumocócica y sí la EPOC que está íntimamente ligada al consumo de tabaco.

En nuestro trabajo el TAR fue el factor con mayor efecto protector frente a la infección neumocócica en el análisis multivariado, lo que concuerda con la mayoría de estudios previos tanto casos y controles [45,46] como estudios de cohortes [47,53,70].

3.2.2. EFICACIA DE LA VACUNA POLISACÁRIDA EN LA PREVENCIÓN DE ENFERMEDAD NEUMOCÓCICA EN PACIENTES CON INFECCIÓN POR VIH

La conclusión más importante del estudio fue que la PPV era un factor protector independiente frente a la infección neumocócica en pacientes con infección por VIH, lo que concuerda con las conclusiones de otras cohortes [57,96,189] y de otros estudios de casos y controles [46,47,93,95].

El principal argumento en contra de la PPV en pacientes con infección por VIH viene del único trabajo prospectivo y randomizado que comparaba la PPV frente a placebo en ugandeses VIH e inmunodepresión avanzada sin TAR, que no sólo demostró una falta de eficacia en la prevención de neumonía neumocócica y en la mortalidad, sino que los vacunados tenían más neumonías por "cualquier causa" [100]. Pero un seguimiento posterior a seis años, demostró una reducción de la mortalidad y de las neumonías por cualquier causa en los vacunados, sin diferencias significativas respecto a los que recibieron placebo [101]. Aun así, sus conclusiones no serían aplicables a nuestro medio con pleno acceso al TAR.

En nuestro estudio la tasa de vacunación de pacientes VIH era muy baja (20%) a pesar de las recomendaciones, similar a la objetivada por Dworkin en pacientes VIH americanos (37%) [47] y Grau en pacientes VIH españoles (7%-25%) [57] y menor a la tasa reportada en 2005 en mayores de 65 años de Cataluña, que era del 35%, [190].
Una explicación para la pobre cobertura vacunal es la controversia en la eficacia de la PPV y su menor inmunogenicidad en pacientes inmunocomprometidos [87,143,189,191,192]. Otra explicación podría ser la percepción que la enfermedad neumocócica no es un problema importante desde la era TAR y que es más coste efectivo la mejora del cumplimiento y de la eficacia del TAR [193].

En trabajos previos el efecto protector de la vacuna se limitaba a determinados grupos de pacientes VIH, como la raza blanca [95] o CD4 mayores a 200 céls/μL [46,93]. En nuestro trabajo el efecto protector se mantenía, también en aquellos con inmunodepresión avanzada (CD4<200 céls/μL) y además persistía más allá de los 5 años de la vacunación, lo que añade más incertidumbre sobre si la revacunación sería útil y si sería el intervalo de 5 años el periodo más adecuado para la revacunación [86,87].

3. 3. IMPACTO DE LA VACUNACIÓN PREVIA EN EL PRONÓSTICO DE ADULTOS CON INFECCIÓN POR VIH HOSPITALIZADOS POR ENFERMEDAD NEUMOCÓCICA INVASIVA

Entre 1996 y 2000 se diagnosticaron 179 episodios de EIN en 165 pacientes VIH.
Se recogió el número de episodios y no el de pacientes ya que 15 individuos tuvieron más de un episodio (35), en 6 el paciente había recibido PPV y en 29 aún no había sido vacunado.

En 23 casos el paciente había sido vacunado previamente con PPV y en 7 la vacuna había sido administrada hacía más de 5 años.
En el grupo de vacunados había un sólo paciente con enfermedad hepática crónica frente a 27 en el grupo de los no vacunados, diferencia no estadísticamente significativa. Los vacunados tenían un recuento de CD4 mayor que los no vacunados (325 céls/µL vs. 209 céls/µL, p: 0,014) y mayor porcentaje tomaban TAR (59,1% vs. 34,5%, p: 0,034). Tabla 1.
El síndrome más frecuente de EIN fue la neumonía neumocócica bacteriémica en ambos grupos y ningún paciente que había sido vacunado previamente presentó meningitis. Tabla 2.

Se serotiparon 101 de los aislados de neumococo, 84 eran serotipos incluidos en la PPV (83,2%), sin diferencias significativas entre vacunados y no vacunados (76,9% vs. 84,1%).

Ninguno de los 23 episodios en pacientes vacunados necesitaron UCI o murieron en el hospital en comparación con los 34 episodios (24,5%) en pacientes no vacunados (p: 0,004). La estancia hospitalaria fue significativamente más corta en pacientes vacunados (8,5 vs. 13,3 días, p: 0,011).

Al analizar el subgrupo de pacientes con neumonía bacteriémica los vacunados tenían menor proporción de neumonía grave (clases IV y V de Fine) que los no vacunados (16,7% vs. 37,4%) y ninguno de los vacunados desarrolló empiema vs. el 9,3% de los no vacunados, sin diferencias significativas.

Se realizó el análisis excluyendo los pacientes con enfermedad hepática crónica, ninguno de los vacunados murió en el hospital o precisó ingreso en UCI vs. el 22% de los no vacunados (p: 0,013), 14% murieron en el hospital y 18% precisaron ingreso en UCI. La estancia hospitalaria fue menor en los vacunados (8,5 vs. 14,3 días, p: 0,007).

Tabla 1. Características basales dependiendo del estado de vacunación frente a neumococo.

VARIABLES	VACUNADOS (N=23)	NO VACUNADOS (N=139)	P
Edad Media (±DE)	38,5 (±9,1)	38,5 (±8,6)	0,99
Sexo masculino	20 (87%)	106 (76%)	0,41
ADVP o ex-ADVP	13 (56%)	103 (74%)	0,13
Consumo de tabaco	17 (74%)	90 (65%)	0,48
Abuso alcohol	4 (17%)	31 (22%)	0,79
Hepatopatía crónica	1 (4.3%)	27 (19%)	0,13
EPOC	2 (8.7%)	9 (6.5%)	0,66
Neo hematológica o esplenectomía	4 (17%)	4 (3%)	0,02
Neoplasia sólida	0 (0%)	2 (1%)	1
Hospitalización previa	4 (17%)	40 (29%)	0,32
Media CD4 (±DE)	325 (±244)	209 (±192)	0,01
CD4≥200 céls/µL	15 (71%)	61 (44%)	0,03
CV <50copias/mL	5 (25%)	13 (12%)	0,01
TAR	13 (59%)	48 (36%)	0,03
Cotrimoxazol	5 (23%)	40 (29%)	0,62
Estadío C	9 (39%)	58 (42%)	0,82

Tabla 2. Características clínicas y microbiológicas dependiendo del estado de vacunación frente a neumococo.

	VACUNADOS (N=23)	NO VACUNADOS (N=139)	P
Neumonía bacteriémica	21/23 (91%)	113 /139 (81%)	0,37
Bacteriemia primaria	2 /23 (9%)	6/139 (4%)	0,32
Meningitis	0/23 (0%)	10/139 (7%)	0,36
Peritonitis	0/23 (0%)	10/139 (7%)	0,36
Serotipo incluido en PPV	10/13 (77%)	74/88 (84%)	0,45
Cepa resistente a penicilina	5/23 (22%)	58/139 (42%)	0,10
Cepa resistente a cefotaxima	1/20 (5%)	21/108 (19%)	0,19
Cepa resistente a Cotrimoxazol	5/20 (25%)	63/133 (47%)	0,09

Tabla 3. Variables pronósticas dependiendo del estado de vacunación frente a neumococo.

	VACUNADOS (N=23)	NO VACUNADOS (N=139)	P
Muerte o necesidad UCI	0/23 (0%)	34/139 (24%)	0.00
Mortalidad intrahospitalaria	0/23 (0%)	25/139(18%)	0.03
Necesidad UCI	0/23 (0%)	21/139 (15%)	0.05
Intubación oro traqueal	0/23 (0%)	15/139 (11%)	0.13
Shock	1/21 (5%)	16/114(14%)	0.47
Empiema	0/21 (0%)	10/108 (9%)	0.36
Neumonía alto riesgo (IV,V Fine)	3/18 (17%)	40/107 (37%)	0.11
Media estancia hospitalaria (±DE)	8,48 (±6,14)	13,27 (±14,62)	0.01
Media días a defervescencia (±DE)	2,55 (±2,72)	3,21 (±4,28)	0.48

DISCUSIÓN

La conclusión del estudio fue que los pacientes con infección por VIH vacunados con PPV que desarrollaban EIN (lo que podría considerarse un fracaso de la vacuna), tenían un mejor pronóstico de ésta con menor mortalidad, menos complicaciones y menor estancia hospitalaria.

Trabajos anteriores ya habían descrito mejor evolución de las neumonías en pacientes no VIH previamente vacunados frente a neumococo.
Mykietiuk et al. en 554 adultos hospitalizados por NAC vio que los vacunados con PPV tenían menor bacteriemia, menor mortalidad, más rápida resolución de los síntomas y menor estancia hospitalaria [104].
Fisman et al. en 62.918 adultos hospitalizados por NAC evidenció que los que habían recibido PPV tenían menor estancia hospitalaria y menos complicaciones y una de reducción de la mortalidad del 40-70% [105].
Johnstone et al. en una cohorte prospectiva de 3.415 adultos hospitalizados por NAC observó que los vacunados con PPV tenían menor mortalidad y menor necesidad de ingreso en UCI [103].

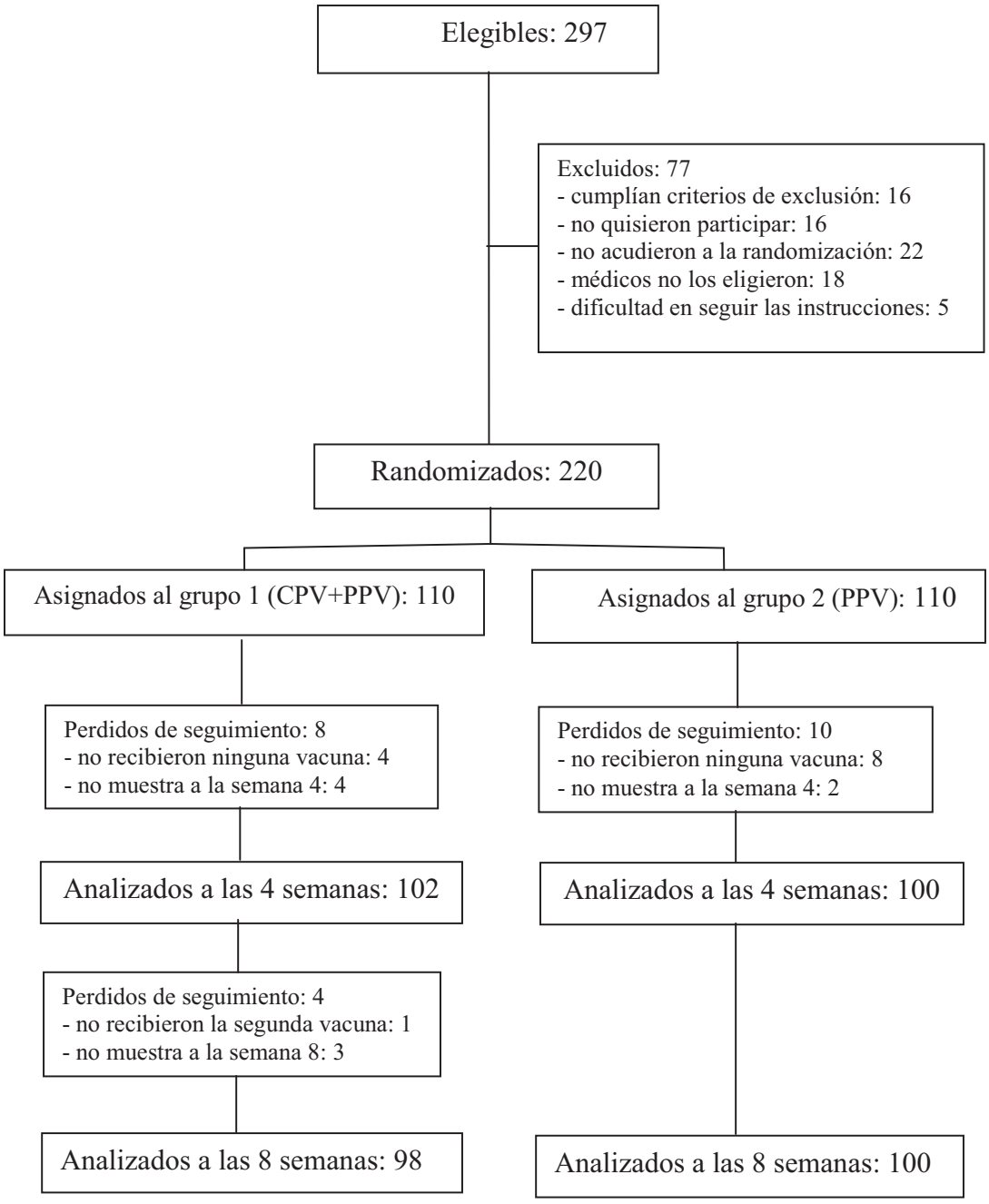
Una posible explicación de éstos efectos adidionales de la PPV sería que, aunque la respuesta vacunal no sea suficiente para evitar la infección neumocócica, la respuesta parcial podría atenuar la inflamación inicial y prevenir la mortalidad precoz y las complicaciones, ya que la vacuna además de la inducción de anticuerpos, estimula la opsonización, activa el complemento y promueve la fagocitosis [195,196].

En 7 pacientes la PPV se había administrado hacía más de 5 años, ninguno murió ni precisó ingreso en UCI, lo que indica que estos efectos adicionales se mantienen más allá del tiempo recomendado para la revacunación [92].

Los pacientes vacunados tenían CD4 más altos y mayor proporción estaban tomando TAR, variables relacionadas con menor incidencia de EIN [46, 47,50, 57,60, 70, 71]. En el mismo sentido sólo uno de los pacientes con hepatopatía crónica había recibido PPV, factor de riesgo de infección neumocócica, pero también de peor respuesta inmune a la vacuna [196] y entre los vacunados menor proporción eran ADVP, también factor de riesgo de EIN, lo que indica que aún queda un gran esfuerzo para vacunar a la población VIH que precisamente tiene mayor riesgo de EIN.
De acuerdo con otros estudios los serotipos más frecuentemente implicados en la EIN estaban incluidos en la PPV [70,197].

3. 4. UNA ESTRATEGIA DE VACUNACIÓN SECUENCIAL FRENTE A NEUMOCOCO CONVACUNAS CONJUGADA Y POLISACÁRIDA FRENTE A LA VACUNA POLISACÁRIDA EN PACIENTES CON INFECCIÓN POR VIH.

Se randomizaron 220 pacientes, 110 se asignaron a recibir CPV y PPV a las 4 semanas y 110 a recibir PPV. Figura 1.



No había diferencias en los dos grupos excepto en el TAR, 100 pacientes en el grupo CPV+PPV vs. 91 pacientes en el grupo de PPV tomaban TAR; y en el consumo activo de cocaína, 2 pacientes en el primer grupo vs. 8 pacientes en el segundo grupo consumían cocaína.

Tabla 1.Características basales en los dos grupos

Variables	CPV+PPV: 102	PPV: 100	p
Edad media ± DE	43 ± 10	44 ± 8	0,57
Sexo masculino (%)	73 (72%)	72 (72%)	0,95
Transmisión ADVP (%)	38 (37%)	27 (27%)	0,12
Transmisión heterosexual (%)	32 (31%)	39 (39%)	0,12
Transmisión homosexual (%)	24 (23%)	28 (28%)	0,12
Transmisión desconocida (%)	5 (5%)	6 (6%)	0,12
Estadío C (%)	39 (38%)	35 (35%)	0,63
TAR (%)	100 (98%)	91 (91%)	0,027*
Carga viral indetectable (%)	84 (82%)	80 (80%)	0,67
Profilaxis cotrimoxazol (%)	16 (16%)	15 (15%)	0,89
Tabaco (%)	62 (61%)	56 (56%)	0,49
Cannabis (%)	22 (22%)	1 (13%)	0,11
Cocaína (%)	2 (2%)	8 (8%)	0,048**
Alcohol (%)	14 (14%)	20 (20%)	0,23
Asma (%)	4 (4%)	5 (5%)	0,71
EPOC (%)	8 (8%)	5 (5%)	0,41
Neumonía previa (%)	24 (23%)	21 (21%)	0,67
Coinfección VHB (%)	6 (6%)	6 (6%)	0,97
Coinfección VHC (%)	47 (46%)	33 (33%)	0,057
Diabetes	4 (4%)	2 (2%)	0,42
CD4 inclusión, media ± SD (céls/µL)	368+86	351+84	0,15
CD4 nadir, media ± SD (céls/µL)	158+120	155+122	0,89

*OR: 0,20 (CI95%: 0,43-0,96)
**OR: 4,34 (CI95%: 0,90-21)

El 34% de pacientes que recibieron la CPV se quejaron de efectos secundarios vs. el 20% de los que recibieron la PPV, diferencias no significativas (p: 0,07), todos leves y autolimitados en 1 o 2 días, los más frecuentes fueron dolor en el lugar de inoculación (20% tras la CPV y 12% tras la PPV), fiebre (6% tras la CPV y 3% tras la PPV), astenia y artromialgias (6% tras la CPV y 3% tras la PPV).

Los pacientes que tenían Ac basales protectores (tomando como nivel protector mayor o igual a 1µg/mL) no tenían más efectos secundarios ni a la CPV ni la PPV que aquellos con niveles por debajo del umbral protector.

El porcentaje de pacientes con IgG mayor a 1µg/mL previo a la vacunación variaban entre el 8% en el primer grupo y el 10% en el segundo grupo para el serotipo 9V y entre el 75% y el 72% para el serotipo 6B, sin diferencias entre los dos grupos vacunales. Tabla 2.

Tabla 2. Porcentaje de pacientes con niveles de anticuerpos "protectores" (≥1µg/mL) antes de la vacunación para cada serotipo.

Serotipos	CPV+PPV (n:102)	PPV (n:100)	p
4	13%	16%	0,51
14	43%	52%	0,21
19F	27%	24%	0,57
23F	34%	31%	0,62
6B	75%	72%	0,57
18C	33%	41%	0,26
9V	8%	10%	0,59

La proporción de pacientes con Ac naturales por encima de 1µg/mL era similar en aquellos con o sin neumonía previa y en hombres y mujeres. Aunque había una tendencia a mayor porcentaje de Ac naturales protectores en fumadores las diferencias sólo fueron significativas para el serotipo 4.

Los pacientes con EPOC también tenían una tendencia a mayor proporción de Ac naturales protectores pero las diferencias sólo fueron significativas para los serotipos 18C y 9V.

Las GMC de los anticuerpos específicos eran similares en los dos grupos de vacunación para todos los serotipos tanto basales como a las 4 y 8 semanas de la vacunación. Tabla 3.

Tabla 3. Concentración Media Geométrica (µg/mL) para cada serotipo en los dos grupos de vacunación antes, a las 4 semanas y a las 8 semanas.

	Basal			Semana 4			Semana 8		
Serotipos	CPV+PPV (n:102)	PPV (n:100)	p^1	CPV+PPV (n:102)	PPV (n:100)	p^2	CPV+PPV (n:98)	PPV (n:100)	p^3
4	0,15	0,19	0,24	0,28	0,34	0,39	0,31	0,34	0,70
14	0,73	0,90	0,34	2,86	3,50	0,45	3,13	3,50	0,68
19F	0,39	0,39	0,97	0,59	0,63	0,81	0,64	0,63	0,96
23F	0,49	0,51	0,89	0,92	0,80	0,54	1,02	0,80	0,29
6B	3,30	2,23	0,06	5,37	3,98	0,21	6,12	3,98	0,08
18C	0,56	0,63	0,61	2,25	2,08	0,78	2,62	2,08	0,43
9V	0,18	0,20	0,74	0,43	0,55	0,33	0,45	0,55	0,44

p^1 significación estadística de la comparación de la media de los logaritmos de las concentraciones de IgG al comparar los dos grupos de vacunación antes de la vacunación.

p^2 significación estadística de la comparación de la media de los logaritmos de las concentraciones de IgG al comparar los dos grupos de vacunación a la semana 4 (4 semanas tras la CPV en el grupo 1 y 4 semanas tras la PPV en el grupo 2)

p^3 significación estadística de la comparación de la media de los logaritmos de las concentraciones de IgG al comparar los dos grupos de vacunación a la semana 8 (4 semanas tras la PPV en ambos grupos)

Tomando el aumento de las IgG mayor a dos veces su valor a las 8 semanas como criterio de respuesta vacunal, no se encontraron diferencias entre las dos estrategias vacunales. Tabla 4.

Tomando el aumento de las IgG mayor a dos veces su valor y alcanzar la concentración de ≥1µg/mL a las 8 semanas como criterio de respuesta vacunal, tampoco se encontraron diferencias entre las dos estrategias excepto para el serotipo 23F en el que un 26% de los que recibieron dos vacunas respondieron vs. un 14% de los que recibieron una sola dosis de PPV (p: 0,028). Tabla 5.

Tabla 4. Porcentaje de pacientes respondedores de acuerdo con el primer criterio: aumento de dos veces el valor de las IgG tras la vacunación.

	Semana 4			Semana 8		
	CPV (n:102)	PPV (n:100)	P	CPV+PPV (n:98)	PPV (n:100)	P
Serotipo 4	36%	34%	0,73	43%	34%	0,20
Serotipo 14	59%	61%	0,75	66%	61%	0,44
Serotipo19F	24%	29%	0,38	31%	29%	0,80
Serotipo23F	29%	28%	0,82	39%	28%	0,11
Serotipo6B	23%	31%	0,17	33%	31%	0,80
Serotipo18C	64%	55%	0,21	66%	55%	0,10
Serotipo 9V	44%	54%	0,16	52%	54%	0,78

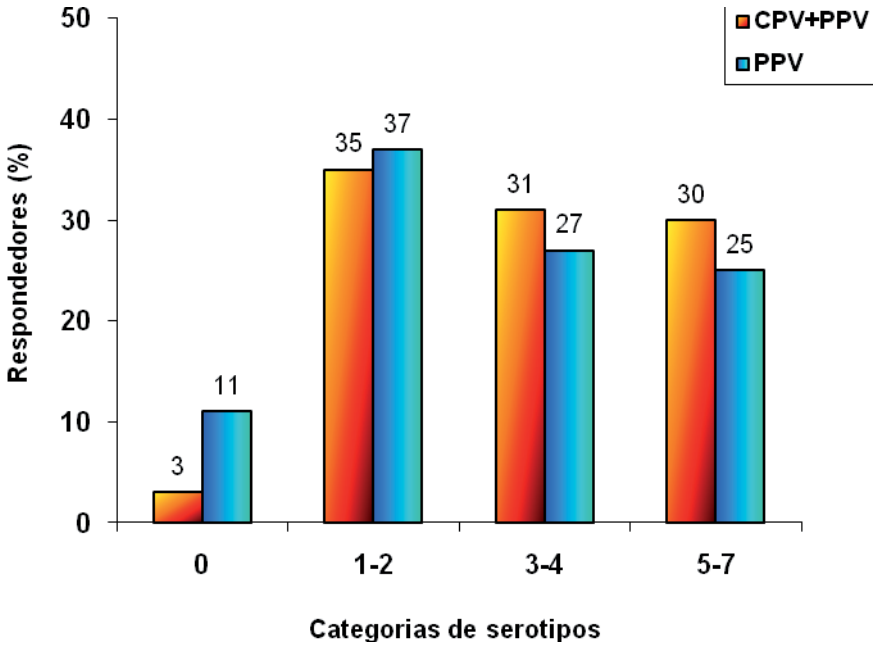
Tabla 5. Porcentaje de pacientes respondedores de acuerdo con el segundo criterio: aumento de dos veces el valor de las IgG y alcanzar la concentración de ≥1µg/mL tras la vacunación.

	Semana 4			Semana 8		
	CPV+PPV (n:102)	PPV (n:100)	P	CPV+PPV (n:98)	PPV (n:100)	P
Serotipo 4	10%	11%	0,78	13%	11%	0,62
Serotipo 14	47%	49%	0,78	50%	49%	0,89
Serotipo19F	12%	17%	0,29	17%	17%	0,95
Serotipo 23F	19%	14%	0,37	26%	14%	0,028*
Serotipo 6B	22%	30%	0,17	32%	30%	0,80
Serotipo18C	50%	47%	0,67	55%	47%	0,25
Serotipo 9V	24%	27%	0,57	27%	27%	0,93

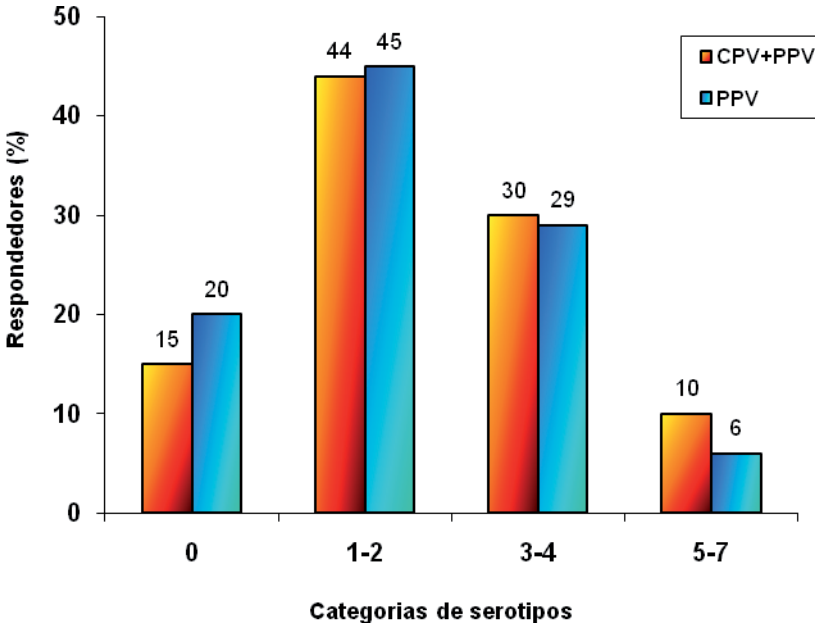
*OR: 2,2 (CI95%: 1,07-4,56)

La proporción de pacientes que respondieron a 0, 1-2, 3-4 o 5-7 serotipos a la semana 8, utilizando cualquiera de los dos criterios de respuesta, fue similar en ambos grupos vacunales.

Figura 2. Porcentaje de respondedores a las cuatro categorías de acuerdo con el primer criterio de respuesta: aumento de los anticuerpos específicos IgG dos veces su valor a la semana 8



Porcentaje de respondedores a las cuatro categorías de acuerdo con el segundo criterio de respuesta: aumento de los Ac específicos IgG dos veces su valor y ≥1µg/mL a la semana 8.



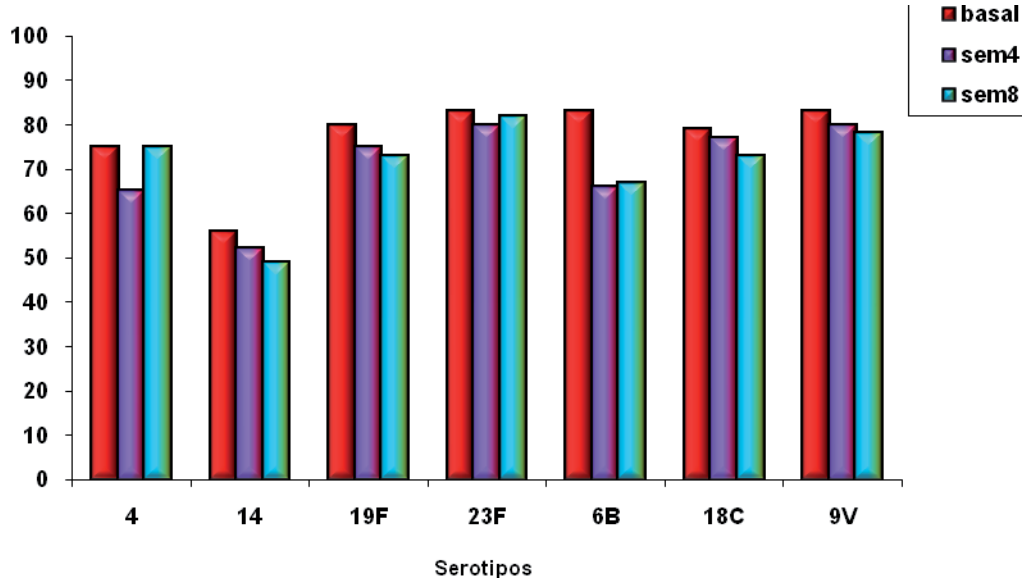
En la regresión logística bivariante y multivariante tomando el aumento de dos veces el valor de las IgG y alcanzar la concentración mínima de IgG≥1µg/mL a un mínimo de cuatro serotipos, tener unos CD4 nadir mayores o iguales a 200 céls/µL y no haber presentado neumonía previa se asociaba con respuesta. Ninguna variable se asociaba a respuesta a un mínimo de 5 serotipos y sólo no haber presentado neumonía previa se asociaba a respuesta a un mínimo de 3 serotipos en ambos análisis (OR: 2,91; CI 95%: 1,31-6,48 p: 0,007).

Tabla 6. Variables asociadas a respuesta a un mínimo de 4 serotipos de acuerdo con el segundo criterio de respuesta en la regresión logística.

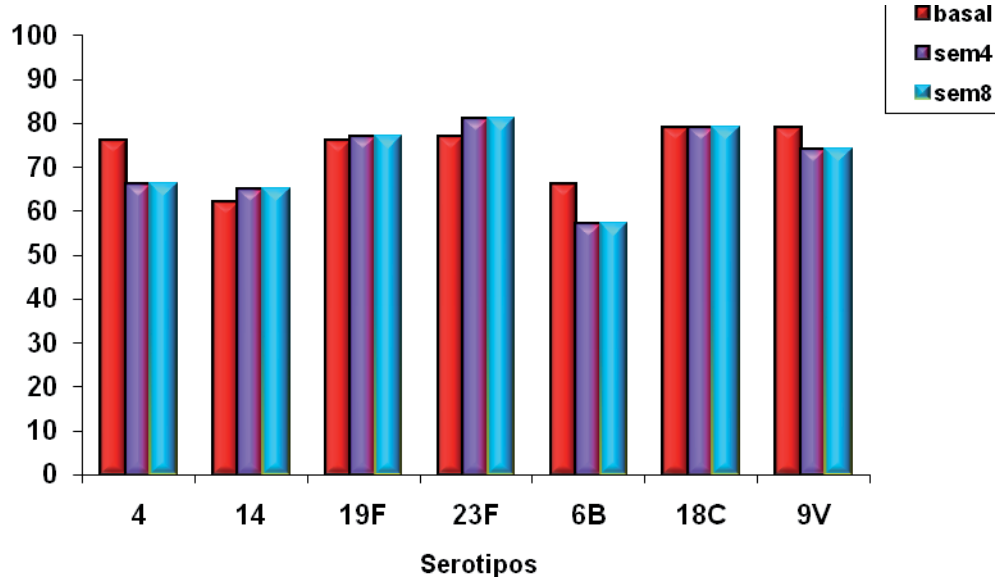
Variables	Respondedores	Bivariada		Multivariada	
		p	odds ratio	p	odds ratio
Edad media ± DE respondedores vs. no respondedores	45 ±10 vs.44 ± 9	0,19	1,03 (0,99-1,07)		
Hombres vs. mujeres	20% vs. 20%	0,74	1,15 (0,49-2,65)		
CPV+PPV vs. PPV	22% vs. 18%	0,30	1,49 (0,69-3,19)		
Transmisión ADVP vs. otras	21% vs. 20%	0,59	1,43 (0,39-5,18)		
Fumadores vs. no fumadores	21% vs. 19%	0,69	1,18 (0,37-1,91)		
Abuso alcohol vs. no alcohol	25% vs. 19%	0,39	1,54 (0,24-1,74)		
TAR vs. no TAR	19% vs. 36%	0,28	0,38 (0,45-15,14)		
Estadío C vs. estadío A o B	15% vs. 23%	0,81	0,89 (0,38-2,14)		
CD4 inclusión ≥350céls/µL vs. <350céls/µL	22% vs. 18%	1,00	1,00 (0,45-2,24)		
CD4 nadir≥200céls/µL vs. <200céls/µL	30% vs. 15%	0,019	2,34 (1,15-4,77)	0,02	2,34 (1,14-4,81)
CVdetectable vs. CVindetectable	21% vs. 20%	0,45	1,58 (0,18-2,12)		
Coinfección VHC vs. no VHC	19% vs. 21%	0,89	0,92 (0,27-3,08)		
EPOC vs. no EPOC	23% vs. 21%	0,72	1.33 (0,28-6.22)		
Neumonía previa vs. no neumonía	9% vs. 23%	0,034	3.49 (1.10-11.08)	0.048	3.05 (1.01-9.18)

Los índices de avidez prevacuna eran muy heterogéneos en cada serotipo (10-100%) y no aumentaron tras las vacunaciones en ninguna de las estrategias.

Media de Índices de Avidez en el primer grupo (CPV+PPV) basalmente y a las 4 y 8 semanas.



Media de Índices de Avidez en el segundo grupo (PPV) basalmente y a las 4 y 8 semanas.



No se encontró correlación entre las concentraciones de IgG y su avidez ni basalmente ni a las 4 u 8 semanas en ninguno de los dos grupos vacunales.Tampoco había diferencias en los índices de avidez dependiendo de si habían tenido neumonía previa, o de si tenían EPOC, cirrosis, coinfección con VHB o VHC, o si eran fumadores, alcohólicos, ADVP, o tomaban TAR, o del estadío CDC o de los CD4 nadir o de los CD4 en el momento de la vacunación.

DISCUSIÓN

3.4.1. EVALUACIÓN DE LA RESPUESTA INMUNE A LAS DOS VACUNACIONES

La vacunación con CPV y PPV producía una respuesta de Ac frente a los serotipos de la CPV similar a la PPV sola en pacientes VIH con inmunodepresión moderada. Aunque escogimos los niveles de respuesta más aceptados en vacunación neumocócica en adultos (duplicación de IgG o duplicación y alcanzar 1µg/mL), esos niveles no se asocian necesariamente con protección individual como observaron O'brien et al. [198] y tampoco con eficacia clínica, aunque ningún paciente desarrolló neumonía o infección neumocócica en las 8 semanas de seguimiento.

La población del estudio era en su mayoría masculina, fumadores, con abuso de alcohol, ex-ADVP, en estadío C y coinfectados por VHC, todos ellos factores de riesgo de infección neumocócica, lo que podría explicar la alta proporción de pacientes con Ac naturales protectores, tomando el nivel más utilizado como protector en infección neumocócica en adultos, IgG≥1µg/mL. Este hecho podría disminuir la posibilidad de observar pequeñas diferencias entre las dos estrategias vacunales.

Más pacientes en el grupo de dos vacunas tomaban TAR, variable asociada a mejor respuesta a la vacunación en algunos estudios [88,199,200], lo que pudo influir en una leve mejor respuesta a la CPV+PPV aunque no encontramos asociación entre TAR y respuesta inmune ni a la CPV ni a la PPV. Se ha descrito que los varones, los fumadores, los pacientes con EPOC, y los que habían tenido neumonía previa tienen mayor proporción de Ac protectores, en nuestro estudio no encontramos dicha relación, aunque los fumadores y los pacientes con EPOC tenían una tendencia a mayor proporción de Ac protectores.

Este estudio sólo reflejaba la respuesta inicial a las dos estrategias vacunales, más interesante será ver la persistencia de los Ac en el tiempo y el intervalo al que descienden a niveles "no protectores", lo que podría indicar el tiempo adecuado para la revacunación. El estudio de Hung et al. con PPV [89], evidenció la pérdida de Ac protectores entre 1 y 3 años según los CD4 en el momento de la vacunación. También sería interesante evaluar las vacunaciones en pacientes con inmunodepresión avanzada (CD4<200céls/µL) que son los de mayor riesgo de infección neumocócica y los que más se beneficiaría de una mejor prevención.

En nuestro trabajo la PPV tras la CPV no produjo mayores incrementos de IgG, por lo que no se demostró el efecto “priming” de la CPV para la PPV visto en los trabajos de Lesprit y Kroon en pacientes VIH [143,144], Chan y Orthopoulos en inmunodeprimidos [201,202], Nachman y Rose en niños [123,128] o Roux en ancianos[118]; tampoco evidenciaron efecto "priming" de la CPV ni Feikin ni Chen en pacientes VIH [59,142], ni Stanford ni Store en asplénicos [135,203].

3.4.2. SEGURIDAD DE LAS VACUNAS FRENTE A NEUMOCOCO.

Ninguna de las vacunas causó efectos secundarios graves. Aunque de manera no significativa, una mayor proporción de pacientes refería efectos secundarios tras la CPV que tras la PPV (34% vs. 20%).

En trabajos previos se había observado que aquellos pacientes que tenían basalmente Ac protectores, es decir, IgG≥1µg/mL, referían efectos adversos con mayor frecuencia [204], en cambio nosotros no pudimos encontrar relación alguna entre presencia de Ac protectores y más reacciones adversas a ninguna de las vacunas y tampoco encontramos relación cuando definimos Ac protectores niveles mayores a 0,5 µg/mL.

El hecho que el ensayo clínico no fuera doble ciego podía haber influido en los posibles efectos adversos que referían los pacientes pero es poco probable que afectara la determinación de los niveles de IgG o de su avidez.

3.4.3. VARIABLES ASOCIADAS A BUENA RESPUESTA A LAS VACUNACIONES.

Trabajos previos evidenciaron, en pacientes con infección por VIH, mejores respuestas a la vacunación antineumocócica en aquellos con TAR [88,200], CV indetectable [88,89,200], CD4 mayores a 200 céls/µL en el momento de la vacunación [87-89,141,200], ausencia de coinfección con VHC [144], mayores niveles de IgG pre vacunales [121,200], o vacunación con CPV frente a PPV [59,144].

En nuestro ensayo clínico no se encontró asociación alguna entre esas variables y respuesta inmune a cualquiera de las vacunas, ni para cada serotipo por separado ni para un mínimo de 3, 4 o 5 serotipos.

Tan sólo al escoger como criterio de respuesta el aumento de IgG específicas dos veces su valor alcanzando el nivel de 1µg/mL a un mínimo de 4 de los 7 serotipos, el no haber presentado neumonía previa y los CD4 nadir mayores a 200 céls/µL se asociaban a mejor respuesta.
Tampoco Falco et al. y Tasker et al. [58,86] encontraron asociación entre respuesta inmune a la PPV y TAR, CD4, CV VIH, sexo o edad.

3.4.4. CAPACIDAD FUNCIONAL DE LOS ANTICUERPOS TRAS LAS VACUNACIONES.

Aunque las avideces eran altas antes de la vacunación, eran muy heterogéneas dentro de cada serotipo (10-100%), como se ha descrito previamente ya que la población de Ac en muestras humanas es muy variable en su unión al Ag. No sólo no aumentaron tras las vacunaciones sino que sorprendentemente, disminuyeron para dos de los siete serotipos en los dos grupos.

La avidez de los Ac madura tras la exposición al Ag y los Ac muestran mayores avideces tras exposiciones repetidas (infecciones o vacunaciones) como concluyeron Usinger, Romero Steiner y Wuorimaa [205-207]. En nuestro estudio la avidez no sólo no aumentó tras la segunda vacuna (PPV) en el grupo de dos vacunas, sino que disminuyó en uno de los serotipos.

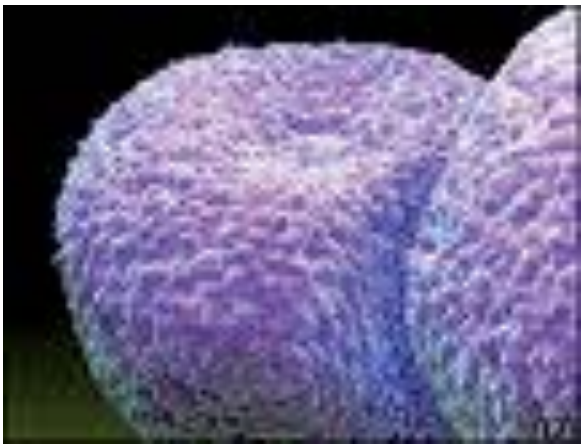
En concordancia con nuestros resultados Spoulou evidenció que las avideces de los Ac aumentaban tras la vacunación con dos dosis de CPV y una de PPV en niños sanos, pero disminuía con las vacunaciones en niños con infección por VIH [140].

Los incrementos de las avideces de los Ac tras la inmunización reflejan un buen efecto "priming" y la generación de memoria inmune. Así las vacunas conjugadas a proteínas podrían inducir Ac con mayor avidez pero en este estudio, aunque el seguimiento era muy corto (8 semanas), no se pudo demostrar generación de memoria inmune.

Trabajos previos estudiaron la correlación entre concentración de Ac y su capacidad funcional medida por actividad opsonofagocítica en diferentes esquemas de vacunación antineumocócica, así en niños sanos Rose [128] y en adultos sanos Antilla, Musher, Romero Steinert, Wuorimaa evidenciaron buena correlación [206,208-210]. Pero estudios realizados en pacientes VIH vieron que la correlación era mala tanto en niños [130,137,199,211] como en adultos [59,84,90,212], aunque mejoraba tras las vacunaciones [90,211].

Menos estudiada está la correlación entre la concentración de Ac y su avidez, en nuestro estudio no evidenciamos correlación alguna ni antes ni a las 4 u 8 semanas tras las vacunaciones, al igual que Spoulou, Antilla y Wuorimmaa [140,207,208], en cambio sí observaron buena correlación Romero Steiner y Usinger [205,206].

CONCLUSIONES



4. CONCLUSIONES

La colonización y la infección por neumococo es un proceso dinámico entre los factores de virulencia del neumococo y los mecanismos de defensa del huésped, cuya interrelación no está completamente aclarada.

El neumococo es capaz de expresar determinados factores de virulencia o de cambiar la apariencia de la cápsula según las características del medio.

La PCR, a diferencia de la MBL, sí se comporta como reactante de fase aguda en pacientes con neumonía neumocócica y se mantiene más elevada en aquellos con comorbilidad asociada.

La MBL y la PCR no sirven como marcadores de gravedad en la neumonía neumocócica. Aunque cuando existe bacteriemia asociada los niveles de PCR son más altos y hay una tendencia a niveles mayores de MBL, no existe correlación entre ambas.

Los pacientes con infección por VIH tienen más riesgo de enfermedad neumocócica que los individuos sanos, mayor cuanto más avanzada es la inmunodepresión, especialmente en aquellos con CD4 menores a 200 céls/µL.

Los factores de riesgo asociados a enfermedad neumocócica en los pacientes con infección por VIH son tener CD4 menores a 200 céls/µL, el alcoholismo y las comorbilidades EPOC y cirrosis, y existe una tendencia a mayor riesgo en aquellos con cargas virales mayores a 5000 copias/mL y los ADVP.

Los factores protectores de enfermedad neumocócica en los pacientes con infección por VIH son el TAR y la vacunación previa frente a neumococo con PPV.

La vacuna polisacárida, aunque menos eficaz que en inmunocompetentes, es beneficiosa en pacientes con infección por VIH, ya que disminuye el riesgo de enfermedad neumocócica y no sólo en aquellos pacientes con CD4 mayores a 200 céls/µL, en los que está recomendada, sino también en aquellos con CD4 menores a 200 céls /µL, en los que su uso es más controvertido.

Aunque se recomienda la revacunación frente a neumococo en pacientes con infección por VIH cada 5 años, y se ha visto que en ellos los Ac descienden a niveles por debajo del umbral protector en 2-3 años, el efecto protector de la vacuna polisacárida mantiene más allá de los 5 años.

Aunque existen "fallos" de la vacuna, es decir pacientes vacunados que adquieren infección por neumococo, ésta confiere una serie de beneficios adicionales ya que los pacientes con enfermedad neumocócica que habían sido vacunados previamente tienen una resolución más rápida de los síntomas, menor estancia hospitalaria, menos complicaciones y menor mortalidad que aquellos no vacunados.

La respuesta inicial de anticuerpos frente a los serotipos de neumococo incluidos en la vacuna conjugada, es similar tras la vacunación con las dos vacunas, conjugada seguida de polisacárida, que con una sola dosis de vacuna polisacárida.

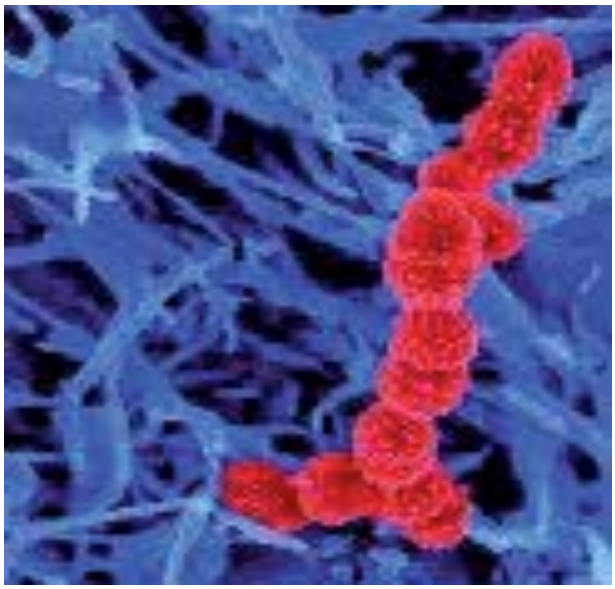
La vacunación frente a neumococo es segura en pacientes con infección por VIH tanto con vacuna polisacárida como con vacuna conjugada, los efectos secundarios son poco frecuentes y leves.

En pacientes con infección por VIH la vacunación previa con CPV no induce una mayor respuesta inmune a la vacunación posterior con PPV.

El TAR, la ausencia de comorbilidades o un recuento más alto de CD4 no influye en una mejor respuesta a la vacunación frente a neumococo.

La capacidad funcional de los Ac medida por su avidez a los polisacáridos no mejora tras la vacunación frente al neumococo, independientemente del tipo de vacunación.

RECAPITULACIÓN



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Mannose-binding lectin does not act as an acute-phase reactant in adults with community-acquired pneumococcal pneumonia

M. Perez-Castellano,* M. Peñaranda,† A. Payeras,‡ J. Milà,* M. Riera,† J. Vidal,† F. Pujalte,* A. Pareja,‡ C. Villalonga† and N. Matamoros*

**Service of Immunology, †Infectious Diseases Unit Hospital Son Dureta, and ‡Internal Medicine Service, Hospital Son Llàtzer, Palma de Mallorca, Spain*

Summary

The objective of this work was to study the role of mannose-binding lectin (MBL) and C-reactive protein (CRP) in pneumococcal pneumonia, to determine whether MBL acts as an acute-phase reactant and whether the severity of the disease correlates with MBL levels. The study comprised 100 patients with pneumococcal pneumonia. The pneumonia severity score was calculated and graded into a risk class of mortality (Fine scale). The MBL genotypes and the levels of MBL and CRP at the acute and recovery phases were determined. Fifty patients with the wild-type MBL genotype showed higher MBL levels in each phase ($P < 0{\cdot}001$) and an increased risk to developing bacteraemia, odds ratio (OR) 2·74, 95% confidence interval (CI) 1·01–7·52) ($P = 0{\cdot}02$), but this did not correlate with the pneumonia severity class. CRP levels in the acute phase, 79·53 mg/l [standard deviation (s.d.) 106·93], were higher in the subjects with positive blood cultures ($P = 0{\cdot}003$), and remained higher [20·12 mg/l (s.d. 31·90)] in the group of patients with an underlying disease ($P = 0{\cdot}01$). No correlation was observed between the levels of MBL and CRP in each phase, or with the pneumonia severity score. We cannot conclude that MBL acts uniformly as an acute-phase reactant in pneumococcal pneumonia. MBL levels do not correlate well with the severity of the pneumonia. The risk of developing bacteraemia could be enhanced in individuals with the wild-type MBL genotype.

Keywords: acute phase proteins, community-acquired pneumonia, C-reactive protein, mannose-binding lectin, *Streptococcus pneumoniae*

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Correspondence: Antoni Payeras, Internal Medicine Service, Hospital Son Llàtzer, Carretera Palma-Manacor Km 4, 07198 Palma de Mallorca. Spain.
E-mail: apayeras@hsll.es

Introduction

The estimated incidence of community-acquired pneumonia (CAP) in Spain is about 162 cases per 100 000 individuals, with 53 000 hospital admissions per year [1]. *Streptococcus pneumoniae* is the most important CAP aetiological agent and elicits a powerful inflammatory and immune response that relies upon a close relationship between innate and adaptive components of the host's immune system, with a release of inflammatory mediators, such as interleukin (IL)-6, which is the main inducer of the synthesis in hepatocytes of an acute-phase protein, the C-reactive protein (CRP) [2]. CRP plays a significant role in the defence against *S. pneumoniae*, binding the C polysaccharide of the cell wall, activating the classical complement pathway and favouring the opsonophagocytosis of these bacteria [3,4].

Interest in CRP has increased during recent years because changes in its concentration have been used as a marker related to the risk of future coronary events or as a measure of the activity of some rheumatological disorders. In the case of CAP, higher plasma levels of CRP have been encountered in patients with bacterial pneumonia, in contrast to viral aetiology, and have been related to the severity of the disease, suggesting that CRP levels may help to evaluate a patient's management and to monitor response to antibiotic therapy [5–8]. However, a recently published systematic review has concluded that testing for CAP is neither sensitive nor specific enough to rule in or rule out a radiological infiltrate or the bacterial aetiology of the respiratory tract infection [9].

Mannose-binding lectin (MBL) is a plasma lectin with a high affinity for N-acetyl glucosamine, a component of peptidoglycan, present on the surface of microbes but not on human cells. MBL interacts with both the innate and

adaptive immune system, and has some homologies with CRP: it is synthesized by hepatocytes, takes part in the opsonophagocytosis of bacteria and initiates the complement cascade (via the lectin pathway). MBL has also been suggested to act as an acute-phase reactant, and that heat shock and proinflammatory cytokines could stimulate its production *in vitro* [10,11]. The role of MBL in the risk of developing bacterial infections has been studied with conflicting results. It seems that MBL gene mutations determine a susceptibility to infection in patients with systemic lupus erythematosis and HIV infection, influence the severity of lung disease in patients with cystic fibrosis and increase the number of episodes of acute respiratory infection in children [12–15]. Moreover, homozygotes for MBL variants could be at increased risk of developing invasive infections by *S. pneumoniae,* as has been demonstrated previously [16], although this relationship was not encountered in another published work [17].

The objective of the present study was to evaluate the role of MBL in pneumococcal pneumonia by comparing the levels of MBL and CRP, to determine whether MBL really acts as an acute-phase reactant in this setting, and to establish whether the severity of the disease correlates with lower MBL levels. The latter issue gains in importance, as MBL therapy could be a possible option to treat deficiency states, as has been demonstrated in cystic fibrosis [18].

Materials and methods

The study was conducted in Son Dureta Hospital, a 900-bed tertiary referral centre, and Son Llàtzer Hospital, a 350-bed community centre (Mallorca, Spain).

Selection of patients and definitions

From 1 June 2003 to 30 June 2005 we studied all consecutive adult patients (> 18 years old) with pneumococcal pneumonia with clinical features and/or X-ray findings. The microbiological criteria used to confirm the pneumococcal aetiology were: (1) at least one blood or pleural fluid culture positive for *S. pneumoniae*, (2) one sputum culture positive for the same bacteria in addition to a positive determination of rapid immunochromatographic urinary antigen test or (3) in those cases in which bronchoscopy procedures were carried out, a quantitative bacterial recount of at least 10^3 colony-forming units (CFU)/ml for telescope catheter culture; 10^4 CFU/ml for bronchoalveolar lavage or 10^5 CFU/ml for bronchoaspirate were considered significant.

Patients were recruited from the emergency departments of either of the two participant hospitals and a blood sample (acute phase) was obtained immediately after the microbiological diagnosis was confirmed. When a patient with a confirmed microbiological pneumococcal pneumonia did not meet the criteria for hospital admission they were requested by telephone to attend the out-patient clinic to provide a blood sample. At least 4 weeks after the resolution of the acute pneumococcal infection, the patients were requested to visit the out-patient clinic to provide another blood sample (recovery phase).

Demographic variables and comorbidities were collected for each patient. In each episode the pneumonia severity score was calculated and graded into a risk class of mortality by using the Fine scale [19], considering three groups: low risk (classes I, II and III), moderate risk (class IV) and high risk (class V).

Patients with nosocomial infections, primary immunodeficiencies (routine immunological tests were carried out to evaluate their immunological status), those who died before the microbiological diagnosis was obtained, or who did not turn up at the out-patient clinic during the acute phase and those who refused to sign the informed consent were excluded. For patients with recurrent pneumonia episodes, only the first episode was considered.

Laboratory tests

Blood samples were collected aseptically into plain and ethylenediamine tetraacetic acid (EDTA) tubes in the first 48 h of hospital admission. For all samples, serum was separated immediately and transferred into cryovials and preserved at −80°C for further testing. EDTA blood samples were used for genomic DNA isolation. DNA isolation was carried out using the proteinase K method.

CAP was determined by nephelometry (BNAII, Dade Behring® Marburg, Germany) using a highly sensitive commercial kit (Dade-Behring®). The cut-off point used to detect abnormal values was > 3 mg/dl, as suggested by the CRP manufacturer.

Mannose-binding lectin (MBL) serum concentrations were determined by enzyme-linked immunosorbent assay (ELISA) performed in microwells coated with a monoclonal antibody against the MBL carbohydrate-binding domain in a commercial kit (oligomerized mannan-binding lectin; AntibodyShop®, Gentofte, Denmark). MBL concentrations in serum were expressed as ng/ml.

For the MBL genotype, genotyping was perfomed by polymerase chain reaction with sequence-specific primers (PCR–SSP). Primers and conditions for amplification of MBL mutations were used according to Steffensen *et al.*'s [20] method, with a personal modification of the temperature conditions for amplification of exon 1 with mix B (20 s at 60°C instead of 58°C). Analysis was accomplished by subjecting samples of genomic DNA from each individual to a maximum of 17 PCR reactions carried out under three different PCR conditions with various MgCl2 concentrations. The genotypes were determined by the presence or absence of a specific band after electrophoresis in a 2% agarose gel stained with ethidium bromide visualized by ultraviolet light and photographically recorded (data not shown).

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Ethics

All the patients were required to sign an informed consent and the study was approved by the Ethics Committee of the Comunitat de les Illes Balears.

Statistical analysis

First, a descriptive analysis of the population of the study was made. In the bivariate analysis, we used χ^2 tests to compare qualitative variables. Comparison of the quantitative variables was carried out using non-parametric tests (Mann–Whitney test). For the correlation analysis we calculated Pearson's coefficient. Statistical significance was taken as a *P*-value less than 0·05. The risk of bacteraemia associated with MBL genotypes was estimated using the calculation of odds ratios (OR) with 95% confidence interval (CI). Statistical power was at least 80%.

The analysis was carried out with SPSS 12·0 and GraphPad Prism4 software.

Results

One hundred patients (68 male, 32 female) with pneumococcal pneumonia (53 with bacteraemia) were included. Fifty-one patients had at least one comorbidity, mainly chronic obstructive pulmonary disease (COPD) (33 cases), and 15 subjects were infected by HIV-1. Forty-three patients were classified into the low-risk mortality class and 57 into the moderate–high-risk classes. Forty-three patients' samples of the recovery phase were processed. Demographic and general characteristics of the study population are summarized in Table 1.

Fifty patients presented the wild-type MBL genotype (AA), 43 were heterozygous (AO) and four homozygous (OO) for the MBL variants. In three cases it was not possible to determine the genotype. The patients with the AA genotype showed higher levels of MBL than the two mutated groups (AO/OO) considered together, in the acute ($P < 0{\cdot}001$) and recovery phases ($P < 0{\cdot}001$). Non-statistical differences were observed between the MBL genotype and the levels of CRP (Table 2).

Table 1. Demographic and general characteristics of the study population.

	n = 100
Male/female	68/32
Age (years; mean/range)	58·27 (21–96)
Comorbidity	51
COPD	33
Diabetes	9
Cardiac failure	9
Hepatopathy	7
Corticosteroids/immunosuppressors	4
Neutropenia	2
Renal insufficiency	2
Solid neoplasm	2
HIV-1 infection	15
HAART	8
CD4 cell count/cell/μl (median/range)	135 (36–500)
Smokers	46
Alcoholism	4
Drug abuse (active)	8
Bacteraemia	53
Mortality risk class (Fine)	
Low I	15
Low II	11
Low III	17
Moderate IV	42
High V	15

COPD: chronic obstructive pulmonary disease; HAART: highly active anti-retroviral therapy.

Table 2. Mannose-binding lectin (MBL) and C-reactive protein (CRP) levels according to the MBL genotype. The number of patients, grouped by risk class, with very low levels of MBL in the acute phase is also shown.

	Genotype AA	Genotype AO/OO	*P*-value
MBL acute (ng/ml)			
Mean (s.d.)	4298·81 (2109·34)	863·83 (675·12)	< 0·001
Median (range)	4225 (1150–10000)	700 (35–2500)	
MBL recovery (ng/ml)			
Mean (s.d.),	3576·19 (1246·25)	695·56 (523·37)	< 0·001
Median (range)	4000 (1200–5750)	650 (35–2100)	
MBL acute < 500 ng/ml			
Low-risk	–	7 cases	–
Moderate–high-risk	–	6 cases	
CRP acute (mg/l)			
Mean (s.d.)	67·69 (105·28)	54·95 (70·37)	n.s.
Median (range)	34 (1–578)	22 (1–265)	
CRP recovery (mg/l)			
Mean (s.d.)	10·12 (14·76)	12·50 (26·92)	n.s.
Median (range)	4·50 (1–67)	3 (0–125)	

Table 3. Mannose-binding lectin (MBL) genotypes in relation to bacteraemia and risk class of mortality (Fine scale).

	Genotype AA	Genotype AO/OO	Total
Blood cultures*			
(a) Positive	32	18	50
(b) Negative	13	20	33
Risk class*			
(a) Low	18	22	40
(b) Moderate–high	32	15	47

*In 14 episodes blood cultures were not performed and in three cases MBL genotypes were not performed.

The relation between MBL genotypes and the presence or absence of bacteraemia is shown in Table 3. Patients with the AA genotype showed a higher risk of developing bacteraemia, OR 2·74 (95% CI 1·01–7·52, $P = 0·02$). No statistical differences were found between risk class of mortality (Fine scale) and MBL genotypes (Table 3). Thirteen patients with the AO/OO genotype presented MBL levels lower than 500 ng/ml (seven in the low- and six in the moderate–high-risk groups) in the acute phase, but no differences were detected when compared with those having the MBL acute level above that value.

Although the mean and median of the MBL levels, both in acute and in recovery phases, were higher for the moderate–high-risk group, these differences did not reach statistical significance. The mean values of CRP in acute phase were similar for both groups, and only the CRP level in the recovery phase was statistically higher ($P = 0·005$) in the moderate–high-risk group (Table 4). When we compared the MBL levels with the severity group for each of the genotypes (AA *versus* AO/OO) in the acute and recovery phases, these differences remained non-significant (Table 4). Figure 1 represents the individual MBL and CRP concentrations of the 43 patients with two paired samples (acute and recovery phases). Fifty-four patients were lost to follow-up, 28 were AA and 26 were AA/OO, without differences in comparison with patients whose paired samples were obtained.

At each phase, the mean levels of MBL of the patients with bacteraemia were higher than those with negative blood cultures [acute: 3038·49 (s.d. 2218·70) ng/ml *versus* 2532·17 (s.d. 2590·70) ng/ml; recovery: 2751·94 (s.d. 1781·36) ng/ml *versus* 1923·12 (s.d. 1751·47) ng/ml] but

Table 4. Mannose-binding lectin (MBL) and C-reactive protein (CRP) concentrations in the acute and recovery phases in accordance with the mortality risk group (Fine scale) for the whole study population. MBL concentration in each phase and risk group for the different MBL genotypes.

	Low-risk*	Moderate–high-risk**	*P*-value
The whole study population			
MBL acute (ng/ml)			
Mean (s.d.)	2380·69 (2174·29)	2990·00 (2451·03)	n.s.
Median (range)	1450 (35–7250)	2600 (90–10000)	
MBL recovery (ng/ml)			
Mean (s.d.)	1988·57 (1536·02)	2391·20 (1871·05)	n.s.
Median (range)	1850 (100–5250)	2400 (35–5750)	
CPR acute (mg/l)			
Mean (s.d.)	68·29 (113·11)	57·04 (70·94)	n.s.
Median (range)	22 (1–578)	31 (1–294)	
CRP recovery (mg/l)			
Mean (s.d.)	4·24 (6·36)	15·77 (26·19)	0·005
Median (range)	2 (0–25)	6 (1–125)	
Restricted to AA genotype			
MBL acute (ng/ml)			
Mean (s.d.)	4525·00 (1745·95)	4185·71 (2291·04)	n.s.
Median (range)	4500 (1400–7250)	3750 (1150–10000)	
MBL recovery (ng/ml)			
Mean (s.d.)	3107·14 (1316·06)	3810·71(1188·43)	n.s.
Median (range)	2400 (1700–5250)	4000 (1200–5750)	
Restricted to AO/OO genotypes			
MBL acute (ng/ml)			
Mean (s.d.)	835·47 (704·97)	897·50 (659·16)	n.s.
Median (range)	675 (35–2500)	750 (90–2200)	
MBL recovery (ng/ml)			
Mean (s.d.)	870·00 (678·60)	584·55 (393·06)	n.s.
Median (range)	720 (100–2100)	580 (35–1300)	

*Includes: I, II and III Fine categories. **Includes: IV and V Fine categories.

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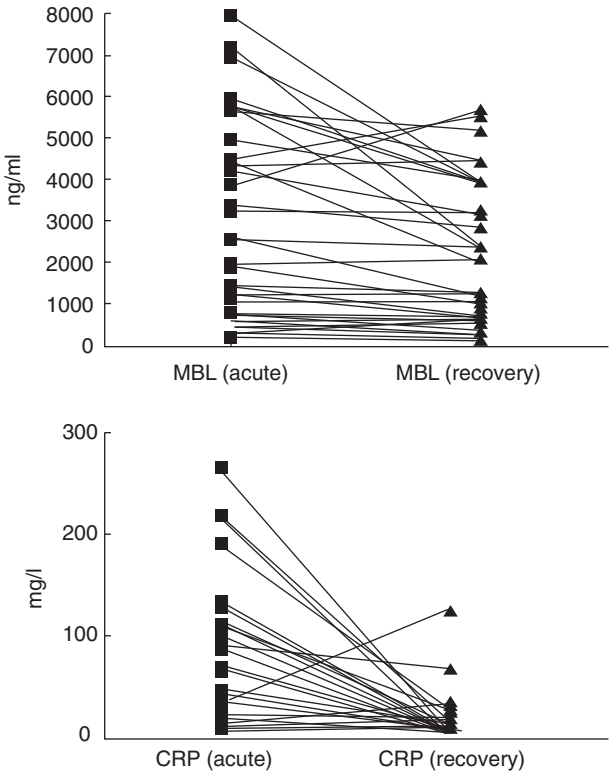

Fig. 1. The mannose-binding lectin and C-reactive protein concentrations of each of the 43 patients with two paired samples in the acute and recovery phases.

without statistical significance. In the acute phase, the levels of CRP were significantly higher in the subjects with positive blood cultures [79·53 (s.d. 106·93) mg/l *versus* 36·68 (s.d. 63·77) mg/l ($P = 0{\cdot}003$)], but in the recovery phase these differences disappeared [6·03 (s.d. 7·58) mg/l *versus* 12·78 (s.d. 17·02) mg/l].

The levels of CRP in the recovery phase remained higher in the group of patients with an underlying disease in comparison with the previously healthy ones [20·12 mg/l (s.d. 31·90) *versus* 5·77 mg/l (s.d. 7·42) ($P = 0{\cdot}01$)].

There was no correlation between the levels of CRP and MBL either in the acute phase ($P = 0{\cdot}87$) or in the recovery phase ($P = 0{\cdot}53$). Finally, no correlation was observed between the pneumonia severity score at hospital admission and the acute values of CRP ($P = 0{\cdot}78$) or MBL ($P = 0{\cdot}21$).

One patient died, in relation to the pneumococcal pneumonia. She was an elderly woman with chronic hepatopathy and cardiac failure, classified at admission as high risk. She presented the AO genotype, and the values of CRP and MBL in acute phase were 138 mg/l and MBL 215 ng/ml, respectively. Four additional patients died in the 3 months following the acute episode, two with exacerbated COPD, one with a non-Hodgkin lymphoma and one with diffuse pleural carcinomatosis. When we compared the levels of CRP and MBL in the acute phase of the deceased cases with the remaining 95 surviving subjects no statistical differences were detected.

The previously exposed statistical analysis was repeated excluding the 15 patients with HIV-1 infection, also excluding the 51 patients with comorbities, and the results obtained were comparable with those of the whole population (data not shown).

Discussion

The properties of MBL as an acute-phase reactant have been studied in different scenarios since the first description [10], that MBL synthesis is induced as a part of the acute response. Neth *et al.* observed a higher MBL concentration in children with malignancy than in healthy individuals [21]. Two other studies have analysed the behaviour of MBL in patients undergoing major surgery, with discordant results. In the first study, in sequential blood samples of 11 patients after major hip surgery, increases in MBL concentration between 1·5 and threefold were observed [22]. In the same study, a rise in MBL levels was observed in five patients after a malaria attack. In another study including patients who underwent gastrointestinal resections for malignant disease, the MBL levels did not rise immediately after surgery, but lower MBL levels were associated with the occurrence of postoperative infections [23]. However, although both studies were conducted in the postoperative setting the population analysed was different, and in the second study the observation period was shorter, with only two blood samples tested (1 and 3 days after the surgery).

A study of critically ill patients admitted to an intensive care unit (ICU) concluded that, even though the MBL levels on day 5 of admission and on the last day of the ICU stay were higher in the non-survivors, these levels were not related to the outcome in the multivariate analysis when adjusted for all risk factors upon admission [24]. The authors observed that the MBL concentration increased during the ICU stay; however, no correlation was observed with CRP levels at any point. In another study including patients with severe infection and proven sepsis (bloodstream infection or community-acquired pneumonia), MBL concentrations behaved as an acute-phase reactant, defined as a 25% increase or decrease from baseline levels, in 31·3% and 27·3% of the cases, respectively, with 41·4% of the patients exhibiting steady-state levels throughout the study period. The authors concluded that the MBL concentrations demonstrated a variable acute-phase response [25].

The present study has failed to establish a correlation between MBL levels and the severity of pneumococcal pneumonia, evaluated with a well-validated scale. Moreover, no differences between MBL levels during the acute episode and in the recovery phase were detected, and no correlation was observed with the CRP levels at any of the time-points. So, we agree with the conclusions reached in the previous study [25], that MBL does not act uniformly in all patients as an acute-phase reactant. Furthermore, in the group of patients

with underlying disease the CRP concentration in the recovery phase remained significantly higher, probably indicating a persistent inflammatory state. The same was not observed for MBL levels, supporting the absence of parallelism between both proteins.

As expected [26–28], MBL concentrations were lower for patients with the mutated MBL genotype. In our study it was not possible to establish a relationship with the severity of the pneumonia, nor for the subgroup of cases with very low levels of MBL, although the number of patients in this situation was limited. Although levels below 500 ng/ml have been observed more frequently among non-survivors, these differences disappeared when corrected for the risk factors at admission [24].

Interestingly, the patients with the wild-type MBL genotype had a greater risk of developing bacteraemia. When the total study population was considered, in patients with positive blood cultures the MBL levels tended to be higher, although without reaching statistical significance. To explain this finding, which seems to render bloodstreams more susceptible to invasion, it can be speculated that, as has been reported [29], the N-acetylglucosamine of the cell wall of Gram-positive bacteria is a biologically relevant ligand for MBL and that MBL inhibits peptidoglycan-induced production of proinflammatory cytokines, suggesting that MBL may down-regulate macrophage-mediated inflammation and, furthermore, that MBL enhances phagocyte recruitment, thus hypothetically favouring the bloodstream entrance of the bacteria by proper cell-surface anchoring.

CRP levels in the acute phase did not correlate with the severity of the pneumonia; the data were not concordant with those reported previously [8]. Some factors can justify these differences: our study was restricted only to episodes of well-documented pneumococcal pneumonia attended at hospital emergencies, and the CRP level was determined not immediately after admission, but only when a microbiological confirmation was obtained; because the plasma half-life of CRP is about 19 h [30], the stimulus for its increased production could have ceased after the antibiotic treatment was initiated, thus not reflecting the real values at admission. However, patients with pneumococcal bacteraemia presented higher CRP values in the acute phase than those with negative blood cultures, a circumstance that has also been emphasized by other authors [5].

There are some possible limitations to our study. As it was conducted in the ambit of hospital emergencies, less severe episodes of pneumonia attended normally in the primary care setting could not be included, although 43 of the episodes were grouped into the low-risk classes, with an estimated risk of mortality under 1%. We used restrictive criteria to warrant the aetiology of the pneumonia, excluding other possible pneumococcal episodes such as those without a confirmed microbiological diagnosis, with an isolated positive urinary antigen or an isolated sputum smear. Determination of MBL and CRP levels was made when the microbiological confirmation was obtained. This delay, as commented above, could have influenced the drop in the CRP concentrations, but probably did not affect the MBL concentrations because the half-life of circulating MBL, as observed from infusion studies in MBL-deficient humans, has been estimated to be as long as 5–7 days [31].

Although 42 and 15 patients in our series were grouped into moderate- and high-risk classes (estimated risk of mortality 9·3% and 27%, respectively) only one patient died in relation to the acute episode, so the analysis was extended to cover mortality within the following 3 months. It is possible that some patients with pneumococcal pneumonia could not be included because they died before a microbiological diagnosis was obtained.

In summary, with the data presented here we cannot conclude that the MBL acts as an acute phase reactant in pneumococcal pneumonia. Although the study was not designed to decide the need for hospitalization of patients with community-acquired pneumococcal pneumonia, it seems that MBL cannot be used for this purpose, as the levels of this lectin do not correlate with the severity of the pneumonia. The role of MBL could be different in pneumonias of another aetiology or in other clinical situations (i.e. immunosuppressed patients), as the antimicrobial mechanism of MBL will depend on the nature of the infecting organism and the immunological state of the host.

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MAJOR ARTICLE

Effectiveness of Polysaccharide Pneumococcal Vaccine in HIV-Infected Patients: A Case-Control Study

Maria Peñaranda,[1] Vicenç Falco,[4] Antoni Payeras,[2] Queralt Jordano,[3] Adria Curran,[4] Antoni Pareja,[2] Gloria Samperiz,[1] David Dalmau,[3] Esteve Ribera,[4] and Melcior Riera[1]

[1]Hospital Son Dureta, [2]Hospital Son Llatzer, and [3]Mutua de Terrasa, Palma de Mallorca, and [4]Hospital Vall d'Hebron, Barcelona, Spain

Background. Polysaccharide pneumococcal vaccine (PPV) is recommended among human immunodeficiency virus (HIV)–infected patients, although its effect in reducing the incidence of pneumonia or invasive pneumococcal disease is not well established. Our objective was to determine the effectiveness of 23-valent PPV in HIV-infected adults and the risk factors for pneumococcal pneumonia or invasive pneumococcal disease.

Methods. We performed a retrospective case-control study in 4 Spanish hospitals for the period from January 1995 through December 2005 using the HIV database from each hospital to identify case patients with *Streptococcus pneumoniae* disease and control subjects without a history of pneumococcal infection.

Results. A total of 184 case patients and 552 control subjects were identified. The factors associated with pneumococcal disease in bivariate analysis were active injection drug use (odds ratio [OR], 3.33; 95% confidence interval [CI], 2–5.55), alcoholism (OR, 3.03; 95% CI, 1.86–4.91), chronic obstructive pulmonary disease (OR, 2.58; 95% CI, 1.3–5.1), cirrhosis (OR, 6.05; 95% CI, 3.2–11.4), antiretroviral therapy (OR, 0.23; 95% CI, 0.16–0.32), trimethoprim-sulfamethoxazole prophylaxis (OR, 0.66; 95% CI, 0.45–0.97), viral load <5000 copies/mL (OR, 0.38; 95% CI, 0.26–0.54), and previous PPV (OR, 0.39; 95% CI, 0.24–0.65). Risk factors for pneumococcal disease in multivariate analysis were cirrhosis (OR, 5.64; 95% CI, 2.53–12.53), chronic obstructive pulmonary disease (OR, 2.90; 95% CI, 1.21–6.94), and alcoholism (OR, 2.15; 95% CI, 1.11–4.19), whereas protective factors were receipt of antiretroviral therapy (OR, 0.23; 95% CI, 0.14–0.36) and receipt of pneumococcal vaccine (OR, 0.44; 95% CI, 0.22–0.88), even in patients with CD4 lymphocyte counts <200 cells/μL.

Conclusions. Antiretroviral therapy and PPV have a significant, independent protective effect against pneumococcal disease, regardless of CD4 lymphocyte count; thus, all patients with HIV infection should be vaccinated with PPV to prevent pneumococcal disease.

In the HAART era, cases of bacterial pneumonia still occur in HIV-infected patients [1, 2]. As it is in the general population, *Streptococcus pneumoniae* is the most common cause of bacterial pneumonia among HIV-infected adults, who have rates of bacteremia that are higher than those observed in non–HIV-infected subjects [3–6].

Although some studies have reported a decrease in the incidence of invasive pneumococcal disease among HIV-infected patients after the widespread introduction of HAART [3, 7–10], other studies did not find this tendency [11, 12]. Nevertheless, even in the former studies, the incidence of invasive pneumococcal disease among HIV-infected adults is still higher than the incidence among similarly aged non–HIV-infected adults [5, 6, 10]. Several factors, such as injection drug use, chronic liver disease, alcohol abuse, cigarette smoking, and poor adherence to antiretroviral therapy, have been associated with the risk of pneumococcal disease [7,11]. For these reasons, 23-valent polysaccharide pneumococcal vaccine (PPV) is currently recommended for HIV-infected patients, particularly those with CD4 lymphocyte counts >200 cells/μL [8, 13, 14]. However, the evidence supporting this recommendation is controversial, and the case-control studies [7–9, 13, 15, 16] and the only published randomized study [17] to have investigated the effect of this vaccine in preventing invasive pneumococcal disease produced conflicting results.

The aim of our study was to determine the effec-

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Reprints or correspondence: Dr. Maria Peñaranda Vera, Infectious Diseases Unit, Hospital Son Dureta, Andrea Doria, n. 55, 07084, Palma de Mallorca, Spain (mpenaranda@hsd.es).

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tiveness of the 23-valent PPV in preventing pneumococcal pneumonia or invasive pneumococcal disease among HIV-infected adults. Secondary objectives were to investigate the risk factors for pneumococcal disease among HIV-infected adults.

PATIENTS AND METHODS

Study population. A retrospective case-control study including HIV-infected adults with previous pneumococcal disease was performed during the period from 1 January 1995 through 31 December 2005 in 4 Spanish hospitals: Hospital Son Dureta (a 900-bed tertiary care teaching hospital that treats 2000 HIV-infected patients; Palma de Mallorca, Spain), Hospital Vall d' Hebron (a 1200-bed tertiary care teaching hospital that treats 1700 HIV-infected patients; Barcelona, Spain), Fundació Son Llatzer (a 350-bed secondary care teaching hospital that treats 500 HIV-infected patients; Palma de Mallorca, Spain), and Mutua de Terrasa (a 580-bed secondary care teaching hospital that treats 400 HIV-infected patients; Palma de Mallorca, Spain).

Case patients and control subjects. Case patients were identified from the databases of HIV-infected patients for each hospital. Case patients were defined as HIV-infected adults (age, $\geqslant$18 years) with a previous diagnosis of pneumococcal pneumonia or invasive pneumococcal disease from 1995 through 2005. Case patients for whom the CD4 lymphocyte count was unknown at the time of diagnosis or within 3 months before the diagnosis were excluded.

Three patient groups were defined: (1) the definite pneumococcal pneumonia group, which included patients with clinical symptoms and radiological signs of pneumonia and *S. pneumoniae* isolation from blood, pleural fluid, or sputum cultures with positive urinary pneumococcal antigen test results or $\geqslant 10^3$ cfu/mL in bronchoalveolar lavage samples obtained from fiberoptic bronchoscopy; (2) the presumptive pneumococcal pneumonia group, which included patients with clinical symptoms and radiological signs of pneumonia and *S. pneumoniae* isolation from sputum culture or a positive urinary antigen test result; and (3) the other pneumococcal infections group, which included patients with *S. pneumoniae* isolation from normally sterile sites.

Three control subjects without pneumococcal infection or bacterial pneumonia of unknown etiology were selected for each case patient and matched for the following variables: sex, age (age of the case patient at the time of pneumococcal disease diagnosis $\pm$ 5 years), CD4 lymphocyte count (CD4 lymphocyte count of the case patient at the time of pneumococcal disease diagnosis $\pm$ 50 cells/μL; if the CD4 lymphocyte count of the case patient was >500 cells/μL, control subjects were selected from among HIV-infected adults with CD4 lymphocyte counts >500 cells/μL), and , HIV infection risk factor (divided into injection drug use and other, including male-male sex, heterosexual sex, receipt of a blood transfusion, and unknown transmission mechanism).

To identify the matched control subjects for each case patient, we divided the HIV databases from each hospital into different groups according to sex, CD4 lymphocyte count interval, and transmission mechanism. We then selected 3 control subjects for each case patient. For each stratum, we selected control subjects who followed the case patient in alphabetical order. Information about vaccination status was not visible at the moment of control subject selection.

Study variables. Once case patients and control subjects were identified, we reviewed clinical records and collected the following variables: age, sex, 23-valent PPV administration (Pneumo23; Sanofi Pasteur), date of vaccination (before the pneumococcal disease diagnosis for case patients and at any time during the study period for control subjects), risk factor for HIV infection, active injection drug use, current smoking (cigarettes), and active alcohol abuse (>80 g of alcohol ingested per day), CD4 lymphocyte count, HIV load, AIDS-defining illnesses, receipt of trimethoprim-sulfamethoxazole (TMP-SMZ) prophylaxis, and macrolide prophylaxis, antiretroviral therapy (ART), hepatitis B virus and hepatitis C virus coinfection, presence of underlying diseases (such as chronic obstructive pulmonary disease [COPD], sickle cell anaemia, or cirrhosis), date of pneumococcal infection diagnosis for case patients, type of infection (definite pneumococcal pneumonia, presumptive pneumococcal pneumonia, or other pneumococcal infection), and results of blood or sputum cultures and urinary antigen tests.

Statistical analysis. Variables were analyzed with the statistical software package SSPS, version 11.0 (SPSS). First, we performed an analysis of the characteristics of the entire study population and separately performed another description in which the study population was analyzed in 2 groups: case patients and control subjects. We then conducted a bivariate analysis using χ^2 test with a significance level of .05 and a multivariate analysis using a forward-conditional binary logistic regression method that incorporated the variables associated with pneumococcal infection in the bivariate analysis.

RESULTS

From 1 January 1995 through 31 December 2005, 736 subjects were included in the study (184 case patients and 552 control subjects). The 23-valent PPV was administered to 151 (20%) of the 736 study subjects (20 [11%] of 184 case patients and 131 [24%] of 552 control subjects).

The characteristics of case patients and control subjects are summarized in table 1. Among the case patients, there were 117 definite pneumococcal pneumonia (64%), 44 presumptive pneumococcal pneumonia (24%) and 23 other pneumococcal infections (12%). In 127 (69%) of the case patients, there was

Table 1. Baseline characteristics of case patients and control subjects.

Variable	Case patients (n = 184)	Control subjects (n = 552)	P
Age, median years	38	38	1
Male sex	134 (73)	402 (73)	1
Smoking	137 (75)	235 (72)	.43
Alcohol abuse	36 (19.6)	41 (7.4)	<.001
Active injection drug use	37 (26.4)	41 (9.8)	.03
CD4 lymphocyte count, median cells/μL	204	210	.60
CD4 lymphocyte count ⩾200 cells/μL	95 (51.6)	291 (52.7)	.79
HIV load, median log copies/mL	4.5	2.8	.04
HIV load, <5000 copies/mL	60 (37.7)	309 (61.4)	<.001
AIDS-defining illness	70 (38)	190 (34)	.37
TMP-SMZ use	43 (23.4)	174 (31.5)	.04
Macrolide use	0	4 (0.7)	.24
ART use	79 (42.9)	422 (76.6)	<.001
HBV infection	15 (8.2)	33 (6)	1
HCV infection	130 (70.7)	390 (70.7)	1
COPD	16 (8.7)	19 (3.6)	.005
Cirrhosis	29 (15.8)	16 (3)	<.001
Receipt of 23-valent PPV	20 (10.9)	131 (23.8)	<.001

NOTE. Data are no. (%) of patients, unless otherwise indicated. ART, antiretroviral therapy; COPD, chronic obstructive pulmonary disease; HBV, hepatitis B virus; HCV, hepatitis C virus; PPV, polysaccharide pneumococcal vaccine; TMP-SMZ, trimethoprim-sulfamethoxazole.

an associated bloodstream infection. The percentage of patients with positive microbiological test results is shown in table 2.

Factors associated with pneumococcal infection in bivariate analysis were active injection drug use (OR, 3.33; 95% CI, 2–5.55), active alcohol abuse (OR, 3.03; 95% CI, 1.86–4.91), COPD (OR, 2.58; 95% CI, 1.3–5.1), cirrhosis (OR, 6.05; 95% CI, 3.2–11.4), receipt of antiretroviral therapy (OR, 0.23; 95% CI, 0.16–0.32), receipt of TMP-SMZ prophylaxis (OR, 0.66; 95% CI, 0.45–0.97), viral load <5000 copies/mL (OR, 0.38; 95% CI, 0.26–0.54), and previous receipt of 23-valent PPV (OR, 0.39; 95% CI, 0.24–0.65). There was no relation between pneumococcal infection and sex, cigarette smoking, Centers for Disease Control and Prevention HIV infection stage, CD4 lymphocyte count, and hepatitis B virus or hepatitis C virus coinfection. Results are shown in table 3.

Factors associated with an increased risk of pneumococcal disease in the multivariate analysis were cirrhosis (OR, 5.64; 95% CI, 2.53–12.53), COPD (OR, 2.90; 95% CI, 1.21–6.94), and active alcohol abuse (OR, 2.15; 95% CI, 1.11–4.19), whereas protective factors were current receipt of HAART (OR, 0.23; 95% CI, 0.14–0.36) and having received 23-valent PPV (OR, 0.44; 95% CI, 0.22–0.88) (table 3).

When the study population was stratified by CD4 lymphocyte count (⩾200 cells/μL vs. <200 cells/μL; median CD4 lymphocyte count, 325 cells/μL vs. 89 cells/μL), the 23-valent PPV was protective in both groups and achieved a stronger protective effect for pneumococcal infection in the group with a CD4 lymphocyte count <200 cells/μL (OR, 0.15; 95% CI, 0.46–0.50), compared with those with a CD4 lymphocyte count ⩾200 cells/μL (OR, 0.55; 95% CI, 0.31–0.99). The protective effect of vaccination with PPV was observed in all vaccinated patients independently of the time of vaccination, not only in those vaccinated ⩽5 years earlier (OR, 0.36; 95% CI, 0.17–0.77) but also in those vaccinated >5 years earlier (OR, 0.55; 95% CI, 0.34–0.98).

DISCUSSION

Although some studies have found a decrease in the incidence of pneumococcal disease in HIV-infected patients since the widespread use of HAART [3, 5, 9, 18], it remains one of the

Table 2. Microbiological data for HIV-infected patients with pneumococcal disease.

Test	Result
Sputum culture	42/94 (44.7)
Urinary antigen test	36/46 (78)
Blood culture	127/162 (78.4)
Pleural fluid culture	1/1
BAL culture	10/12
CSF culture	5/5
Ascitic fluid culture	4/4

NOTE. Data are expressed as no. of patients with positive result/no. of patients tested (%). BAL, bronchoalveolar lavage.

Table 3. Risk factors related to pneumococcal disease in HIV-infected adults.

	Bivariate analysis		Multivariate analysis	
Risk factor	OR (95% CI)	*P*	OR (95% CI)	*P*
Male sex	1 (0.68–1.45)	1		
Smoking	0.84 (0.56–1.28)	.43		
Alcohol use	3.03 (1.86–4.91)	<.001	2.15 (1.11–4.19)	.02
Active injection drug use	3.33 (2–5.55)	.03		.46
CD4 lymphocyte count >200 cells/μL	1.04 (0.75–1–46)	.79		
HIV load <5000 copies/mL	0.38 (0.26–0.54)	<.001		.24
CDC HIV infection stage	0.85 (0.60–1.20)	.37		
TMP-SMZ use	0.66 (0.45–0.97)	.04		.80
Receipt of ART	0.23 (0.16–0.32)	<.001	0.23 (0.14–0.36)	<.001
HBV infection	0.71 (0.38–1.35)	.30		
HCV infection	1 (0.69–1.44)	1		
COPD	2.58 (1.3–5.1)	<.001	2.90 (1.21–6.94)	.02
Cirrhosis	6.05 (3.2–11.4)	<.001	5.64 (2.53–12.53)	<.001
Receipt of 23-valent PPV	0.39 (0.24–0.65)	<.001	0.44 (0.22–0.88)	.02

NOTE. ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; COPD, chronic obstructive pulmonary disease; HBV, hepatitis B virus; HCV, hepatitis C virus; PPV, polysaccharide pneumococcal vaccine; TMP-SMZ, trimethoprim-sulfamethoxazole.

most common causes of hospital admission in these patients, and the incidence is higher among this group than among similarly aged individuals without HIV infection [1, 4–6, 10–12, 19]. This is especially true in patients with advanced HIV infection [3, 10].

It is worth noting that, despite actual recommendations regarding PPV vaccination, our study found that the rate of vaccination was low (~80% of our patients had not been vaccinated). This finding is in concordance with data documented by other authors, such as Dworkin et al. [7], who reported a vaccination rate in an American population of 37%, and Grau et al. [9], who reported an observed vaccination rate in a Spanish population of 7%–25%. In 1999, a pneumococcal vaccination program was begun in Spain, and within 18 months, vaccination coverage among the elderly population reached 35%, which is higher than that for HIV-infected patients [20].

The reasons for these low vaccination rates are probably related to the lack of evidence of the efficacy of the 23-valent PPV in HIV-infected patients, compared with the general population, including not only a lack of clinical efficacy but also a lower immunological response to vaccination in HIV-infected patients, compared with healthy control subjects, as has been demonstrated by other authors [21–25]. Another reason could be the belief that pneumococcal infection is not an important problem in HIV-infected patients in the developed world because of the widespread use of HAART and that it could be more cost-effective to concentrate efforts on strategies to improve adherence to antiretroviral therapy [18].

The most important conclusion in our study is that 23-valent PPV shows a significant, independent protective effect for pneumococcal disease in all HIV-infected patients, even in patients with CD4 lymphocyte counts <200 cells/μL. These results are in concordance with those observed in other cohorts [9, 25, 27] and case-control studies [7, 8, 13, 16]. However, in previous studies, this protection was limited to specific groups of patients, such as patients who were white [16] or those with CD4 lymphocyte counts $\geqslant$200 cells/μL or $\geqslant$500 cells/μL [7, 8, 13]. In our study, the protective effect of the 23-valent PPV in patients with CD4 lymphocyte counts <200 cells/μL, although showing a tendency to be stronger, is not statistically different from that observed in those with CD4 lymphocyte counts $\geqslant$200 cells/μL. This is, to our knowledge, the first report to establish a stronger protective effect of PPV vaccination in patients with CD4 lymphocyte counts <200 cells/μL.

The main argument against the use of PPV comes from the only published randomized, double-blind, placebo-controlled trial, which involved HIV-infected patients from Uganda. This study demonstrated not only a lack of efficacy of the vaccine in preventing invasive pneumococcal disease, pneumococcal pneumonia, or death but also an increased risk of all-cause pneumonia in the group of vaccinated patients [17]. However, the results of this study would not be applicable in a developed country with full access to antiretroviral therapy; in our population, for example, 49% and 76% of case patients and control subjects, respectively, were receiving antiretroviral therapy.

The main objective of PPV vaccination should be to avoid pneumococcal infection. Recent data from hospitalized adults with pneumonia suggest that there are other objectives that could be achieved with PPV. In an observational study, Fisman et al. [26] found that prior pneumococcal vaccination was associated with a 40%–70% reduction in risk of in-hospital death in a large cohort of consecutive hospitalized individuals with

community-acquired pneumonia. The explanation for this phenomenon is that early death due to pneumococcal infection despite receipt of adequate antibiotic therapy may be caused by the release of cell wall components from killed pneumococci, which results in a cytokine-mediated inflammatory cascade that causes death [28]. It is possible that prior vaccination may contribute to preventing the development of such early inflammatory response and, consequently, may reduce early mortality and complications of pneumococcal infection. Although these data should be confirmed in future studies involving HIV-infected patients, they support the recommendation for pneumococcal vaccination.

Antiretroviral therapy demonstrated the strongest protective effect on pneumococcal infection in the multivariate analysis in our population. This result is in concordance with the findings of the majority of case-control [8, 11] and cohort studies [1, 3, 7].

Most other risk factors have been associated with pneumococcal disease in HIV-infected patients in previous reports, such as black race [7, 11, 19], smoking [1, 12], alcohol abuse [7, 9], injection drug use [7, 11], CD4 lymphocyte count <200 cells/μL [8, 11, 12], previous pneumonia or previous hospitalization [7–9], TMP-SMZ prophylaxis [1], or underlying conditions, such as lymphoma [12, 19], cirrhosis [9, 11, 12], COPD [12], or low albumin level [11]. In our study, only alcohol abuse and, in particular, comorbidities such as cirrhosis (OR, 5.64) and COPD (OR, 2.9) were important risk factors for pneumococcal disease.

Neither injection drug use nor TMP-SMZ prophylaxis reached a statistically significant level in the multivariate analysis of factors associated with pneumococcal disease in HIV-infected patients, although these risk factors were more frequent in case patients than in HIV-infected control subjects. Only Kohli et al. [1] found that TMP-SMZ prophylaxis was associated with a lower risk of bacterial pneumonia in a prospective cohort of women; this finding was in contrast with the findings of Jordano et al. [12], who not only did not find any protective effect against invasive pneumococcal disease, but also found that it was associated with increased rates of infection with TMP-SMZ–resistant and penicillin-resistant pneumococci.

The main limitation of our study is related to its retrospective design. Although we are aware of this limitation, we believe that our results are in concordance with those obtained in other geographical areas. As a consequence, some epidemiological data, such as data pertaining to smoking or alcohol abuse, which were collected retrospectively, may not be complete in clinical records. This could be the reason why smoking cigarettes was not a risk factor for pneumococcal disease, as was COPD, which is intimately associated with smoking cigarettes. Another limitation of our study is the fact that serotypes were not determined for pneumococcal isolates, and we could not conclude whether case patients were infected with pneumococcal serotypes included in the 23-valent PPV.

A future randomized trial must answer some of the questions that remain as to the real effectiveness of the 23-valent PPV, the recommendation for revaccination every 5 years [24, 29], and the role that conjugate heptavalent pneumococcal vaccine will play. This vaccine is recommended for infants in the United States and in the majority of European countries, and its use has been associated with a reduction in incidence of invasive pneumococcal disease, not only in infants [30–32], but even in the elderly population [33] and among HIV-infected individuals [34]. Moreover, other strategies of sequential immunization with both the polysaccharide and the conjugate [35] vaccines should be explored. In the meantime, in our opinion, all HIV-infected patients must be vaccinated with the 23-valent PPV, even those with CD4 lymphocyte counts <200 cells/μL.

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ORIGINAL RESEARCH

Impact of prior pneumococcal vaccination on clinical outcomes in HIV-infected adult patients hospitalized with invasive pneumococcal disease

A Imaz,[1] V Falcó,[1] M Peñaranda,[2] Q Jordano,[3] X Martínez,[4] C Nadal,[2] A Curran,[1] AM Planes,[5] D Dalmau,[3] E Ribera,[1] M Riera,[2] E Ruiz de Gopegui[6] and A Pahissa[1]

[1]Infectious Disease Department, Hospital Universitari Vall d'Hebron, Autonomous University of Barcelona, Barcelona, Spain, [2]Infectious Disease Unit, Hospital Universitari Son Dureta, Palma de Mallorca, [3]Internal Medicine Department, Hospital Mutua de Terrasa, Barcelona, Spain, [4]Preventive Medicine Department, [5]Microbiology Department, Hospital Universitari Vall d'Hebron, Autonomous University of Barcelona, Barcelona, Spain and [6]Microbiology Department, Hospital Universitari Son Dureta, Palma de Mallorca, Spain

Background

Recent studies in hospitalized patients with community-acquired pneumonia have found a lower risk of bacteraemia and better clinical outcomes in patients who had previously received the 23-valent pneumococcal polysaccharide vaccine (PPV) in comparison with unvaccinated individuals. The aim of this study was to assess the influence of prior PPV on clinical outcomes in HIV-infected adult patients hospitalized with invasive pneumococcal disease (IPD).

Methods

This was an observational study of all consecutive HIV-infected adults hospitalized with IPD from January 1996 to October 2007 in three hospitals in Spain. Baseline characteristics and clinical outcome-related variables were compared according to prior PPV vaccination status.

Results

A total of 162 episodes of IPD were studied. In 23 of these (14.2%), patients had previously received PPV. In both vaccinated and unvaccinated patients, most of the causal serotypes were included in the 23-valent PPV (76.9% and 84.1%, respectively). Overall, 25 patients (15.4%) died during hospitalization, 21 patients (13%) required admission to an intensive care unit (ICU) and 34 patients (21%) reached the composite outcome of death and/or admission to the ICU. None of the 23 patients who had previously received PPV died or required ICU admission, in comparison with 25 (18%; $P = 0.026$) and 21 (15.1%; $P = 0.046$), respectively, of the unvaccinated patients. The length of hospital stay for vaccinated patients was significantly shorter (8.48 *vs.* 13.27 days; $P = 0.011$).

Conclusions

Although 23-valent PPV failed to prevent IPD in some HIV-infected patients, vaccination produced beneficial effects on clinical outcomes by decreasing illness severity and mortality related to IPD.

Keywords: HIV infection, invasive pneumococcal disease, pneumococcal pneumonia, pneumococcal vaccine, 23-valent polysaccharide vaccine

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Introduction

Since the widespread introduction of highly active antiretroviral therapy (HAART), decreasing rates of invasive pneumococcal disease (IPD) in HIV-infected patients [1–7] have been reported. Despite these data, the incidence of IPD in persons with HIV infection remains significantly higher than in similarly aged non-HIV-infected adults [1,2,5]. Advanced immunodeficiency and the association with other comorbidities appear to be the main risk factors for IPD [6–11], but a high incidence of IPD has also been reported, even in HIV-infected patients with CD4 counts >200 cells/μL [2].

Correspondence: Dr Arkaitz Imaz, Infectious Diseases Department, Hospital Universitari Vall d'Hebron, Autonomous University of Barcelona, P° Vall d'Hebron 119-129, 08035 Barcelona, Spain. Tel: + 34 93 274 60 90; fax: + 34 93 489 40 91; e-mail: aimaz@vhebron.net

Studies assessing the effectiveness of the 23-valent pneumococcal polysaccharide vaccine (PPV) in preventing pneumococcal pneumonia and IPD in HIV-infected adults have yielded controversial results. Several cohort and case–control studies have found that the vaccine produces a beneficial effect by decreasing the incidence of IPD, particularly in patients receiving HAART and with higher CD4 counts but also in patients with CD4 counts below 200 cells/μL [4,6,7,12–14]. In contrast, other studies, including the only published randomized double-blind placebo-controlled study, have not found a clear benefit of the 23-valent PPV in preventing IPD in HIV-infected patients [10,15–18]. In any case, because the impact of pneumococcal infections on morbidity and mortality remains high, vaccination with 23-valent PPV is currently recommended in HIV-infected patients, particularly in those with CD4 counts >200 cells/μL [19,20].

Recent studies in hospitalized patients with community-acquired pneumonia (CAP) have found a lower risk of bacteraemia and better clinical outcomes, including a faster resolution of pneumonia symptoms, a shorter length of hospital stay and a lower mortality rate, in patients who had previously received the 23-valent PPV compared with unvaccinated individuals [21–23]. Furthermore, among patients with documented pneumococcal pneumonia and patients with major risk factors for pneumococcal pneumonia (i.e. older patients and nursing home residents) the association of prior vaccination with 23-valent PPV and better clinical outcomes appeared to be more significant [21]. To our knowledge there are no published data about these additional effects of 23-valent PPV in HIV-infected patients. The aim of the present study was to assess the influence of prior 23-valent PPV on clinical outcomes in HIV-infected adults hospitalized with IPD.

Patients and methods

Study population and setting

We performed an observational study of all consecutive HIV-infected adults (age ≥ 18 years) hospitalized with IPD from January 1996 to October 2007 in three hospitals in Spain: University Hospital Vall d'Hebron (a 1200-bed tertiary care teaching hospital that treats 1900 HIV-infected patients; Barcelona), Hospital Son Dureta (a 900-bed tertiary care teaching hospital that treats 2000 HIV-infected patients; Palma de Mallorca) and Hospital Mutua de Terrasa (a 580-bed secondary care teaching hospital that treats 400 HIV-infected patients; Barcelona). During the study period, 179 episodes of IPD were diagnosed in 165 HIV-infected hospitalized adults. Seventeen episodes were excluded because data on the patients' vaccination status were not available. Thus, 162 cases were finally included in the study. Fifteen patients had more than one episode of IPD: one patient had four episodes, three patients had three episodes and 11 patients had two episodes. Among this group of 15 patients with repeated episodes of IPD (overall 35 episodes), in six episodes the patient had received PPV prior to the pneumococcal infection episode and in 29 episodes the patient had not been previously vaccinated. Three patients received 23-valent PPV after the first episode of IPD. Because the different vaccination status of the same patient in different episodes of IPD could influence the outcomes, we decided to choose the overall number of episodes and not patients for the analysis. The study was approved by the Commission of Medical Ethics of the Vall d'Hebron Hospital where all data were centralized.

Study variables and data collection

The patients' records from 1996 to 1999 were reviewed retrospectively, and from 2000 onwards all data were collected prospectively. The following variables were recorded: (1) demographic and epidemiological data (age, gender, active or prior injecting drug use, current tobacco smoking and current alcohol abuse); (2) prior vaccination with 23-valent PPV; (3) clinical conditions associated with higher risk for pneumococcal disease (cirrhosis or chronic liver disease, chronic pulmonary disease, solid neoplasm, haematological malignancy and splenectomy); (4) HIV infection-related data (HIV infection risk factors, CD4 lymphocyte count, HIV-1 viral load, current use of HAART, current or prior AIDS-defining illnesses and trimethoprim-sulfamethoxazole prophylaxis); (5) clinical syndrome (pneumonia, meningitis, peritonitis and primary bacteraemia); (6) pneumonia severity assessed with the Pneumonia Severity Index (PSI) at the moment of admission to the Emergency Department; (7) microbiological data (serotype and antibiotic resistance pattern of the *Streptococcus pneumoniae* causal strain); and (8) variables related to clinical outcome [in-hospital mortality, intensive care unit (ICU) admission, orotracheal intubation requirement, time to defervescence and length of hospital stay].

Pneumococcal vaccination status

Baseline characteristics and variables related to clinical outcome were compared according to prior PPV vaccination status. In our setting, primary care physicians do not administer PPV to HIV-infected patients and the vaccines are all administered in the hospital in which these patients are managed. In our hospitals, all HIV-infected patients who receive the 23-valent PPV are recorded in a database by the Infectious Disease Department and/or the Preventive

Medicine Department. Informed consent is obtained before the inclusion of the data in the database. Both databases were checked to ascertain the vaccination history of each patient. We considered that a patient was vaccinated if he or she had ever received the 23-valent PPV prior to the IPD episode.

Definitions

IPD was defined as isolation of *S. pneumoniae* from a normally sterile site (blood, cerebrospinal fluid, pleural fluid or peritoneal fluid). Invasive pneumococcal pneumonia was diagnosed when a patient had consistent clinical findings plus a new pulmonary infiltrate on chest radiography and isolation of *S. pneumoniae* in blood and/or pleural fluid cultures. HAART was defined as the use of an antiretroviral agent combination based on current guidelines for HIV infection management. Chronic liver disease was defined on the basis of the presence of typical clinical, laboratory and/or ultrasonography signs and/or the presence of histological findings in liver biopsy. Chronic obstructive pulmonary disease (COPD) was defined on the basis of clinical and/or functional test-based criteria. Pneumococcal serotypes related to those serotypes contained in the 23-valent PPV were not considered as vaccinal serotypes, because cross-immunity between related serotypes has not been demonstrated [24]. 'Days to defervescence' was the number of days from admission to the disappearance of fever. In-hospital mortality was defined as deaths that occurred during the hospital stay for IPD.

Microbiological procedures

S. pneumoniae strains were identified by Gram staining, optoquin susceptibility, bile solubility testing and latex agglutination testing. The antibiotic susceptibility was assessed, according to current Clinical and Laboratory Standards Institute (CLSI) recommendations, using Mueller–Hinton agar supplemented with 5% horse blood (Kirby Bauer diffusion method) and Rosco disks (neo-Sensifab; Rosco Diagnostica, Tastrup, Denmark). Minimum inhibitory concentrations (MICs) of penicillin G, cefotaxime and cotrimoxazole were determined by Etest (bioMérieux SA, Marcy l'Etoile, France) Isolates were classified as penicillin-susceptible (MIC ≤ 0.06 mg/L), penicillin-intermediate (MIC 0.12–1 mg/L), or penicillin-resistant (MIC ≥ 2 mg/L). Intermediate or resistant isolates were considered to be nonsusceptible. All microbiological tests except for serotyping were performed with the same methods at each hospital. Isolates were serotyped at the Spanish Pneumococcal Reference Laboratory (Instituto de Salud Carlos III, Madrid, Spain) with standard antiserum. The serotype identification was only performed on patients admitted to two of the three hospitals, so only data from 101 cases were available.

Outcome-related variables

The main measured outcomes were in-hospital mortality and ICU admission. In previous studies a composite of the two variables was used to assess the severity of CAP, so we decided to choose the composite variable 'mortality and/or ICU admission' as our primary outcome [22,25]. Secondary outcomes were: in-hospital mortality alone, ICU admission, orotracheal intubation requirement, severity of pneumonia at presentation (PSI class IV or V), development of septic shock or empyema, length of hospital stay and time to defervescence.

Statistical analysis

All variables were compared according to the prior vaccination status of the patients in each episode of IPD analysed (patients who had been vaccinated with the 23-valent PPV and unvaccinated patients). Statistical analyses were performed using the statistical software package SPSS version 12.0 (SPSS, Chicago, IL, USA). Categorical variables were compared using the χ^2 test or Fisher's exact test and continuous variables using Student's *t*-test. Differences were considered significant at $P < 0.05$.

Results

A total of 162 episodes of IPD were studied. The mean age of patients was 38.5 years, and 126 (77.8%) episodes occurred in men and 36 (22.2%) in women. In 23 episodes (14.2%), the patient had previously received the 23-valent PPV, and in seven episodes the vaccine was administered more than 5 years before the episode of IPD.

Baseline characteristics of the patients stratified by 23-valent PPV vaccination status are shown in Table 1. There were some differences between vaccinated and unvaccinated patients at baseline. There was only one patient (4.3%) with chronic liver disease among the vaccinated patients, in comparison with 27 (19.4%) of 139 unvaccinated patients, although the difference was not significant. Prior vaccine recipients had significantly higher CD4 counts at the time of pneumococcal infection (325 cells/μL in vaccinated patients *vs.* 209 cells/μL in unvaccinated patients; $P = 0.014$) and were more likely to be on HAART (59.1% *vs.* 34.5%; $P = 0.034$).

The most common clinical presentation of IPD was bacteraemic pneumonia in both groups of patients and, interestingly, none of the patients who had received the 23-valent PPV had meningitis (Table 2). We could determine

Table 1 Baseline characteristics of patients according to pneumococcal vaccination status

	Vaccinated patients ($n = 23$)	Unvaccinated patients ($n = 139$)	*P*
Age (years) [mean (SD)]	38.5 (9.1)	38.5 (8.6)	0.993
Sex [*n* (%)]			
Male	20/23 (87)	106/139 (76.3)	0.416
Female	3/23 (13)	33/139 (23.7)	
Tobacco use [*n* (%)]	17/23 (73.9)	90/138 (65.2)	0.482
Alcohol abuse [*n* (%)]	4/23 (17.4)	31/138 (22.5)	0.786
Chronic liver disease [*n* (%)]	1/23 (4.3)	27/139 (19.4)	0.132
COPD [*n* (%)]	2/23 (8.7)	9/139 (6.5)	0.657
Haematological malignancy and/or splenectomy [*n* (%)]	4/23 (17.4)	4/139 (2.9)	0.015
Solid neoplasm [*n* (%)]	0/23	2/139 (1.4)	1
Prior hospitalization* [*n* (%)]	4/23 (17.4)	40/138 (29)	0.318
Current or previous injecting drug use [*n* (%)]	13/23 (56.5)	103/139 (74.1)	0.132
CD4 lymphocyte count [mean (SD)]	325 (244.2)	209 (192.1)	0.014
CD4 ≥ 200 cells/μL [*n* (%)]	15/21 (71.4)	61/138 (44.2)	0.033
HIV-1 viral load <50 copies/mL [*n* (%)]	5/20 (25)	13/113 (11.5)	0.148
HAART use [*n* (%)]	13/22 (59.1)	48/139 (34.5)	0.034
Prophylactic TMP-SMZ [*n* (%)]	5/22 (22.7)	40/138 (29)	0.619
Previous AIDS-defining illness [*n* (%)]	9/23 (39.1)	58/138 (42)	0.824

*Hospitalization in the previous 3 months.
COPD, chronic obstructive pulmonary disease; HAART, highly active antiretroviral therapy; SD, standard deviation; TMP-SMZ, trimethoprim-sulfamethoxazole.

Table 2 Clinical and microbiological characteristics according to pneumococcal vaccination status

	All patients ($n = 162$; 100%)	Vaccinated patients ($n = 23$; 14.4%)	Unvaccinated patients ($n = 139$; 85.6%)	*P*
Bacteraemic pneumonia	134/162 (82.7%)	21/23 (91.3%)	113/139 (81.3%)	0.376
Primary bacteraemia	8/162 (4.9%)	2/23 (8.7%)	6/139 (4.3%)	0.317
Meningitis	10/162 (6.2%)	0/23	10/139 (7.2%)	0.360
Peritonitis	10/162 (6.2%)	0/23	10/139 (7.2%)	0.360
23-valent PPV included serotype*	84/101 (83.2%)	10/13 (76.9%)	74/88 (84.1%)	0.455
Penicillin nonsusceptible strain*	60/157 (38.2%)	5/23 (21.7%)	58/139 (41.7%)	0.104
Cefotaxime nonsusceptible strain*	22/128 (17.2%)	1/20 (5%)	21/108 (19.4%)	0.194
Cotrimoxazole nonsusceptible strain*	68/153 (44.4%)	5/20 (25%)	63/133 (47.4%)	0.090

Values shown are number/total tested (%).
*Proportion among patients for whom data were available.
PPV, pneumococcal polysaccharide vaccine.

pneumococcal serotype in 101 cases. In 84 of them (83.2%) the serotype of the pneumococcal strain was included in the 23-valent PPV without significant differences between vaccinated and unvaccinated patients (76.9% *vs.* 84.1%).

In Table 3 we show the outcome variables related to pneumococcal infection according to prior pneumococcal vaccination. Overall, in 21 episodes (13%) patients required ICU admission, 25 patients (15.4%) died during their hospital stay, and in 34 episodes (21%) patients reached the composite outcome of death and/or admission to the ICU. In none of the 23 episodes that occurred in patients who had previously received the 23-valent PPV was the composite outcome of death and/or admission to the ICU reached, in comparison with 34 episodes (24.5%) that occurred in unvaccinated patients ($P = 0.004$). None of the vaccinated patients died or required ICU admission, in contrast with 25 ($P = 0.026$) and 21 ($P = 0.046$) of the unvaccinated patients, respectively. Moreover, the mean length of hospital stay was significantly shorter in vaccinated patients (8.48 *vs.* 13.27 days; $P = 0.011$). When we examined the subgroup of patients with bacteraemic pneumonia, we found that those who had received the 23-valent PPV were less likely to have severe pneumonia at clinical presentation (PSI class IV or V) than unvaccinated patients (16.7 and 37.4%, respectively) and none of the vaccinated patients developed empyema compared with 9.3% of the unvaccinated patients, although these differences were not significant.

Only one of the 24 patients with chronic liver disease had received 23-valent PPV. As chronic liver disease has been described as a risk factor for pneumococcal infection and this condition could influence the results, favouring a worse prognosis in unvaccinated patients, we attempted to avoid a possible selection bias by performing a new analysis excluding all patients with chronic liver disease (Table 3). In this subgroup of patients the difference between vaccinated and unvaccinated patients remained significant when the composite outcome of death or admission to the ICU was measured. None of the previously vaccinated patients reached this composite outcome, in contrast with 22.3% of the unvaccinated patients ($P = 0.013$). The proportion of unvaccinated patients who died during their hospital stay (14.3%) or required ICU admission (17%) remained higher than that for vaccinated patients, where there were no deaths or ICU admissions, although differences in mortality rates were not statistically significant. The length of hospital stay was also significantly shorter for previously vaccinated patients than for unvaccinated patients (8.5 *vs.* 14.29 days; $P = 0.007$).

Discussion

Recently, some authors have suggested that prior pneumococcal vaccination is associated with improved outcomes in vaccinated patients who develop CAP [22–24].

Table 3 Outcome variables related to invasive pneumococcal disease according to prior vaccination status; on the right side of the table, patients with chronic liver disease have been excluded

		All patients included			Patients with chronic liver disease excluded*		
(*n* = 162)	All patients (*n* = 162)	Vaccinated (*n* = 23)	Unvaccinated (*n* = 139)	*P*	Vaccinated (*n* = 22)	Unvaccinated (*n* = 112)	*P*
Death and/or ICU admission	34/162 (21)	0/23	34/139 (24.5)	0.004	0/22	25/112 (22.3)	0.013
In-hospital mortality	25/162 (15.4)	0/23	25/139 (18)	0.026	0/22	16/112 (14.3)	0.073
ICU admission	21/162 (13)	0/23	21/139 (15.1)	0.046	0/22	19/112 (17)	0.042
Orotracheal intubation	15/162 (9.3)	0/23	15/139 (10.8)	0.132	0/22	13/112 (11.6)	0.126
Shock	17/135 (12.6)	1/21 (4.8)	16/114 (14)	0.471	1/20 (5)	14/96 (14.6)	0.463
Empyema	10/129 (7.8)	0/21	10/108 (9.3)	0.365	0/20	10/95 (10.5)	0.206
PSI high risk classes†	43/125 (34.4)	3/18 (16.7)	40/107 (37.4)	0.111	2/17 (11.8)	29/95 (30.5)	0.146
Days of hospital stay [mean (SD)]	12.62 (13.87)	8.48 (6.14)	13.27 (14.62)	0.011	8.5 (6.29)	14.29 (15.88)	0.007
Days to defervescence [mean (SD)]	3.11 (4.09)	2.55 (2.72)	3.21 (4.28)	0.484	2.62 (2.76)	3.17 (3.52)	0.497

Values shown are number/total tested (%), unless otherwise stated.
*28 patients with chronic liver disease were excluded.
†Pneumonia Severity Index (PSI) class IV or V. PSI was available for 125 patients with bacteraemic pneumococcal pneumonia.
ICU, intensive care unit; SD, standard deviation.

Our findings in HIV-infected patients are consistent with those previously published in non-HIV-infected patients. The composite outcome of death or admission to the ICU was significantly lower in previously vaccinated patients compared with unvaccinated patients. In fact, none of the vaccinated patients died or required admission to the ICU during their hospital stay for IPD. These data suggest that, despite the failure to prevent pneumococcal infection, prior PPV administration has a favourable impact in HIV-infected patients who develop IPD by improving clinical outcomes. The length of hospital stay was also significantly shorter in vaccinated patients. Regarding the presence of shock and also the severity in terms of clinical presentation and the development of empyema, among patients with bacteraemic pneumonia, the differences did not reach statistical significance but appeared to be clinically relevant.

In previous studies in non-HIV-infected patients, similar results were obtained. In a cohort of 554 adults hospitalized with community-acquired pneumococcal pneumonia (CAP), Mykietiuk *et al.* [23] reported a lower risk of bacteraemia and also better clinical outcomes, including a faster resolution of pneumonia symptoms, a lower rate of mortality and a shorter length of hospital stay, in prior PPV recipients compared with unvaccinated patients. Fisman *et al.* [22] studied a large cohort of 62,918 adults hospitalized with CAP and found that the prior receipt of pneumococcal vaccine was associated with decreased length of hospital stay and lower rates of severe complications and death during hospitalization. More recently, Johnstone *et al.* [21] analysed prospectively a cohort of 3415 adults hospitalized with CAP and found that prior 23-valent PPV vaccination was associated with a reduction in the rate of death or ICU admission in hospitalized adults with CAP. In this last study, a prespecified sensitivity analysis restricted to patients who presented with bacteraemic pneumococcal pneumonia was conducted. Among 95 patients with bacteraemic pneumococcal pneumonia, none of the 10 previously vaccinated patients died or was admitted to the ICU, in comparison with 27 (32%) of 85 patients who had not received pneumococcal vaccine ($P = 0.06$).

To our knowledge, these additional effects of the 23-valent PPV in patients who develop IPD have not previously been studied in HIV-infected patients. Our study focused on a cohort of HIV-infected patients with IPD in whom this effect of PPV might be expected to be particularly beneficial because of the high risk of complications related to IPD in this population.

In HIV-infected patients, as in the general population, the most common pneumococcal serotypes involved in IPD are those included in the 23-valent PPV [3,26]. In this study, it is worth noting that, in both the vaccinated and the unvaccinated groups, the most common causal serotypes were those included in the 23-valent PPV. This finding demonstrates that the vaccine failed to prevent pneumococcal infection in some prior vaccinated patients. However, the better clinical outcomes found in patients who had received the 23-valent PPV suggest that pneumococcal vaccination in HIV-infected patients could have significant clinical benefits, despite not conferring protection against IPD. It has been shown in animal models that a vaccine-generated immune response may facilitate opsonization, activate complement and promote bacterial phagocytosis. Therefore, it has been suggested that, although the antibody response following vaccination

may not be sufficient to prevent pneumococcal infection and pneumonia, the partial immune response generated could attenuate the early inflammatory response and prevent early mortality and complications of pneumococcal infection [27,28]. In seven patients the 23-valent PPV was administered more than 5 years before the episode of IPD. None of these seven patients died or was admitted to the ICU, suggesting that the 23-valent PPV could retain this additional effect for a longer time.

Clinical guidelines recommend that re-immunization should be considered 5 years after the first dose of 23-valent PPV, specifically if the initial vaccination was given when the CD4 count was <200 cells/μL [20], although there are no available data supporting the clinical efficacy of revaccination. Moreover, some studies in patients with CD4 counts above 200 cells/μL and patients receiving HAART showed that revaccination did not improve the immune response over that achieved after the initial vaccination [29,30].

As a nonrandomized study, this observational study carries the limitations related to possible selection bias and confounding factors. The patients' records from 1996 to 1999 were reviewed retrospectively, and from 2000 onwards all data were collected prospectively. All 47 cases (29%) included in the period from 1996 to 1999 were studied retrospectively but clinical charts were carefully reviewed and the protocol was exactly the same throughout the study period, so we feel that the results have not been influenced by this fact. One problem for the analysis was the presence of recurrent episodes in some patients, which could influence the results. Three of these patients were vaccinated after the first episode of IPD, so the same patient could be counted as unvaccinated in one episode and as vaccinated in another. Because the different vaccination status of the same patient in different episodes of IPD could influence the outcomes, we decided to analyse each episode of IPD separately. In any case, in order to assess a possible selection bias related to the inclusion of patients with recurrent episodes, we carried out a new analysis after excluding all patients with repeated episodes of IPD (127 episodes in 127 patients) and the results did not change significantly (data not shown).

We found that patients who received PPV had higher CD4 cell counts and were more likely to be receiving HAART. The effectiveness of PPV in severely immunosuppressed patients has been broadly questioned and pneumococcal vaccination in HIV-infected patients is recommended in current guidelines, particularly in those with CD4 counts above 200 cells/μL. Although we only recorded the CD4 cell count at IPD presentation, it is possible that patients with severe immunosuppression were less likely to have received PPV. Nevertheless, we did not find significant differences in the proportion of patients with undetectable viral load. A decreased incidence of IPD associated with the use of HAART and immunological improvement has been reported [1–7]. In contrast, some studies showed that clinical presentation, severity of illness and mortality related to IPD were not associated with CD4 cell count or the use of HAART [6]. Thus, we believe that the beneficial effect of prior pneumococcal vaccination may not be attributable to a different severity of pneumococcal infection in patients with higher CD4 lymphocyte counts or those using HAART.

Among patients who had received PPV, there was only one patient with chronic liver disease. Chronic liver disease has been described as a risk factor for both IPD and a low response to PPV [31], so it could influence the clinical outcomes of IPD and the differences found could be overestimated. Because none of the previously vaccinated patients died or required ICU admission, we could not perform a multivariate analysis, so we attempted to avoid this possible selection bias by performing a new analysis excluding all patients with chronic liver disease. The differences between vaccinated and unvaccinated patients according to the composite outcome of death or admission to the ICU, admission to the ICU and length of hospital stay remained significant. Regarding the other clinical outcomes measured, the differences did not reach statistical significance, which could be explained by the reduction in the size of the sample. However, these differences appear to be clinically relevant.

Another difference between vaccinated and unvaccinated patients is that those who received the pneumococcal vaccine were less likely to be current or previous injecting drug users. Injecting drug use has been associated with a higher risk of IPD, and these findings, although they did not reach statistical significance, suggest that efforts should be made to increase vaccination rates in HIV-infected patients with other underlying high-risk conditions such as injecting drug use or chronic liver disease.

In summary, this study suggests that, as has been shown in non-HIV-infected patients, prior vaccination with PPV may provide some beneficial effects in HIV-infected patients by improving clinical outcomes in those who eventually develop IPD. Although 23-valent PPV fails to prevent IPD in some HIV-infected patients, previously vaccinated patients may have less severe illness, a lower risk of ICU admission, reduced in-hospital mortality and a shorter length of stay in hospital. We believe that these results support the current guidelines that recommend pneumococcal vaccination of HIV-infected adults, and emphasize the importance of this recommendation in order to decrease the morbidity and mortality related to pneumococcal infections in the HIV-infected population.

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Conjugate and polysaccharide pneumococcal vaccines do not improve initial response of the polysaccharide vaccine in HIV-infected adults

Maria Peñaranda[a], Antoni Payeras[b], Ana Cambra[c], Joan Mila[c], Melcior Riera[a], the Majorcan Pneumococcal Study Group

This is a randomized trial to compare the immunoglobulin G response and the antibody avidity after two pneumococcal vaccinations, conjugated pneumococcal vaccine (CPV) and polysaccharide pneumococcal vaccine (PPV) 4 weeks after vs. PPV alone in 202 HIV-infected adults. There were no differences in the two strategies, either in the percentage of immunoglobulin G two-fold increase for the CPV included serotypes or immunoglobulin G two-fold increase, reaching the level of 1 μg/ml except for serotype 23F (26% responded after conjugated pneumococcal vaccine + PPV vs. 14% after PPV). No avidity increases were seen in any strategy.

The polysaccharide pneumococcal 23-valent vaccine (PPV) is recommended for HIV-infected patients and has shown to be immunogenic in this population [1]; moreover, in those with a CD4 cell count above 200 cells/μl and those under HAART [2], although its clinical effectiveness is still controversial. The immunogenicity of the conjugated pneumococcal vaccine (CPV) has been widely demonstrated in children [3–6] but with no advantage over PPV in elderly [7], or immunosuppressed populations [8]. Studies in HIV-infected adults with both vaccines found a superior response with the CPV in antibody concentration [9,10] and in functional activity [11], although others found no difference [12].

The main objective of our study was to determine whether a pneumococcal vaccination strategy combining the CPV followed by the PPV produce higher levels of specific immunoglobulin G (IgG) antibodies against the CPV included serotypes, as compared with the recommended PPV in HIV-infected adults with moderate immunosuppression. Secondary objectives were to determine those factors associated with pneumococcal vaccination response, to compare the antibody avidities before and after vaccination and between the two vaccination strategies, to assess the correlation between avidity and antibody concentration, and finally to evaluate the safety of both vaccines.

A randomized, open label and multicentric study was conducted between December 2007 and April 2008 including those HIV-infected adults who were never vaccinated against *Streptococcus pneumoniae* with moderate immunosuppression (CD4 cell count between 200 and 500 cells/μl) and a HIV viral load under 5 log copies/ml, from two Spanish hospitals, Son Dureta Hospital and Son Llatzer Hospital. Patients allocated to group 1 received one dose of CPV and one dose of PPV after 4 weeks . Patients allocated to group 2 received a single dose of PPV.

Blood samples were extracted before CPV (basal), before PPV (4weeks), and at 8 weeks in patients allocated in the group 1; and before PPV (basal) and at 4 weeks in patients allocated to group 2. Secondary adverse events due to both vaccines were recorded by telephone interview 3 days after the vaccination.

IgG against the CPV included serotypes, which was performed in all the samples using the methodology described by Wernette *et al.* [13]. Avidity for each antibody was also determined in all the samples using the methodology described by Romero-Steiner *et al.* [14]. The percentage of responders to each serotype in both groups at 8 weeks were compared using two vaccination response criteria: the first was specific antibody duplication and the second was specific antibody duplication, reaching the level of 1 μg/ml.

A total of 220 HIV-infected adults were randomized to receive CPV along with PPV 4 weeks after ($n = 110$) or to receive one PPV ($n = 110$), 18 patients were lost to follow-up, eight in group 1 and 10 in group 2. Finally, 202

Table 1. Percentage of patients responders using the first criteria, immunoglobulin G duplication after vaccination.

	Week 4			Week 8		
	CPV (%; $n=102$)	PPV (%; $n=100$)	P	CPV + PPV (%; $n=98$)	PPV (%; $n=100$)	P
Serotype 4	36	34	0.73	43	34	0.20
Serotype 14	59	61	0.75	66	61	0.44
Serotype 19F	24	29	0.38	31	29	0.80
Serotype 23F	29	28	0.82	39	28	0.11
Serotype 6B	23	31	0.17	33	31	0.80
Serotype 18C	64	55	0.21	66	55	0.10
Serotype 9V	44	54	0.16	52	54	0.78

CPV, conjugated pneumococcal vaccine; PPV, polysaccharide pneumococcal vaccine.

patients were included in the analysis at 4 weeks ($n=102$ and 100) and 198 in the analysis at 8 weeks ($n=98$ and 100). Median age was 44 years in both groups and 72% were men, there were no differences between the two vaccination groups in the following variables: tobacco (61% in group 1 and 56% in group 2 were smokers), alcohol (14% and 20%), CD4 cells count at inclusion (368 and 351 cells/μl), nadir CD4 cells count (158 and 155 cells/μl), undetectable viral load at inclusion (82% and 80%), C-HIV stage (39% and 35%), HIV transmission mechanism (37% and 27%, IDU), cotrimoxazole prophylaxis (16% and 15%), previous pneumonia episodes (23% and 21%), chronic obstructive pulmonary disease (8% and 5%), hepatitis B (6 and 6%) or hepatitis C coinfection (45% and 33%). By contrast, 98% of patients in group 1 were taking HAART vs. 91% in group 2 ($P=0.027$), and two patients in group 1 vs. eight patients in group 2 were cocaine users ($P=0.046$).

The 34% of patients receiving CPV complained of secondary effects vs. 20% receiving PPV ($P=0.07$), all mild and self limited. The most frequent were local pain (20% after CPV vs. 12% after the PPV), fever (6% vs. 3%), and asthenia and myalgias (6% vs. 3%). The geometric mean concentration of specific antibodies was similar in the two vaccination groups as pre vaccination after 4 and 8 weeks. As taking the first response criteria (duplication of specific antibodies at week 8) and the second response criteria (duplication of specific antibodies and IgG ≥ 1 μg/ml at week 8), there were no differences between the two strategies except for serotype 23F when the second response criteria was used in which 26% of patients who received two vaccines responded vs. 14% of patients who received the PPV [odds ratio (OR) 2.2, 95% confidence interval (CI) 1.07–4.56, $P=0.03$] Tables 1 and 2.

In the bivariate and the multivariate linear regression taking duplication and IgG at least 1 μg/ml to a minimum of four serotypes as response criteria solely nadir CD4 cells count of at least 200 cells/μl (OR 2.34, 95% CI 1.14–4.81, $P=0.02$) and not reporting previous pneumonia (OR 3.05, 95% CI 1.01–9.18, $P=0.04$) were associated with response. Also, not reporting previous pneumonia was the only variable associated with response to a minimum of three serotypes in the bivariate and multivariate linear regression (OR 2.90, 95% CI 1.30–6.46, $P=0.01$). No variable was associated with response to a minimum of five serotypes.

Before vaccination, the avidity indexes were very heterogeneous in each serotype (from 10% to 100%). No increases in avidity after 8 weeks were seen for any serotype in both vaccination groups. There was no correlation between avidity and antibody concentration either before vaccination or at 4 or 8 weeks for any serotype.

Although the present study only reflects the initial response to the two vaccination strategies, the CPV followed by PPV showed no advantage over the recommended PPV in IgG concentration or avidity

Table 2. Percentage of patients responders using the second criteria, immunoglobulin G duplication and immunoglobulin G at least 1 μg/ml after vaccination.

	Week 4			Week 8		
	CPV (%; $n=102$)	PPV (%; $n=100$)	P	CPV + PPV (%; $n=98$)	PPV (%; $n=100$)	P
Serotype 4	10	11	0.78	13	11	0.62
Serotype 14	47	49	0.78	50	49	0.89
Serotype 19F	12	17	0.29	17	17	0.95
Serotype 23F	19	14	0.37	26	14	0.028[a]
Serotype 6B	22	30	0.17	32	30	0.80
Serotype 18C	50	47	0.67	55	47	0.25
Serotype 9V	24	27	0.57	27	27	0.93

CPV, conjugated pneumococcal vaccine; PPV, polysaccharide pneumococcal vaccine.
[a]Odds ratio 2.2 (95% CI 1.07–4.56).

against the CPV included serotypes in HIV-infected adults with moderate immunosuppression. Nevertheless, more interesting will be the persistence of specific antibodies in each group, the interval at which antibodies decrease to the prevaccine level and whether it could be a good indicator for revaccination. We can conclude that a sequential vaccination with both vaccines, CPV and PPV, does not improve, in terms of specific antibodies and avidity, the PPV in HIV-infected patients. As more data are known, CPV should not be recommended instead of PPV in this population.

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Majorcan Pneumococcal Study Group: Bassa A, Cambra A, Campins A, Carratala C, Cifuentes C, Frontera G, García M, Hernandez RM, Homar F, Leyes M, Liebana A, Mila J, Morey C, Murillas M, Ortiz A, Pareja A, Payeras A, Peñaranda M, Ramirez A, Roca A, Ribas MA, Riera M, Samperiz G, Serra A, Serrano A, Villalonga C, Villoslada A.

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There are no conflicts of interest.

[a]Infectious Diseases Division, Internal Medicine Department, Son Dureta University Hospital, [b]Internal Medicine Department, Son Llatzer Hospital, and [c]Immunology Department, Son Dureta University Hospital, Palma de Mallorca, Spain.

Correspondence to Maria Peñaranda, Physician, Infectious Diseases Division, Internal Medicine Department, Son Dureta University Hospital, Palma de Mallorca, Spain.
Tel: +34 971175371; fax: +34 971175228;
e-mail: maria.penaranda@ssib.es

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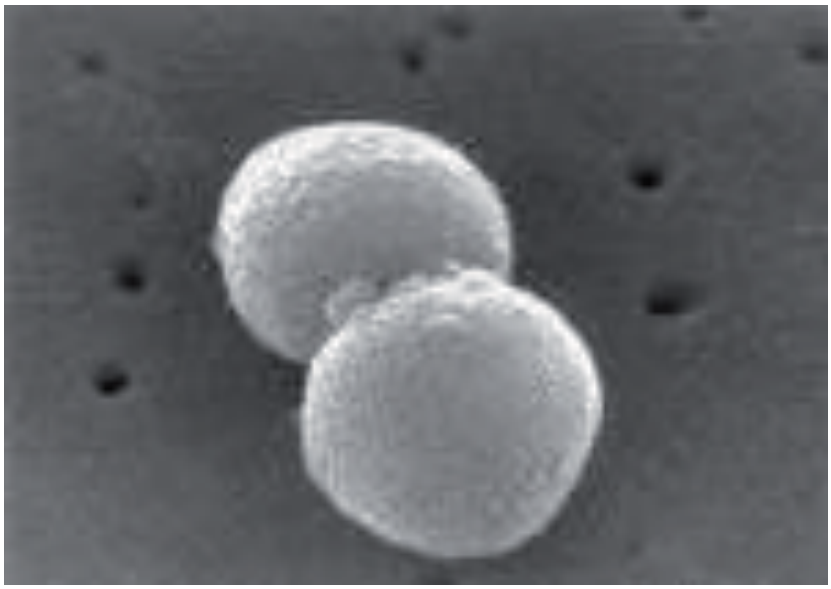

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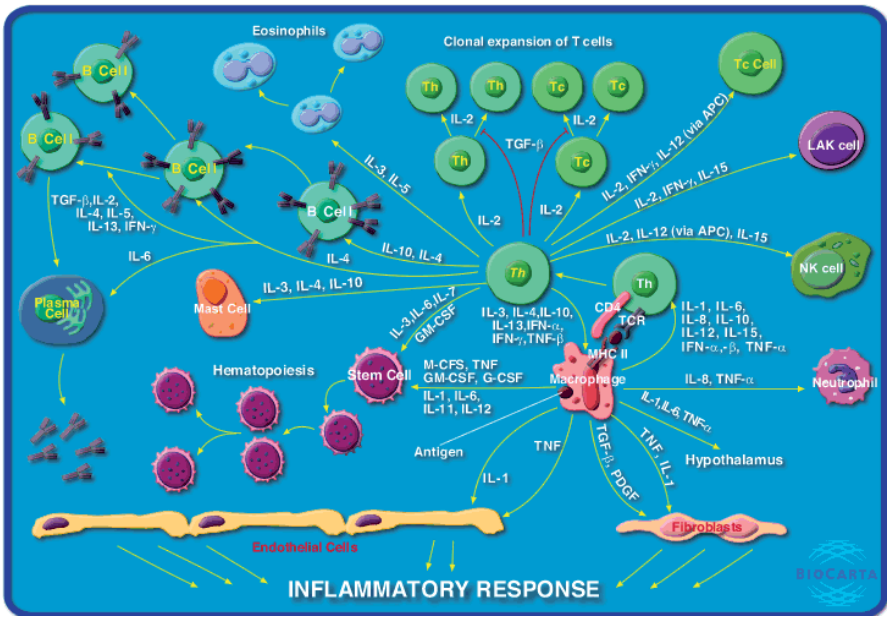
APÉNDICE


Eosinophils
Clonal expansion of T cells
B Cell
Tc Cell
Th
Tc
IL-2
TGF-β
LAK cell
IL-3, IL-5
IL-2, IFN-γ, IL-12 (via APC)
IL-2, IFN-γ, IL-15
TGF-β,IL-2,
IL-4, IL-5,
IL-13, IFN-γ
IL-6
IL-4
IL-10, IL-4
IL-2, IL-12 (via APC), IL-15
NK cell
IL-3, IL-4, IL-10
Plasma Cell
Mast Cell
IL-3,IL-6,IL-7
GM-CSF
IL-3, IL-4,IL-10,
IL-13,IFN-α,
IFN-γ,TNF-β
CD4
TCR
IL-1, IL-6,
IL-8, IL-10,
IL-12, IL-15,
IFN-α,-β, TNF-α
MHC II
Hematopoiesis
Stem Cell
M-CFS, TNF
GM-CSF, G-CSF
Macrophage
IL-8, TNF-α
Neutrophil
IL-1, IL-6,
IL-11, IL-12
IL-1,IL-6,TNF-α
Antigen
TNF
TGF-β, PDGF
TNF, IL-1
Hypothalamus
IL-1
Fibroblasts
Endothelial Cells
INFLAMMATORY RESPONSE
BIOCARTA

7. APÉNDICE

INNATE IMMUNITY IN PNEUMOCOCCAL PNEUMONIA: NEW AND OLD PLAYERS

Antoni Payeras , Joan Milà, Melcior Riera, Maria Peñaranda, Jaime Pons

Unitat de Medicina Interna, Servei d'Immunologia i Unitat Infeccioses

Hospital Son Llàtzer. Hospital Son Dureta

Palma Mallorca. Spain

Abstract

Community-acquired pneumonia is a common disease and *Streptococcus pneumoniae* is the main agent responsible. *Streptococcus pneumoniae* from the nasopharynx, carrier state, can spread to other localizations and develop the whole spectrum of diseases related to the bacteria. Innate immune mechanisms play an important role in controlling the bacteria at the localized surfaces to prevent the infection of the lower respiratory tract. Some components of the innate immune system have been studied for a long time, but the description of new unknown functions of the same old agents, the study of the newly discovered ones, the interactions between them or with the adaptive immune system have focused the interest of recent research. In this review we summarize some of the advances in furthering the knowledge of the role of human collectins (mannose-binding lectin or lung surfactant proteins), C-reactive protein, complement system, cytokine or chemokine mediated responses, and other factors in the context of pneumococcal pneumonia. Toll-like (TRLs) and NOD-like receptors are involved in the recognition mechanisms of the pathogens by the immune system. Certain impaired responses, after receptor signaling, have been related to susceptibility to pneumococcal disease, and also different expression patterns of the same receptors can be modulated by *Streptococcus pneumoniae*. Finally, we discuss some of the mechanisms involved in the risk of developing pneumococcal pneumonia after infection with respiratory viruses, such as viral up-regulation of bacterial adhesion molecules, the receptor for platelet activating factor (PAF-R), and the effect of viral neuraminidase on bacterial adhesion and triggering host inflammatory responses.

Correspondence: Antoni Payeras. Unitat de Medicina Interna. Hospital Son Llàtzer. C. Palma-Manacor, Km 4. 07198 Palma Mallorca. Illes Balears. Spain. E-mail: apayeras@hsll.es.

Introduction

Community-acquired pneumonia (CAP) is a common reason to consult at primary care and at hospital emergency services. The real incidence is difficult to determine, but in European studies rates between 5-11 cases per 1000 inhabitants and year have been described (1, 2). In the United States CAP results in an estimated 350,000 to 620,000 hospitalizations per year among elderly patients (3, 4) and in Spain one study reported a total of 53,000 hospital admissions per year (5).

Streptococcus pneumoniae is the main etiological agent of CAP in hospitalized patients (3, 6, 7, 8), especially affecting older adults, but also other populations such as patients with chronic obstructive pulmonary disease (COPD) or HIV-1 infection (9, 10, 11).

The infection mechanism of *Streptococcus pneumoniae* is usually related to a previous colonization of the nasopharynx, and the later spread of these bacteria to different localizations originating the spectrum of infectious diseases related to pneumococcus:

otitis media (auditory canal), sinusitis (upper respiratory tract), large upper airways (bronchitis), small lower airways (pneumonia) or pneumococcal invasion of sterile sites (bacteremia, meningitis, arthritis, etc.) (12).

Colonization of the mucosal surfaces of the respiratory tract due to *Streptococccus pneumoniae* is a dynamic process in which bacteria are acquired or eliminated during lifetime (13). The rates of nasopharynx colonization vary depending on some factors, but shortness of age is the main one, especially the first age of life or when there is a colonized sibling. Some other situations such as respiratory illness, the time of year, breast-feeding, day care attendance, smoking or crowding, have also been related with higher colonization rates (14, 15, 16).

The innate and adaptive immune systems interact to prevent progression from the carrier state to invasive disease, and the first step to containing the bacteria at mucosal surfaces is represented by the local immune mechanisms.

Among the antigenic determinants of *Streptococcus pneumoniae* the capsular polysaccharide is the better known, and much research has been conducted to measure humoral responses against the different serotypes of the bacteria. These studies have made it possible to progress in the development of vaccines - polysaccharide and, more recently, conjugated vaccines - with epidemiological benefits on the burden of pneumococcal disease in childhood and older adults (17, 18). An overall 60-75% efficacy of polysaccharide vaccine for bacteremia and meningitis in immunocompetent adults has been observed in epidemiological studies, however some controversy exists as to the effectiveness of the pneumococcal vaccine in other risk groups, such as sickle cell disease, and even a significant increase in the rate of pneumonia of any cause was described in a study including HIV-1 infected adults in Uganda (19, 20). More recently, the development of vaccines against other antigenic determinants which are common for all pneumococcal types has focused the research in this field (21, 22).

The potential superiority of the immune responses at the external mucosal surfaces has been suggested by some studies, but only few vaccines via mucosal route have become available, principally against viruses (poliomyelitis, adenovirus, rotavirus or influenza) but also against bacteria (*Salmonella typhi* and *Vibrio cholera*). Vaccination via the mucosal route may have some limitations, such as the difficulty to induce the immune response after local administration of non-replicating agents because of their rapid elimination or inactivation by mucosal enzymes and bacterial flora. Further, the weakness of the contact between the immunizing agents and the cells involved in the antigen uptake and processing, or potential dangers of replicating agents have to be mentioned as other potential limitations. However, other approaches to mucosal immunization have been proposed, such as subunit vaccines, synthetic peptides or vaccine antigens generated by mutagenesis, chemical conjugation and genetic reassortment, DNA vaccines or antigens generated by transgenic

plants (23).

Due to the aforementioned interaction between the bacteria and the mucosa of the nasopharynx it seems important to understand which mechanisms are involved in holding the pneumococcus at the mucosal surface, carrier state, and when a failure of these mechanisms produces the subsequent invasion of the different tissues. A better knowledge of the immune response at the mucosal surfaces would also make it possible to make headway in the prevention of pneumococcal respiratory tract infections.

Some of the recent advances in understanding the innate immune mechanisms involved in the defense against *Streptococcus pneumoniae* as the main responsible of CAP will be discussed in the present review.

Mannose-Binding Lectin (MBL)

Mannose-Binding Lectin (MBL) is a serum protein belonging to a family of Ca2+-dependent collagenous lectins, called collectins (24). Collectins are characterized by the presence of lectin domains - responsible for the recognition and binding to carbohydrates present on the surfaces of the microorganisms - in association with repetitive collagen sequences (25).

Human collectin genes are located on the long arm of chromosome 10 and there is a single functional gene comprising four exons. MBL deficiencies are related with three point mutations within exon 1 at codons 52, 54 and 57. These mutations are also called structural alleles and are designated as D, B or C variants, with A as the wild type. Mutated MBL has an abnormal structure that leads to enzymatic degradation and functional deficiency. Persons who are heterozygous for any codon variant have 20% lower MBL concentrations than those who are homozygous for the wild type alleles. Levels less than 2% or absent are observed in persons with two mutated alleles (homozygotes or heterozygotes for the two different codon mutations), who are designated O/O (26, 27).

Several polymorphisms of the promoter region of the MBL gene (a single base-pair change at position -221) are also related to collectin levels. These polymorphisms, designated as H/L, X/Y or P/Q, and the haplotypes LXP, LYP, LYQ and HYP are the most frequently found ones. The HYP haplotype is related to the highest levels of MBL and, conversely, the LXP haplotype to the lowest levels (26, 27, 28) (Figure 1).

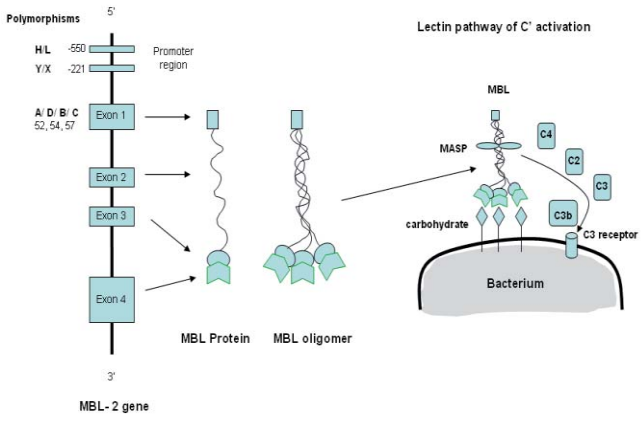


Figure 1.- Mannose-binding lectin (MBL) polymorphisms and biological function.

Although MBL is considered a component of the innate immune system it can also interact with adaptive immunity, because it has some functional homologies with IgM and C1q, mainly, and also with IgG and IgA. MBL can take part in the opsonization and phagocytosis

of bacteria by means of the attachment to the carbohydrates on their surfaces, either directly or through fragments of C3 and C4 released during C1q activation. MBL is the only collectin that is able to activate the complement system by using a different route to the classical or alternative pathways, known as the lectin or MBL pathway (Figure 1).

The carriers of MBL gene mutations related to lower levels of this protein are at risk of developing recurrent infections and autoimmune diseases, especially in conjunction with another deficiency of the adaptive immune system. The increased risk of pneumococcal infection in association with the MBL genotype was described by Roy S, *et al* (29) in a case-control study conducted in the UK, including 337 patients with invasive pneumococcal disease and 1032 ethnically homogeneous healthy controls. Sixty eight percent of the variant homozygotes and 71% of the patients with other genotypes had the diagnosis of pneumococcal pneumonia. The researchers found a substantially increased risk of invasive pneumococcal disease in the homozygotes for MBL codon variants, although the promoter polymorphism did not influence that risk. In the aforementioned study many of the patients with pneumococcal disease were relatively old and did have other non-genetic risk factors such as malignancies or cardiac disease, however, when an adjusted analysis for age was carried out the differences remained significant.

In another study from Denmark (30) including 141 patients with pneumococcal bacteremia, 116 of them with pneumonia, and 250 healthy blood donors or laboratory personnel, no differences were found regarding the risk of bacteremia and its outcome. The sole condition related to the risk of death was the presence of underlying chronic disease. More recently, in a European study including 63 Caucasian adult patients with invasive pneumococcal disease (43 with bacteremic pneumonia), after stratification by sex and age, no significant differences were found between the patient population and the control group in the distribution for MBL codon and promoter variants. Furthermore, the analysis for selected patient subgroups (diagnosed with pneumonia, bacteremia, meningitis and other pneumococcal infections) did not show differences between cases and controls (31).

However, due to the small sample size of some of the studies and the differences in ethnicity and age between the three mentioned studies, Moens L, *et al* made a meta-analysis in which a significantly increased prevalence of the O/O genotype was observed in the group of patients compared with the control population. However, when combining their data with those of the Denmark study and by jointly analyzing the MBL codon variants with promoter variants, grouping by sufficient alleles (AA + YA/O) and insufficient alleles (XAO + O/O), no differences were found between patients and controls (31).

Our group studied 97 adult patients (mean age 58.2 years) with a well documented pneumococcal pneumonia episode, 53 of them with bacteremia and 51 with at least one

comorbidity. No statistical differences were found when the MBL genotype was compared with the severity of the pneumonia, measured with a well validated prognostic scale (Fine severity index). Indeed, no differences were detected in the severity of the pneumonia episode when patients with the AO/OO genotype who presented very low MBL levels - less than 500 ng/ml - were compared with those having the MBL level above that value (32). In the XII Congress of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC), we reported our results comparing the MBL codon and promoter variants - also grouping by sufficient and insufficient alleles - of the 97 cases of pneumococcal pneumonia from our series, with those of 91 healthy control individuals. In this case, in agreement with the abovementioned work, no differences were documented between groups (33).

The results of these works are in concordance with those obtained from an *in vitro* study aimed at looking into the MBL binding capacities of different microorganisms and even differences between organisms of the same genus. This research concluded that *Streptococcus pneumoniae*, beta-haemolytic group B streptococci and *Staphylococcus epidermidis* showed low binding levels, whereas *Candida spp*, *Aspergillus fumigatus*, *Staphylococcus aureus* and beta-haemolytic group A streptococci exhibited strong MBL binding (34). However, it is possible that each serotype of *Streptococcus pneumoniae* may not have the same affinity to bind MBL. For instance, in a clinical study, serotype 14 of pneumococcus which has a repeating subunit of N-acetylglucosamine (a sugar with a high affinity for MBL) at the surface polysaccharide was more frequent in MBL homozygote patients than among heterozygotes (29). Another *in vitro* study also demonstrated higher binding levels of radiolabelled MBL to *Streptococcus pneumoniae* than to other meningitis pathogens such as *Neisseria meningitidis* or *Haemophilus influenzae* (35).

Another remarkable observation in our series of patients with pneumococcal pneumonia is the fact that the patients with the wild-type MBL genotype (A/A) had a greater risk of developing bacteremia, and when the whole study population was considered, the levels of this lectin tended to be higher in patients with positive blood cultures. To explain this finding, which seems to render bloodstreams more susceptible to invasion, it can be speculated that, as has been reported, the N-acetylglucosamine of the cell wall of gram positive bacteria is a biologically relevant ligand for MBL and that MBL inhibits peptidoglycan-induced production of pro-inflammatory cytokines, suggesting that MBL may down-regulate macrophage-mediated inflammation and, furthermore, that MBL enhances phagocyte recruitment, thus hypothetically favoring the bloodstream entrance of the bacteria by proper cell-surface anchoring (36).

For the time being, and taking into account all the published data from the studies regarding the relationship between the MBL genotype and susceptibility to pneumococcal infections, one can conclude that the role of lectin is secondary in patients without another

underlying disease or immunodeficiency. More studies are needed to confirm whether or not high levels of MBL predispose to developing bacteremia once the patient has been infected locally.

This role of MBL deficiency could be more important in other clinical scenarios, as has been observed in patients with systemic lupus erythematosus, HIV-1 infection, cystic fibrosis, during early childhood, or in children with malignancies and infections with other microorganisms capable of stronger binding of MBL (37, 38, 39, 40, 41).

Another matter for concern is the debate as to the function of MBL as an acute-phase reactant. Since the first description by Ezekowitz AB, *et al* (42) that MBL synthesis in the liver is induced as part of the acute response, the acute-phase properties of MBL have been determined in different scenarios. Neth O, *et al* (41) observed a higher MBL concentration in children with malignancy than in healthy individuals. Two other studies have analyzed the behavior of MBL in patients undergoing major surgery, with discordant results. In the first study, in sequential blood samples of eleven patients after major hip surgery, increases of 1.5 to three times the MBL concentration were observed (43). In the same study an increase in the MBL levels was observed in five patients after a malaria attack. Another study including patients who underwent gastrointestinal resections for malignant disease, the MBL levels did not rise immediately after surgery, but lower MBL levels were associated with the occurrence of postoperative infections (44). However, although both studies were carried out in a postoperative setting, the populations analyzed were different, and in the second work the observation period was shorter, with only two blood samples tested (1 and 3 days after the surgery).

In a study of critically ill patients admitted in an intensive care unit (ICU), Hansen TK, *et al* (45) concluded that, even though the MBL levels on day five of admission and on the last day of the ICU stay were higher in the non-survivors, these levels were not related to the outcome in the multivariate analysis when adjusted for all upon-admission risk factors. The authors observed how the MBL concentration rose during the ICU stay, although no correlation was observed with C-reactive protein (CRP) levels at any point. In another study including patients with severe infection and proven sepsis (bloodstream infection or community-acquired pneumonia), the MBL concentrations behaved as an acute phase reactant, defined as a 25% increase or decrease from baseline levels, in 31.3% and 27.3% of the cases respectively, with 41.4% of the patients exhibiting steady-state levels throughout the study period. The authors concluded that MBL concentrations demonstrated a variable acute-phase response (46).

In agreement with these last two studies we were unable to observe differences between MBL levels in patients with pneumococcal pneumonia during the acute episode and those measured at least four weeks after recovery. Furthermore, no correlation was observed between MBL and CRP levels. The

patients with comorbidity showed significantly higher CRP concentrations during recovery, probably rendering a persistent inflammatory state, though this behavior was not observed for MBL levels (32). These data support the absence of parallelism between both proteins (Figure 2).

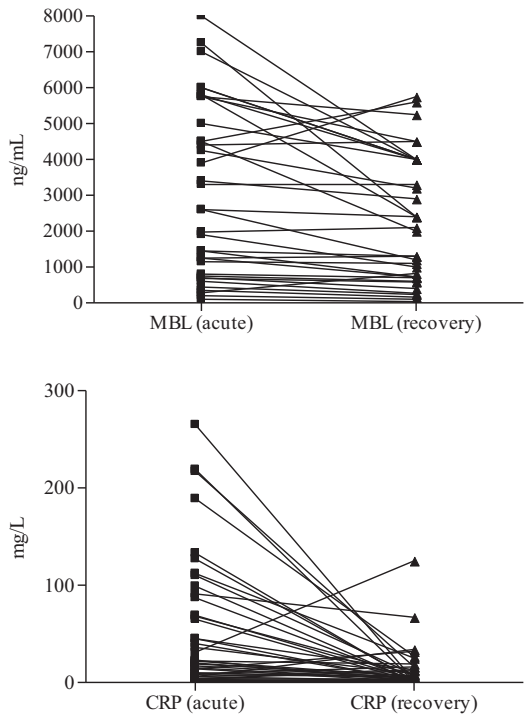


Figure 2.- levels of MBL and CRP in paired samples from patients with pneumococcal pneumonia, during the acute episode and at least 4 weeks after recovery.

Other members of the human collectins are collectin liver protein 1 and lung surfactant proteins A and D (SP-A and SP-D). Recent interest has been focused on SP-D, a collectin produced by alveolar type II cells and non-ciliated Clara cells of the lung. SP-D accumulates in the airspaces and can enhance the uptake of *Streptococcus penumoniae* by neutrophils (47). Recent results indicate that SP-D binds to carbohydrates on the pneumococcal surface through its calcium C-type lectin domains (48). In an *in vivo* study, by comparing the outcome of intranasal infection in SP-D deficient (SP-D-/-) to wildtype (SP-D+/+) mice, SP-D deficiency was associated with enhanced colonization and infection of the upper and lower respiratory tract and earlier onset and longer persistence of bacteremia. These data provide evidence that SP-D plays a significant role in the clearance of pneumococci during the early stages of infection in both pulmonary sites and blood (49). It has also been observed that SP-D may have other functions such as minimizing the inflammatory response in the lung by enhancing the clearance of dead and dying cells from the airspaces, and reducing the basal rate of alveolar macrophage apoptosis (50). Besides, other research has been conducted to attain a better understanding of how lung collectins modulate cellular responses and whether there are interactions with the toll-like receptors (TLR) (51). However, a more complete understanding of the molecular mechanism and further studies in humans are required in order to know the exact role of these collectins in pneumococcal infections.

C-Reactive Protein (CRP)

CRP was first described in 1930 because of its reactivity with the pneumoccocal C-polysaccharide in the plasma of patients with pneumococcal pneumonia (52). Initially it was used as a laboratory marker in patients with pneumonia, but was not extensively used for many years. More recently, due to the development of new commercial automated

tests which are more sensitive than the previously available ones, interest in CRP has been renewed. CRP is an acute-phase protein produced by the liver after the stimulus of the cytokines released during an inflammatory process. Among these cytokines, interleukin-6 (IL-6) seems to be the main inducer in conjunction with IL-1 β and tumor necrosis factor α (TNF-α) (53). CRP is able to bind to phosphorylcholine present on the surface of microorganisms, such as pneumococcus or *Haemophilus influenzae*, or the phospholipid constituents of damaged cells. After binding to bacterial or cell surfaces, CRP can trigger the classical pathway of the complement system by binding C1q and may interact with phagocytic cells, mainly the polymorphonuclear leucocytes, through FcgammaRI and FcgammaRII, thus initiating the elimination of bacteria and damaged cells. Due to these properties, CRP may be considered a component of the innate immune system (54) (Figure 3).

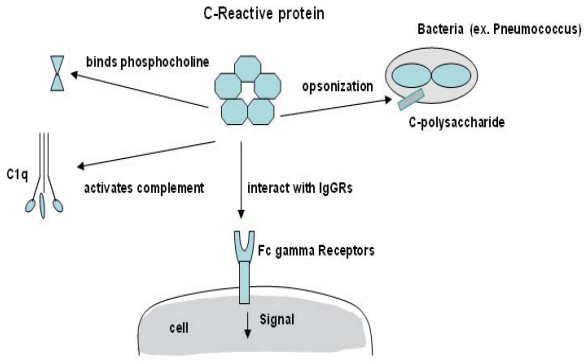


Figure 3.- C-reactive protein (CRP) binds different ligands and have important biological roles.

In one study it was observed that CRP recognition of *Streptococcus pneumoniae* and binding to FcgammaR may enhance the early protective cytokine response to infection (55).

Some works have investigated the possible existence of a genetic susceptibility to developing pneumococcal invasive infections in relation with certain CRP polymorphisms. Studies in mice have observed a protective role of CRP against pneumococcal infection and disease, and also transgenic mice with human CRP had reduced bacteremia and longer survival after infection with pneumococcus (56, 57). A case-control study, conducted in humans, compared the frequency of the dinucleotide repeat polymorphism located in an intron of the C-reactive protein gene in 205 patients with pneumococcal invasive disease (bacteremia, meningitis or arthritis) and 345 blood donors. The authors found that an allele, of 134 base pairs, was more often observed in cases than controls, and homozygotes with 134 base pairs were at a significantly increased risk of disease. The peak concentrations of CRP were not different in the cases with or without allele 134, although variation between patients at time of sampling limited the analysis (58).

The role of CRP as a guide to differentiate patients with CAP from others with respiratory tract infection and to define cut points to discriminate between both diseases has also been investigated. Some works have tried to establish a relationship between the increased CRP levels and the severity of CAP or to differentiate the etiology, with higher CRP values reported for bacterial infections, mainly for *Streptococcus pneumoniae* or *Legionella pneumophila*, and lower ones for Chlamydia or viral infections. In other studies, the

concentrations of CRP have been greater in patients with pneumococcal pneumonia with bacteremia (59, 60, 61, 62, 63, 64, 65, 66, 67). In a Spanish published work CRP levels were higher in patients with CAP who developed empyema and those with *Legionella pneumophila* pneumonia, while pneumococcal pneumonia was associated with a higher erythrocyte sedimentation rate value. The researchers concluded that acute-phase proteins may be useful in the prediction of pleural complications and in the approach to the etiological diagnosis of CAP (68). In a retrospective study, Madhi SA, *et al* analyzed CRP and procalcitonin levels in sera obtained from children who were hospitalized for treatment of clinically diagnosed lower respiratory tract infection, among those participating in a phase 3 pneumococcal conjugate vaccine efficacy trial. They concluded that CRP levels equal to or greater than 40 mg/dL provide a better measure than chest radiographs to assess the effect of the conjugate vaccine in preventing pneumonia (69).

In our experience, CRP levels in the acute phase of pneumococcal pneumonia were not statistically different between patients classified as low and moderate-high risk according to the Fine severity scale, and no positive correlation was observed between CRP levels and the numerical value of the score (Figure 4).

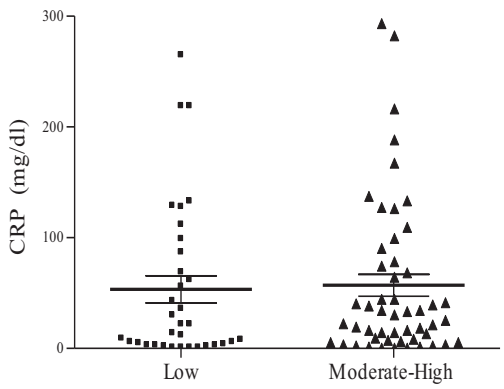


Figure 4.- CRP levels during an acute episode of pneumococcal pneumonia in patients classified as low or moderate-high risk in accordance with the Fine severity scale

However, patients with bacteremia did show greater CRP concentrations, independently of the severity of the episode (Table 1).

Nevertheless, in the recovery phase, at least four weeks after the episode, CRP levels were lower in those patients included in the low risk class or without any comorbidity (Figure 5 and Table 2) (70).

CRP (mg/dl)	Bacteremia	No bacteremia
Acute:		
Mean (SD)	79.53 (106.93)	36.68 (63.77)
Median (range)	40 (1-578)	9 (1-265)

Table 1.- CRP levels during an acute pneumococcal pneumonia episode in bacteremic (n=53) or non bacteremic (n=47) patients (*P*= 0.003).

CRP (mg/dl)	Comorbidity	No comorbidity
Recovery:		
Mean (SD)	20.12 (31.9)	5.77 (7.42)
Median (range)	6 (1-125)	2 (0-27)

Table 2.- CRP levels, at least 4 weeks after a pneumococcal pneumonia episode, in individuals with (n=51) or without (n=48) underlying comorbidity (*P*: 0.01).

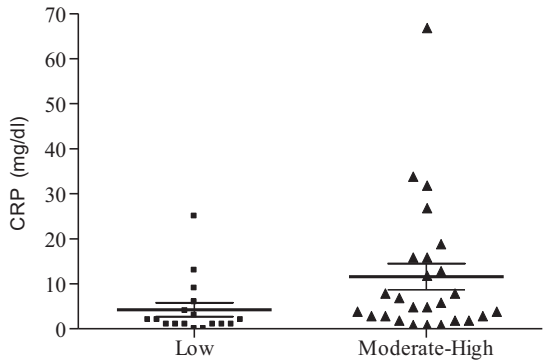


Figure 5.- Recovery phase, after pneumococcal pneumonia, CRP levels between individuals with low and moderate-high risk episodes (*P*=0.005).

In a recently published systematic review with the aim of evaluating the diagnostic accuracy of CRP in detecting radiologically proved pneumonia, and to evaluate how well the bacterial or viral etiology of lower respiratory tract infections can be discriminated, the authors concluded that it was neither sensitive enough to rule out nor specific enough to rule in an infiltrate on chest radiograph and bacterial etiology of lower respiratory tract infections. Another conclusion of this review was the poorness of the methodological quality of the diagnostic studies. Thus, there is neither enough nor consistent enough evidence to support the wide use of CRP as a test to guide prescription of antibiotics (71).

Complement system

The fact that humans with a deficiency in any of the three complement pathways display enhanced sensitivity to pneumococcal infection established the crucial contribution of complement to the innate response to *Streptococcus pneumoniae* (72). The complexity of the immune response, with numerous interacting factors, makes the use of animal models an essential tool. Recently, in mice carrying specific gene deletions, the relative importance of individual complement activation pathways has been assessed (73, 74). The most important pathway for activation of the complement system during innate immunity to *Streptococcus pneumoniae* is the classical pathway, partially mediated by natural IgM antibodies binding to bacteria, possibly to teichoic acid (C polysaccharide) (75). But other activation pathways also contribute, such as CRP, or direct binding of C1q (74). The lectin and alternative pathways are activated directly by binding to bacterial cell surface components and contribute to a lesser extent. The deposition and activation on the bacterial surface of complement factor C3, regardless of the pathway is a key step to eliminating the microbe. Even though pulmonary macrophages and epithelial cells can synthesize and secrete C2, C4, C3, C5, and factor B *in vitro*(76), it has been presumed that complement does not play a major role in anti-pneumococcal defense within the lungs during the early stages of pneumonia, but rather becomes involved when the host is bacteremic. *Streptococcus pneumoniae* inhibits the complement pathway in several ways. The capsule acts by limiting accessibility of surface-bound complement and reducing the amount deposited (77). PhpA a pneumococcal surface protein has been found to possess C3-

degrading activity (78). Pneumolysin, among multiple other biological activities, and by unconfirmed mechanisms, seems to confer protection from complement-mediated clearance *in vitro* and *in vivo*, and is specific to the classical and not the alternative activation pathway (79). The surface proteins PspA and PspC also contribute to complement resistance through the binding of the complement regulator, factor H, and its recruitment onto the pneumococcal surface; PspC can also bind complement component C3 (80, 81).

Toll-like (TLRs) and NOD-like receptors

A prerequisite for the initiation of host responses is the recognition of pathogens by the host immune system. These immunosensors are mainly the transmembrane Toll-like receptors (TLRs) and the cytosolic NOD-like receptors (NLRs) (82).

TLRs signal from the cell surface or endosome upon ligand binding, and the more recently defined NLRs: NODs, NALPs, NAIP and IPAF are activated in the cytosol by characteristic bacterially derived molecules, such as peptidoglycan, RNA, toxins and flagellin (83, 84). Both groups are so-called pathogen recognition receptors (PRRs). Toll-like receptors (TLRs) (with the exception of TLR3), interleukin-1 receptor (IL-1R) and IL-18R induce nuclear factor-κB (NF-κB)-dependent cytokine production through a pathway involving the adaptor molecule myeloid differentiation primary-response gene 88 (MyD88) (85).

Impaired cellular responses post TLR signaling, due to germline mutations in IRAK4 or NEMO have been reported to be associated to increased susceptibility to pneumococcal disease (86, 87). In different infection models, MyD88 -/- mice showed enhanced susceptibility to *Streptococcus pneumoniae* infection (88, 89). The other side of the coin is the microbial ligand recognized by PRRs, called pathogen-associated molecular patterns (PAMPs) which are often structural components of the microorganism that are not subject to much variation. Their recognition by PRRs initiates complex signaling which results in the initial host immunological response (90). To date, ten germline encoded TLRs have been identified in humans, each recognizing different microbial structures. TLR1, -2, -4, -5, -6, and -10 are located on the cell surface and mainly recognize bacterial products that are unique to the invading organism.

Pneumococcus can also modulate the expression pattern of TLRs by increasing the expression of TLR1 and TLR2 in bronchial epithelial cells, but with no effect on TLR4 and TLR6 expression (91).

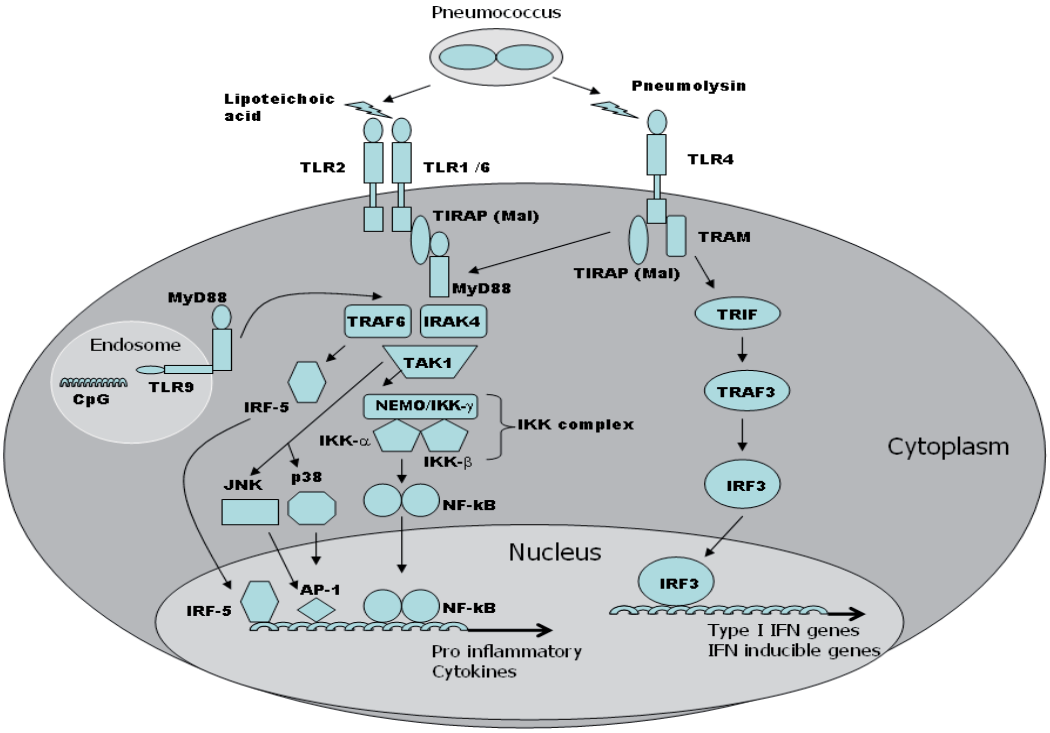
TLR1. TLR1 interacts with TLR2 and TLR6 to discriminate between molecular structures of distinct lipopeptides. TLR1 and -2 are synergistically recognized by pneumococci in cotransfection experiments. *Streptococcus pneumoniae* has been described to activate the human lung epithelial cell line via MyD88 and TRAF6 producing IL-8 release, NF-kappaB (91). Monoclonal antibodies against TLR1 inhibit TNF-□□production induced by pneumococcal lipoteichoic acid (LTA) from human peripheral blood mononuclear cells (92).

TLR2. There are numerous studies in favor of the recognition of LTA and cell wall peptidoglycan

by this receptor (93, 94, 95, 96), although other reports argue for the interaction of peptidoglycan-TLR2 (97). In an animal model, TLR2 gene-deficient mice intranasally inoculated with *Streptococcus pneumoniae* at doses varying from non-lethal (with complete clearance of the infection) to lethal displayed only a modestly reduced inflammatory response in their lungs and an unaltered antibacterial defense when compared with normal wild-type mice (94). These data may suggest that TLR2 plays a limited role in the innate immune response to pneumococcal pneumonia. Results from *Streptococcus pneumoniae* infected epithelial cells indicated a cooperative recognition of these bacteria by TLR1 and TLR2 but not by TLR2 and TLR6 (91).

TLR4. Pneumolysin, the important pneumococcal virulence factor, was found to induce a TLR4 dependent activation of epithelial cells (91, 98). TLR4 mutant mice showed a reduced survival only after infection with low-level bacterial doses, which was associated with a higher bacterial burden in their lungs 48 hours post-infection (99). Recent studies suggest that protection against pneumococcal disease is dependent on the TLR4-mediated enhancement of pneumolysin-induced apoptosis (100). Through its recognition of pneumolysin, according to this study, TLR4 acts in the nasopharynx to limit pneumococcal proliferation.

TLR9. It has been shown that mice deficient in TLR9 but not in TLR1, TLR2, TLR4 and TLR6 or IL-1R/IL-18R are more susceptible to a respiratory tract bacterial infection caused by *Streptococcus pneumoniae*. Intranasal challenge studies revealed that TLR9 plays a protective role in the lungs at an early stage of infection prior to the entry of circulating inflammatory cells. Alveolar and bone marrow-derived macrophages that are deficient in either TLR9 or the myeloid adaptor differentiation protein MyD88 were impaired in pneumococcal uptake and in pneumococcal killing. Our data suggest that, in the airways, pneumococcal infection triggers a TLR9 and MyD88-dependent activation of



phagocytic activity from resident macrophages leading to early clearance of bacteria from the lower respiratory tract (101) (Figure 6).

Figure 6.- Surface-exposed and intracellular pattern recognition receptors (PPRs) and main signalling pathways in pneumococcus infection.

(TRAM) TRIF-related adaptor molecule. (TIRAP) Toll-interleukin 1 receptor (TIR) domain-containing adapter protein. (Mal) MyD88 adapter-like. (TRIF) TIR domain-containing adaptor inducing IFN-β. (TRAF) Tumor necrosis factor receptor-associated factor. (IRF) Interferon regulatory factor. (JNK) JUN N-terminal kinase. (IKKαβγ) Inhibitor of kappa B kinase alfa, -beta, -gamma. (NEMO) nuclear factor- κB essential modulator. (AP-1) Activating protein-1. (IRAK) IL-1R–associated kinase. (CpG) cytosine base paired to a guanine. (MyD88) Myeloid differentiation domain. (NF-kB) Nuclear factor kappa B.

NOD proteins. Recently, two members of a novel class of pattern recognition receptors, the cytosolic proteins nucleotide-binding oligomerization domain 1 (NOD1)/CARD4 and NOD2/CARD15, have been found to detect cell wall peptidoglycans (84, 102). NOD1 is ubiquitously expressed whereas NOD2 is primarily found in antigen presenting cells and epithelial cells. Intracellular pneumococcus is recognized by NOD2 but not NOD1 in epithelial cells and activates the NF-kB pathway following intracellular stimulation (103). Muramyl dipeptide (MDP) moieties from bacterial peptidoglycan are the key components selected by NOD2. Recognition is dependent on internalization of the bacteria. NOD1 and NOD2 expression in pulmonary epithelial cells was up-regulated in mouse lungs infected with pneumococcus (103). Whereas TLRs are involved as the first line receptors for *Streptococcus pneumoniae*, the NOD proteins might play a major role in a subsequent phase of infection against internalized pneumococci (Figure 7).

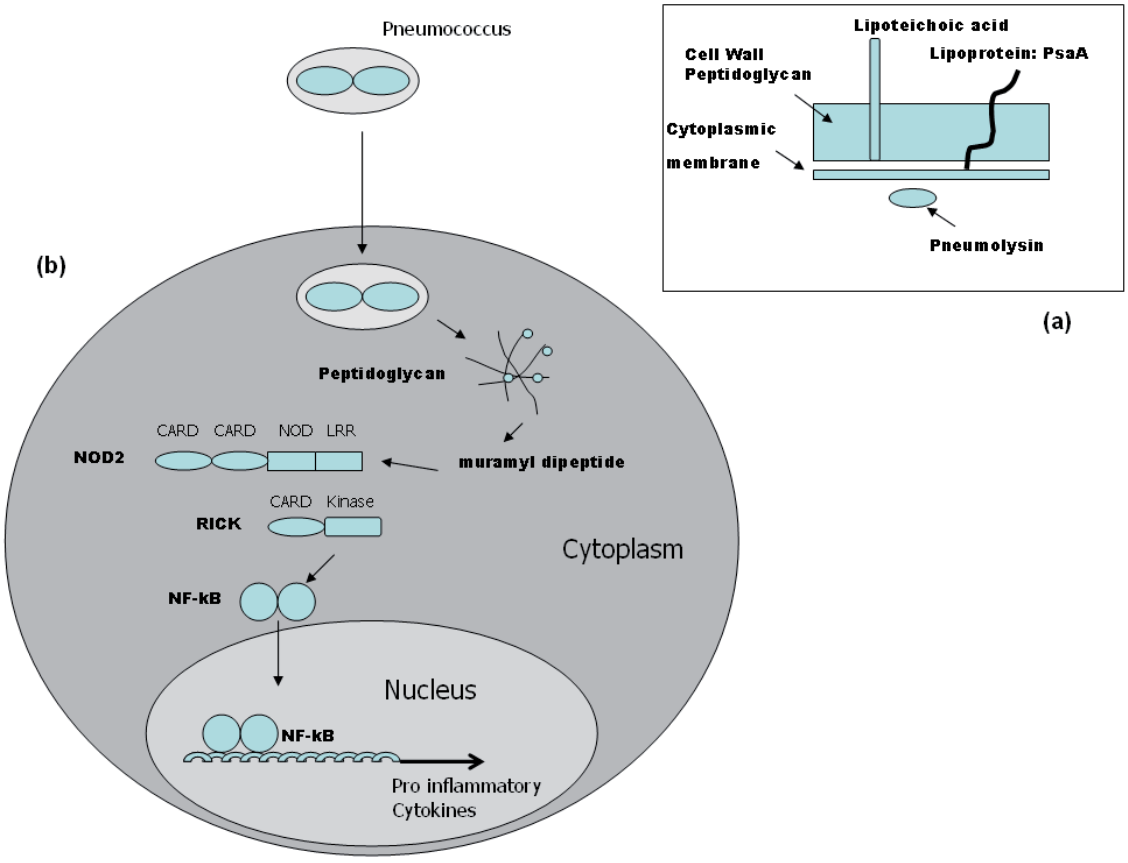


Figure 7.- Structural features of *Streptococcus pneumoniae* (a). Described cytoplasmic bacterial detectors of pneumococcus and their signalling (b).

(NOD2) Nucleotide-binding oligomerization domain 2. (CARD) Caspase-recruitment domain. (LRR) Leucine-rich repeats. (RICK) Receptor-interacting sewerine/threonine kinase. (NF-kB) Nuclear factor kappa B.

Cytokine and chemokine mediated responses

Eradication of bacteria in the lower respiratory tract depends on the coordinated expression of pro-inflammatory cytokines and consequent neutrophilic inflammation, but little is understood as yet about the significance of the different cytokines in protection against extracellular microbes. Furthermore, opposing roles have been proposed for some of them. Inflammatory cytokines induced by bacterial factors may up-regulate expression of host proteins that can subsequently be utilized by the pneumococcus for adhesion (104). Recently, it has been reported that administration of IL-12 resulted in lower bacterial burdens in infected mice and significantly improved survival rates. IFN-□□was found to be essential for IL-12-induced resistance and for neutrophil influx into the lungs, and the observed changes correlated with increased levels of the IL-8 homologue keratinocyte-derived chemokine (KC) (105). In this animal model, exogenous IL-12 was able to improve innate defense in the lung against *Streptococcus pneumoniae* by inducing IFN-□ production, which in turn enhances chemokine expression, and promotes pulmonary neutrophil recruitment into the infected lung. NK cells were the primary cells responsible for IFN-□□production, and IFN-□ in turn was found to increase TNF-α production by bacterium-stimulated alveolar macrophages. However, exogenous IFN-□ treatment before infection did not lead to increased bacterial clearance in the lung. Depletion of neutrophils abrogated the ability of IL-12 to enhance survival after infection but did not affect the number of lung macrophages (105).

Other authors have suggested that IFN-□ plays no role in mediating host defense in response to *Streptococcus pneumoniae* pneumonia (106).

It has been established in a mouse model that IL-10 seems to play an important role in host defense against *Streptococcus pneumoniae* after recovery from influenza infection, in part caused by reduced neutrophil function in the lungs (107).

An increased production of both interleukin IL-10 and IFN-□ was reported during convalescence of invasive pneumococcal disease in human adults (107).

Infection with *Streptococcus pneumoniae* increased the expression of IL-18 mRNA and was associated with elevated concentrations of both precursor and mature IL-18 protein within the lungs. IL-18 knockout mice had significantly more bacteria in their lungs and were more susceptible to progressing to systemic infection at 24 and 48 hours post-inoculation (108). TNF-α is capable of recruiting inflammatory cells to the site of infection both directly and via up-regulation of adhesion molecules. TNF-α is also capable of stimulating the release of chemokines, directly chemotactic for inflammatory cells. Macrophage inflammatory protein 1 alfa (MIP-1α) and MIP-2 are two chemokines known to be important in bacterial pneumonia (109). Following recruitment of phagocytic cells, TNF-α may also promote antimicrobial activity by activating the respiratory burst and by activating the capacity to degranulate.

Epithelial cells play an active role in the host response to respiratory pathogens, such as *Streptococcus pneumoniae,* by releasing chemokines responsible for neutrophil recruitment.

Chemokines are subdivided into 4 distinct subfamilies (CC, CXC, CX3C, and C) depending on the arrangement and number of the onserved N-terminal cysteine molecules.

Chemokines, in particular CCL2, have been associated with survival in invasive pneumococcal disease and correlate with pneumococcal bacterial loads, disease presentation, and outcome (110).

The dynamics of these responses have also been studied using mouse intranasal challenge models. A recent study reports that pneumocyte and nasopharyngeal cell-lines infected with *Streptococcus pneumoniae* D39 or mutants lacking choline-binding protein A (CbpA), pneumococcal surface protein A (PspA), or specific domains, showed a rise in mRNA for the CXC chemokines IL-8, MIP-2α , and MIP-2β and an increase in secretion of IL-8, compared to uninfected control cells (111).

Pulmonary monocyte chemoattractant protein 1 (MCP-1) levels were strongly correlated to bacterial loads during pneumococcal pneumonia in wild-type mice. However, MCP-1 knockout and wild-type mice were indistinguishable with respect to bacterial growth, inflammatory responses and lethality (112) (Figure 8).

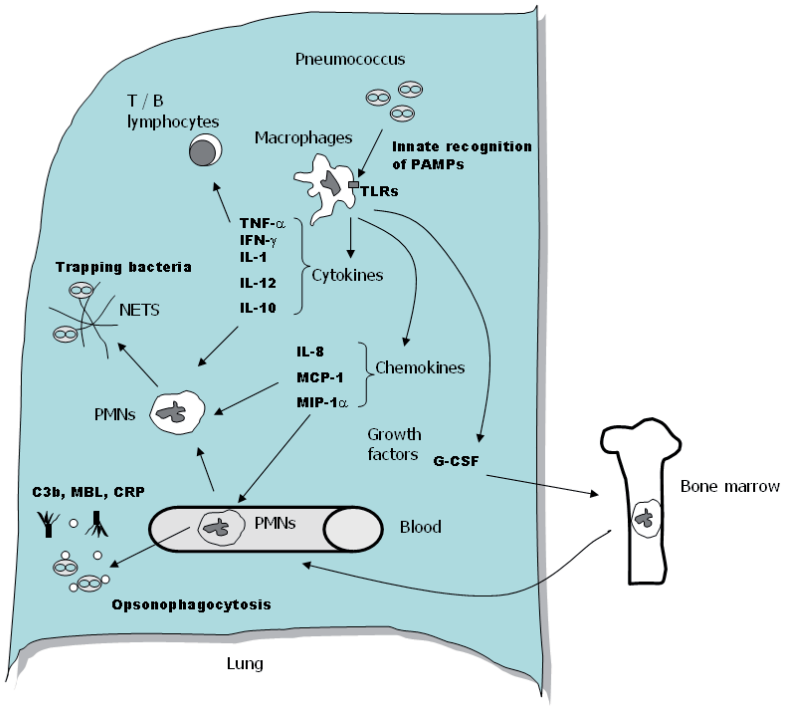


Figure 8.- Cytokine, chemokine and other immunological responses in the lung after pneumococcus infection. (PAMPs) pattern recognition receptors. (TLRs) Toll-like receptors. (TNF-□□□Tumor necrosis factor alfa. (IFN-□) Interferon gamma.(MCP-1) Monocyte chemoattractant protein 1. (MIP-1□) Macrophage inflammatory protein 1 alfa. (G-CSF) Granulocyte colony-stimulating factor. (NETS) Neutrophil intracellular traps. (MBL) Mannose-binding protein. (CRP) C-reactive protein.

Innate immunity after influenza viral infection

The earliest suggestion that viral infections predispose to bacterial diseases has been attributed to Laennec, who observed that the prevalence of pneumonia increased after an epidemic of influenza in 1803. This association became particularly evident after the 1918 influenza pandemic, during which an estimated 40-50 million persons died, many of them from secondary bacterial pneumonia (113). This is particularly important in individuals over the age of 65, as well as in persons with underlying comorbid conditions. Mahdi SA, *et al* (114) observed, in a recently published randomized placebo controlled trial, that pneumococcal conjugated vaccines reduces pneumonia associated with respiratory viral infections, presumably by preventing superimposed bacterial co-infection. Abundant epidemiological and biological evidence indicates that respiratory viruses contribute to bacterial infections through viral destruction of respiratory epithelium, viral up-regulation of bacterial adhesion molecules such as the receptor for platelet activating factor (PAF-R) and (for influenza and rhinovirus) the effect of viral neuraminidase on bacterial adhesion and triggering host inflammatory responses.

Animal models have been established to elucidate the mechanisms of viral/bacterial infiltration and the attendant inflammatory response (115). Mice infected with a non-lethal dose of influenza exhibit 90-100% mortality after challenge with *Streptococcus pneumoniae*; in each instance bacterial infections that are non-lethal by themselves become fatal if they occur at the peak of an ongoing viral infection. The mechanism of this lethal synergy is the much greater bacterial infiltration observed in the virally infected animals. Influenza-infected mice subsequently exposed to *Streptococcus pneumoniae* exhibit high bacterial titters in the lungs and the bacteria rapidly spread systematically, therefore, the primary effect of dual infection appears to be heightened levels of bacterial infiltration/replication, with less effect on virus titters (116).

The influenza virus infects the epithelial cells of the upper and lower respiratory tract. Histological analysis of influenza infection in humans or animal models demonstrated similar pathological findings. Acute influenza infection of the lower respiratory tract leads to desquamation of the epithelium down to the basal cell layer, resulting in clinical tracheobronchitis and bronchiolitis (117). The classic concept that influenza virus-induced epithelial damage provides increased numbers of attachment sites for bacteria is also supported by mouse studies using highly pathogenic mouse adapted viruses. However, non pathological damage to the traqueobronchial tree was seen in studies using unadapted viruses. While pathological studies suggest that epithelial damage is a major factor in human disease with highly virulent viruses such as the 1918 and 1957 pandemic strains, the relative contribution of this mechanism in years when less virulent viruses are circulating may not be as robust.

Bacteria may adhere to the basal membrane after disruption of the airway epithelial layer by the cytophatic effect of the virus, but may also

bind to specific receptors in the airway epithelium induced by influenza virus. Influenza A virus and rhinovirus infection of tracheal epithelial cells similarly increased the number of adherent *Streptococcus pneumoniae*, indicating that the ability to augment bacterial adherence could be a general feature of respiratory viruses (118). Studies in human volunteers demonstrate that influenza A infection enhances the rates of colonization by *Streptococcus pneumoniae* (119). The receptors that pneumococcus uses to adhere and invade the lungs are currently unknown. One proposed mediator is PAF-R. PAF-R is a G protein-coupled receptor that is mainly expressed on macrophages, monocytes and epithelial cells, and has been described to be up-regulated at the epithelial cell surface by inflammatory cytokines and during viral infections. PAF-R is able to bind phosphorylcholine, a cell wall component of *Streptococcus pneumoniae* (120). Using knockout mice which lack the PAF-R entirely, van der Sluijs KF, *et al* (121) showed that the viral clearance was similar in wild type and PAF -/- mice whereas PAF -/- mice displayed a significantly reduced bacterial outgrowth in their lungs, a diminished dissemination of the infection and prolonged survival. Other studies support that influenza virus does not up-regulate PAF-R. Mice treated with a PAF-R antagonist had no reduction in the severity of secondary pneumococcal pneumonia after influenza infections (116).

Viral neuraminidase (NA) is thought to contribute to adherence to epithelial tissues by exposing receptors for pneumococcal adherence and invasion. Viral NA cleaves sialic acid residues on host cell surface carbohydrates generating more bacterial binding sites. Using a mice model of viral-bacterial synergism, NA activity of the virus enhanced adherence of pneumococcus to epithelial cells *in vitro* and predisposed to fatal pneumonia with massive involvement of multiple lobes (122). The administration of the selective NA inhibitor oseltamivir prevented most pneumonias, delaying the development and progression of the pneumonia, restricting this involvement to fewer lobes and improving survival independent of viral replication and morbidity from influenza, suggesting that the effect of NA inhibition is to limit the extent of pneumococcal pneumonia. Despite having no activity against pneumococcal NA, the effect of NA inhibitor could be seen in the severity of pneumococcal pneumonia measured by photon emission and bacterial lung titter (122, 123). Other evidence indicates that NA activity is important in the pathogenesis of pneumococcal infections. Colonization, exposure of receptors on Eustachian-tube epithelium and mouse nasopharyngeal epithelium and virulence in a pneumonia model are decreased in the knockout mutant of pneumococci deficient in Nan A, the major pneumococcal NA gene (124, 125). It has also been demonstrated that either exogenous bacterial NA or pre-infection with influenza A virus may enhance this activity in the chinchilla middle ear model (126). A third method by which pneumococcal adherence might be facilitated is through the fibrin and fibrinogen deposited during the regenerative process. During the initial insult and the inflammatory

changes associated with influenza virus infection the airway undergoes a process of regeneration and remodelling. Although pneumococcus binds poorly to ciliated epithelium, it may adhere more to non-ciliated, differentiating cells involved in the proliferation and regeneration response. Deposition of fibronectin, collagen, and other matrix elements provides further attachment sites for bacteria such as *Streptococcus pneumoniae* and *Staphylococcus aureus* (127).

Viral/bacterial co-infection may push the host immune-response to immunopathological levels (128). Both pneumococcus and influenza virus are recognized by TLRs, generating a cytokine response and triggering an influx of immune effector cells. Because the pathways and intermediate signalling molecules, previously described for pneumococcus in this review, are similar for the influenza virus, it is not surprising that the pro-inflammatory response to the influenza virus mirrors that of the pneumococcus, with induction of IL-1, IL-6, TNF-α, RANTES, MIP-1 alpha, IL-8 and gamma interferon (127, 129). Recent works suggest that the severe lung inflammation seen with the 1918 pandemic strain and the H5N1 avian influenza A viruses may be due to increased levels of pro-inflammatory cytokines and massive influx of neutrophils into the lung. A recently discovered proapoptotic protein, PB1-F2, encoded by most influenza A viruses, could contribute to this inflammatory response and differences in PB1-F2 may contribute to explaining the differences in excess mortality exhibited by the H1N1 and H3N2 strains.

Sequential infection of influenza A and *Streptococcus pneumoniae* resulted in the greatest increase and highest total leukocyte numbers and unaltered macrophage counts in bronchoalveolar lavage following exposure to *Streptococcus pneumoniae*; the immune cell infiltration occurring after dual infection was much greater than with either viral or bacterial infection alone. The cytokines TNF-α and IL-1β were also significantly elevated in the lung from mice previously exposed to influenza A compared to animals receiving either influenza A or *Streptococcus pneumoniae* alone (115).

In knockout mice without PAF-R receptors the effect of PAF on the induction of cytokines and chemokines was also determined. *Streptococcus pneumoniae* infection resulted in enhanced production of TNF-α, IL-6, IL-10 and KC, two days after the infection the IL-10 and KC levels were significantly lower in PAF -/- mice than in wild-type mice (121).

Other innate immological factors

Tables 3, 4 and Figures 6, 7 and 8 summarize a list of other factors that have been involved in the host innate immunological response in pneumococcal pneumonia, including the main described function and the corresponding reference.

Factors	Described functions	Reference number
CD38 (ADP-ribosyl cyclase)	Control of neutrophil chemotaxis to bacterial chemoattractants through its production of cyclic ADP-ribose.	(130)
NETs (Neutrophil extracellular traps)	NETs are involved in host defense during pneumococcal pneumonia. Pneumococci are trapped but, unlike many other pathogens, not killed by NETs.	(131)
MARCO (Macrophage receptor with collagenous structure)	MARCO plays a role in mounting an efficient and appropriately regulated innate immune response against inhaled particles and airborne pathogens (murine model).	(132)
Leptin (adipocyte-derived hormone)	Reduced leptin levels substantially contribute to the suppression of pulmonary antibacterial host defence.	(133)
Fas/FasL system	Blockade of the Fas/FasL system by DcR3-a in the lungs improves clearance of bacteria in mice with pneumococcal pneumonia.	(134)
CD97	Defect in CD97 affects neutrophil migration in a murine model.	(135)
Alveolar macrophages	Clearance of bacteria from the lung during subclinical infection. AM apoptosis contributes to microbiological host defence against pneumococci.	(136)
Galectin-3 (beta-galactoside-binding lectin)	Involved in inflammatory responses as well as in cell adhesion. Plays a role in beta(2) integrin-independent neutrophil extravasation.	(137)

Table 3.- Other factors involved in host innate immunological response in pneumococcal pneumonia (I)

Factors	Described functions	Reference number
MRP1 (Multidrug resistance protein 1)	MRP1(-/-) mice are resistant against pneumococcal pneumonia by a mechanism that involves increased release of LTB(4).	(138)
Complement receptor 3 (CR3; CD18/CD11b)	Recognition and clearance of *Streptococcus pneumoniae* by neutrophils.	(139)
c-Jun-NH2-terminal kinase	*Streptococcus pneumoniae*-induced IL-8 expression by human epithelial BEAS-2B cells is dependent on activation of JNK and recruitment of phosphorylated c-Jun to the IL8 promoter.	(140)
NF-kappaB	RelA (a NF-kappaB subunit) deficiency decreased cytokine expression, alveolar neutrophil emigration, and lung bacterial killing. Killing of *Streptococcus pneumoniae* was also diminished in the lungs of mice expressing a dominant-negative form of IkappaB in airway epithelial cells, implicating this cell type as an important locus of NF-kappaB activation during pneumonia.	(141)
	The combined deficiency of TNF- and IL-1 signaling reduces innate immune responses to *Streptococcus pneumoniae* in the lungs, probably due to essential roles for these receptors in activating NF-kappaB.	(142)
TIRAP (**adaptor protein Mal**)	The Mal S180L variant attenuated TLR2 signal transduction. It seems to have a protective effect against invasive pneumococcal disease.	(143)
p38 MAPK	In pneumococci-infected human pulmonary epithelial BEAS-2B cells, p38 MAPK- and NF-kappaB-controlled COX-2 expression and subsequent PGE(2) release by lung epithelial cells may contribute significantly to the host response in pneumococcal pneumonia	(144)

Table 4.- Other factors involved in host innate immunological response in pneumococcal pneumonia (II)

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