

Biomarcadores na Pneumonia

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Tese para obtenção do grau de Doutor em Medicina

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Para a Beatriz e Madalena

“The beauty of a living thing is not the atoms that go into it,
but the way those atoms are put together.”

Carl Sagan

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AGRADECIMENTOS

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Por fim, à minha família que será sempre o meu melhor abrigo, nos bons momentos e nos menos bons.

LISTA DE ABREVIATURAS

- RNAr – Ácido ribonucleico ribossómico
- PAC – Pneumonia adquirida na comunidade
- PAH – Pneumonia adquirida no hospital
- PAV – Pneumonia associada ao ventilador
- TAV – Traqueobronquite associada ao ventilador
- PCR – Proteína C-reativa
- PCT – Procalcitonina
- COV – Compostos orgânicos voláteis
- DPOC – Doença pulmonar obstrutiva crónica
- IL – Interleucina
- RNAm – Ácido ribonucleico mensageiro
- UCI – Unidade de cuidados intensivos
- PaO₂ – Pressão parcial de oxigénio no sangue arterial
- FiO₂ – fração inspirada de oxigénio
- SOFA – Sequential Organ Failure Assessment
- SAMS – *Staphylococcus aureus* sensível à meticilina
- SAMR – *Staphylococcus aureus* resistente à meticilina
- CPIS – Clinical Pulmonary Infection Score
- sTREM – soluble triggering receptors expressed on myeloid cells-1
- G-CSF – Granulocyte colony-stimulating factor
- MIP – Macrophage inflammatory protein

RESUMO

Esta Tese de Doutoramento é apresentada de acordo com o Regulamento vigente do ciclo de estudos conducente ao grau de Doutor em Medicina da NOVA Medical School|Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Regulamento n.º 519/2015 publicado em Diário da República, 2ª série, Nº 153, a 7 de agosto de 2015. A Tese é apresentada com trabalhos científicos alternativos à Tese de acordo com o Artigo 20º do referido Regulamento.

Os trabalhos científicos propostos são:

- **Artigo 1: Coelho L**, Póvoa P, Almeida E, Fernandes A, Mealha R, Moreira P, Sabino H. Usefulness of C-reactive protein in monitoring the severe community-acquired pneumonia clinical course. Crit Care. 2007;11(4):R92. doi:10.1186/cc6105. **Factor de impacto 6.880.**
- **Artigo 2: Coelho LM**, Salluh JI, Soares M, Bozza FA, Verdeal JC, Castro-Faria-Neto HC, Lapa e Silva JR, Bozza PT, Póvoa P. Patterns of c-reactive protein RATIO response in severe community-acquired pneumonia: a cohort study. Crit Care. 2012 Dec 12;16(2):R53. doi: 10.1186/cc11291. **Factor de impacto 6.880.**
- **Artigo 3:** Salluh JI, Soares M, Coelho LM, Bozza FA, Verdeal JC, Castro-Faria-Neto HC, e Silva JR, Bozza PT, Póvoa P. Impact of systemic corticosteroids on the clinical course and outcomes of patients with severe community-acquired pneumonia: a cohort study. J Crit Care. 2011 Apr;26(2):193-200. doi: 10.1016/j.jcrc.2010.07.014. **Factor de impacto 2.920.**
- **Artigo 4: Coelho L**, Rabello L, Salluh J, Martin-Loeches I, Rodriguez A, Nseir S, Gomes JA, Povoá P; TAVeM study Group. C-reactive protein and procalcitonin profile in ventilator-associated lower respiratory infections. J Crit Care. 2018 Oct 2;48:385-389. doi: 10.1016/j.jcrc.2018.09.036. **Factor de impacto 2.920.**
- **Artigo 5:** van Oort PM, Povoá P, Schnabel R, Dark P, Artigas A, Bergmans D, Felton T, Coelho L, Schultz MJ, Fowler SJ, Bos L. D. The potential role of exhaled breath analysis in the diagnostic process of pneumonia - a systematic review. Journal of

Breath Research 2018, **12(2):024001**. doi: 10.1088/1752-7163/aaa499. **Factor de impacto 3.000**.

De acordo com os critérios quantitativos e em cumprimento da metodologia de cálculo do Artigo 20º do Regulamento n.º 519/2015 de 7 de agosto, o total da soma dos artigos publicados é de 37.78 (Tabela 1).

Tabela 1: Somatório do Factor de Impacto dos Artigos Publicados

Revista	Factor de impacto	N	1º autor	Total
Critical Care	6.880	2	2	27.52
Journal of Critical Care	2.920	2	1	8.76
Journal of Breath Research	3.000	1	0	1.5
Total				37.78

Nota: Score calculado com base na soma dos factores de impacto dos artigos publicados de acordo com a seguinte metodologia: factor de impacto da revista duplicado nos trabalhos em que o doutorando é o primeiro autor; factor de impacto nos artigos com até 10 autores em que o doutorando não é o primeiro autor; nos artigos com mais de 10 autores adiciona-se o factor de impacto dividido por 2. O candidato deve conseguir, segundo a metodologia apresentada, um score de soma de factores de impacto ≥ 20 , devendo obter um score mínimo de 10 como 1.º autor.

Esta Tese de Doutoramento teve por objetivo estudar o papel dos biomarcadores na pneumonia adquirida na comunidade e na pneumonia adquirida no hospital.

ABSTRACT

This doctoral thesis is presented according to the current regulation of the cycle of studies leading to the Doctorate degree of NOVA Medical School | Faculty of Medical Sciences of the New University of Lisbon number 519/2015 published in Diário da República, 2nd series, N^o. 153, August 7. The thesis is presented in accordance with article number 20th with alternative scientific published articles.

The proposed scientific articles are:

- **Article 1:** Coelho L, Póvoa P, Almeida E, Fernandes A, Mealha R, Moreira P, and Sabino H. Usefulness of C-reactive protein in monitoring the severe community-acquired pneumonia clinical course. Crit Care. 2007;11(4):R92. doi:10.1186/cc6105.
Impact factor 6.547
- **Article 2:** Coelho LM, Salluh JI, Soares M, Bozza FA, Verdeal JC, Castro-Faria-Neto HC, Lapa e Silva JR, Bozza PT, Póvoa P. Patterns of C-reactive protein RATIO response in severe community-acquired pneumonia: a cohort study. Crit Care. 2012 Dec 12;16(2):R53. doi: 10.1186/cc11291. **Impact factor 6.547**
- **Article 3:** Salluh JI, Soares M, Coelho LM, Bozza FA, Verdeal JC, Castro-Faria-Neto HC, e Silva JR, Bozza PT, Póvoa P. Impact of systemic corticosteroids on the clinical course and outcomes of patients with severe community-acquired pneumonia: a cohort study. J Crit Care. 2011 Apr;26(2):193-200. doi: 10.1016/j.jcrc.2010.07.014.
Impact factor 2.855
- **Article 4:** Coelho L, Rabello L, Salluh J, Martin-Loeches I, Rodriguez A, Nseir S, Gomes JA, Povoá P; TAVeM study Group. C-reactive protein and procalcitonin profile in ventilator-associated lower respiratory infections. J Crit Care. 2018 Oct 2;48:385-389. doi: 10.1016/j.jcrc.2018.09.036. **Impact factor 2.855**
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Breath Research 2018, **12**(2):024001. doi: 10.1088/1752-7163/aaa499. **Impact factor 3.000.**

According to the quantitative criteria, the total sum of articles published using the methodology cited in article 20th has the total punctuation of 37.78 (Table 1).

Table 1: Total Punctuation of Impact factor of Published Articles

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Critical Care	6.880	2	2	27.52
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Total				37.78

Note: Score calculated based on the sum of the impact factors of articles published according to the following methodology: duplicate journal impact factor in papers in which the doctoral student is the first author; factor of impact in papers with up to 10 authors in which the doctorate is not the first author; in papers with more than 10 authors, the impact factor divided by 2 is added. According to the presented methodology, the candidate must score a sum of impact factors ≥ 20 and must obtain a minimum score of 10 as first author.

The aim of this doctoral thesis was to study the role of biomarkers in community-acquired pneumonia and hospital-acquired pneumonia.

1. PREÂMBULO

O pulmão foi considerado durante muito tempo um órgão estéril. Por isso, a identificação de agentes microbianos nos produtos biológicos, em particular se profundos, eram considerados sugestivos da presença de infecção ou, mais raramente, sobretudo em doentes com doença respiratória crónica estrutural, colonização das vias respiratórias. No entanto, ao contrário do que se acreditava, com o recurso a técnicas de biologia molecular, como por exemplo a identificação e sequenciação de regiões variáveis do gene 16S que codifica o RNAr bacteriano, foi possível verificar que o pulmão não é um órgão estéril, sendo 'habitado' por um conjunto diversificado de microorganismos comensais que interagem com o hospedeiro e entre si, e modulam a resposta imunitária [1].

A pneumonia, a mais grave e mais frequente infecção parenquimatosa pulmonar, resulta do processo inflamatório de resposta à agressão do pulmão por um ou mais agentes microbiológicos (bactérias, vírus, fungos, etc.). Do ponto de vista anatomo-patológico, a pneumonia caracteriza-se por preenchimento alveolar por líquido intersticial rico em proteínas e mediadores inflamatórios, e pelas células inflamatórias envolvidas na resposta do hospedeiro à infecção, conseqüente a um marcado aumento da permeabilidade do capilar pulmonar.

Ainda que este conceito histológico seja considerado o '*gold standard*' para diagnosticar infecção, na prática clínica o diagnóstico assenta nas manifestações clínicas e laboratoriais, e na demonstração da presença de um infiltrado pulmonar na radiografia do tórax [2]. A identificação do agente microbiológico (bactéria, vírus ou fungo) é fundamental para decidir o tratamento antimicrobiano dirigido. No entanto, não é essencial para o estabelecimento do diagnóstico da infecção, ou para decidir qual a terapêutica empírica inicial.

A pneumonia classifica-se consoante o local onde a infecção é adquirida. Designa-se pneumonia adquirida na comunidade (PAC) quando a infecção é adquirida fora do hospital, ou se manifesta nos doentes internados antes das primeiras 48 horas de internamento [2]. Por outro lado, a pneumonia que ocorre depois do segundo dia

de internamento hospitalar, e que não se encontrava em incubação na altura da admissão, é designada pneumonia adquirida no hospital (PAH). A pneumonia que se desenvolve 48-72 horas após a intubação orotraqueal é designada pneumonia associada ao ventilador (PAV) [3], a qual constitui um subtipo da PAH. Quando o doente apresenta um quadro clínico compatível com PAV, mas a radiografia do tórax não apresenta qualquer infiltrado pulmonar *de novo*, classificamos como sendo uma traqueobronquite associada ao ventilador (TAV) [4].

As manifestações clínicas da pneumonia incluem sintomas inespecíficos como a febre, as mialgias e os calafrios, e sintomas mais específicos do atingimento pulmonar tais como a tosse, a expectoração purulenta, a dor torácica tipo pleurítico e a dispneia. Os sinais clássicos de consolidação pulmonar (macicez na percussão, crepitações, aumento da transmissão das vibrações vocais) observam-se em apenas 33% dos doentes internados com PAC confirmada radiologicamente, e em 5-10% dos doentes tratados em ambulatório [5].

O diagnóstico da pneumonia tem-se tornado progressivamente mais difícil. As causas mais prováveis para esta dificuldade crescente são o aumento do número de doentes com doença pulmonar estrutural prévia, com comorbilidades associadas, institucionalizados e imunocomprometidos, associado a um aumento da diversidade de agentes microbiológicos causadores de infecção, e a uma prevalência crescente de organismos multirresistentes [5]. Hoje em dia, é claro que não existem manifestações clínicas ou alterações do exame físico isoladas ou em combinação, capazes de confirmar ou excluir definitivamente o diagnóstico de pneumonia [6]. Apesar da radiografia do tórax ser fundamental no diagnóstico clínico, esta pode não apresentar alterações em cerca de um terço dos doentes [7], nomeadamente nas fases iniciais da doença.

É sabido que a rentabilidade das amostras microbiológicas na pneumonia é baixa. Os exames bacteriológicos da expectoração são frequentemente contaminados com flora da orofaringe e, nos doentes intubados orotraquealmente, apesar de ser mais fácil a obtenção de amostras com melhor qualidade (ex: aspirado traqueal, lavado bronco-alveolar), muitos já se encontram colonizados, sendo mais difícil distinguir

infecção de colonização [8-10]. Além disso, os exames microbiológicos tradicionais não identificam outros agentes considerados menos comuns, como os vírus ou fungos [11, 12].

Outros exames imagiológicos, como a tomografia computadorizada e a ecografia torácicas, podem melhorar a sensibilidade nos casos em que os aspetos observados na radiografia do tórax não são conclusivos, ou na deteção de complicações pulmonares como o empiema ou o abscesso pulmonar [5].

Para ultrapassar estas limitações, diversos biomarcadores têm sido avaliados no sentido de complementar a avaliação clínica e radiológica. Destes, a proteína C-reactiva (PCR) e a procalcitonina (PCT) são os mais estudados e os que se têm mostrado mais úteis na identificação de doentes com infecção, na estratificação da gravidade da pneumonia, e na monitorização da evolução da resposta ao tratamento antibiótico [13].

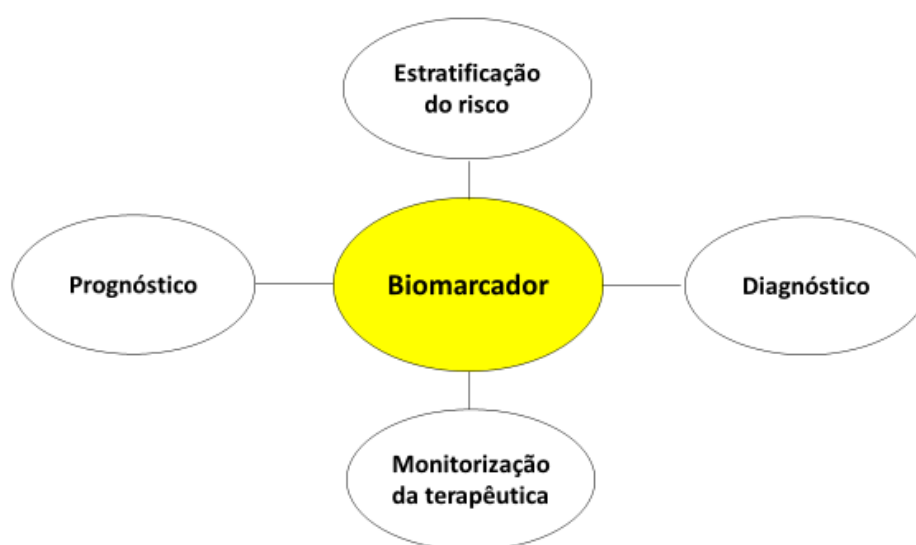
Esta Tese tem como objectivo principal a análise do comportamento destes dois biomarcadores, PCR e PCT, na PAC e na PAV. Na PAC foram analisadas as variações absolutas e relativas da PCR após o início da terapêutica antibiótica, assim como o impacto da terapêutica concomitante com corticóides sistémicos nos valores séricos da PCR durante o tratamento [14-16]. Na PAV foi avaliada a capacidade da PCR e da PCT para distinguirem duas infecções diferentes, a PAV e a TAV [17]. Adicionalmente, foi também realizada uma revisão sistemática sobre o potencial papel dos compostos orgânicos voláteis (COV) no diagnóstico da pneumonia [18].

2. BIOMARCADORES

2.1 Introdução

Um biomarcador é uma molécula biológica medida objectivamente, com exactidão e reprodutibilidade aceitáveis, usada como indicador de um processo fisiológico ou patológico. Numa infecção grave, como a pneumonia, o biomarcador pode ser usado para identificar um grupo de indivíduos com alto risco ou predisposição para a infecção, como marcador de diagnóstico da infecção, para estratificar a gravidade da infecção ou para monitorizar a resposta ao tratamento antibiótico (Figura 1) [19, 20].

Figura 1: Utilidade do Biomarcador



O biomarcador ideal na pneumonia deveria apresentar as seguintes propriedades: 1) fácil de usar e interpretar; 2) ser um teste rápido e reprodutível; 3) ser dinâmico com subidas e descidas rápidas; 4) ter elevada sensibilidade e especificidade, fácil de medir e interpretar; 5) permitir um melhor e mais rápido diagnóstico; 6) não ser modificado por outros tratamentos ou intervenções não dirigidos à infecção (que não sejam a antibioterapia e o controlo do foco infeccioso); 7) permitir a diferenciação entre infecções virais e bacterianas; 8) ter uma boa correlação com a gravidade clínica e a mortalidade; 9) antecipar o diagnóstico da infecção; 10)

apresentar uma grande amplitude de variação; 11) não apresentar um comportamento de exaustão ou fadiga, isto é, numa infeção prolongada ou em infeções sucessivas os seus níveis devem permanecer elevados e responderem sempre aos estímulos infecciosos; e 12) ter baixo custo e com resultados disponíveis rapidamente [21-23].

Tabela 2: Características de um Biomarcador de Infeção Ideal

Critério	Característica
Validação Analítica	<p>Acessível na rotina diária</p> <p>Reprodutível</p> <p>Preciso</p> <p>Boa relação custo-eficácia</p>
Qualificação	<p>Boa sensibilidade e especificidade</p> <p>Valores preditivos elevados</p> <p>Boa correlação com:</p> <ul style="list-style-type: none"> • gravidade da apresentação clínica • disfunção orgânica • controlo do foco • terapêutica antibiótica <p>Preditor da mortalidade</p>
Utilização	<p>Fácil de interpretar</p> <p>Objetivo</p> <p>Dinâmico / Cinética rápida</p> <p>Cinética independente da disfunção de órgão e das terapêuticas concomitantes (ex: corticóides, diálise)</p> <p>Cinética afetada apenas pela antibioterapia dirigida à infeção</p> <p>Minimamente ou não invasivo</p> <p>Variável contínua</p>

Não nos podemos esquecer que as manifestações clínicas típicas da pneumonia podem ser alteradas pela administração prévia de antibióticos, pela administração de outros fármacos (antipiréticos, corticosteroides) ou pelo estado imunitário do doente. Por vezes, a infecção pode iniciar-se e evoluir com poucos sintomas ou imitar uma outra situação clínica dificultando o diagnóstico [24, 25], em particular se desencadeadora de uma agudização de uma doença crónica como a insuficiência cardíaca congestiva ou a doença pulmonar obstrutiva crónica (DPOC). Se as variações do biomarcador dependerem apenas da reacção inflamatória associada à infecção, a informação que se obtém pode ser determinante na confirmação ou exclusão do diagnóstico da infecção, e ajudar na decisão de iniciar ou não a antibioterapia. Por outro lado, após o início do tratamento antibiótico, em muitos casos as melhorias clínica e radiológica são tardias, pelo que, se a antibioterapia for adequada e estiver associada a melhoria clínica, então as concentrações do biomarcador devem diminuir, e a sua monitorização seriada pode ser utilizada para avaliar a resposta clínica e orientar a duração do tratamento. Por outro lado, a persistência de valores elevados do biomarcador, pode ser indicador da ausência de resposta ao antibiótico, seja por inadequação da antibioterapia empírica, seja pelo desenvolvimento de uma complicação (ex: abscesso pulmonar, empiema) [26].

2.2 Proteína C-reactiva

A PCR sérica é uma proteína de fase aguda, sintetizada exclusivamente pelo fígado, sob regulação da interleucina-6 (IL-6) [27]. A concentração sérica da PCR nos indivíduos saudáveis tem uma mediana de 0.08 mg/dL e é inferior a 1 mg/dL em 99% das amostras normais. A secreção da PCR começa 4-6 horas após o estímulo inflamatório, duplica a cada 8 horas e atinge o pico em 36-50 horas. Com um estímulo muito intenso, a concentração da PCR pode subir acima de 50 mg/dL, ou seja, mais de mil vezes o valor de referência. Após o desaparecimento ou a remoção do estímulo, a sua concentração diminui rapidamente com uma semi-vida de 19 horas. A PCR pode permanecer elevada, mesmo por longos períodos, se o estímulo inflamatório persistir.

A sua concentração sérica está dependente apenas da intensidade do estímulo inflamatório e da velocidade de síntese hepática [21, 28].

O nível da PCR é independente da patologia subjacente e da gravidade da doença, das falências orgânicas presentes ou dos tratamentos de suporte instituídos (ex: ventilação mecânica, técnicas de depuração renal), sendo a única exceção a insuficiência hepática aguda grave, na qual não tem subida significativa devido à falência da sua síntese pelo fígado [29]. Apenas as intervenções com efeito sobre o processo inflamatório responsável pela sua elevação vão modificar o nível da PCR [21].

Para além da infecção, existem situações não infecciosas que, com frequência provocam modificações significativas da concentração da PCR, como o trauma grave, intervenções cirúrgicas, queimaduras, doenças auto-imunes (ex: artrite reumatóide) ou neoplasias [27]. Pequenas variações das concentrações da PCR podem também observar-se em situações como o exercício físico intenso, o golpe de calor ou o enfarte agudo do miocárdio [30].

A PCR é um marcador inflamatório de fase aguda inespecífico, pelo que o seu valor pode ser influenciado por múltiplos factores. Assim, as características gerais de um indivíduo, como a idade, o género ou o seu polimorfismo genético, podem influenciar a concentração sérica basal de PCR [30, 31]. Os doentes críticos são frequentemente submetidos a procedimentos invasivos, como sejam a colocação de catéteres venosos centrais ou a ventilação mecânica, associados a falências orgânicas ou a comorbilidades, apresentando por isso uma linha de base ou valor “normal” de PCR geralmente mais elevado quando comparado ao da população geral saudável [27, 31-33]. No entanto, o diagnóstico de todos os estímulos não infecciosos (como trauma, procedimento cirúrgico ou infarto do miocárdio) é habitualmente simples e geralmente não apresenta um desafio diagnóstico. Pelo contrário, a infecção é mais difícil de diagnosticar, pode aparecer silenciosamente ou imitar outra situação clínica.

A PCR apresenta grande amplitude de variação, pelo que é facilmente detectada por métodos laboratoriais de rotina, sendo possível identificar um limiar aproximado para o diagnóstico de inflamação aguda, principalmente infecção. Por outro lado, a avaliação das variações absolutas ou do ‘*slope*’ da PCR, antes do dia de

diagnóstico clínico de infecção, tem sido repetidamente demonstrado que é útil na identificação mais precoce dos doentes em risco de desenvolver uma infecção [34-36].

Diversos trabalhos têm mostrado que o estudo das variações relativas da PCR pode ser mais informativa do que a observação do seu nível absoluto diário na avaliação da resposta ao tratamento antibiótico. O conceito de PCR-ratio foi pela primeira vez descrita pelo nosso grupo e consiste na razão entre a concentração diária de PCR e a sua concentração inicial (medida no dia do diagnóstico da infecção e início da antibioterapia). Como demonstrámos nos nossos trabalhos, a PCR-ratio correlaciona-se com a resolução da infecção, e é mais informativa na monitorização diária das variações deste biomarcador do que a avaliação das variações absolutas [15, 37].

2.3 Procalcitonina

A PCT é uma proteína funcional imunomoduladora com várias funções. A sua existência foi demonstrada nos anos 80 a partir de células de carcinoma medular, onde se mostrou que a calcitonina tinha uma molécula precursora [38]. Nos indivíduos saudáveis, a PCT é produzida nas células C da glândula tiroideia a partir de um gene no cromossoma 11. O produto do RNAm é designado preprocalcitonina e posteriormente modificada para a PCT com 116 aminoácidos. Esta molécula é clivada em 3 moléculas distintas: a calcitonina activa, a katalcalcitonina e a N-terminal PCT [39]. Praticamente, toda a PCT produzida nas células C da glândula tiroideia é convertida em calcitonina, pelo que a quantidade libertada para a circulação sanguínea é mínima. Assim, o nível de PCT nos indivíduos saudáveis é muito baixa (<0.1 ng/mL). Após um estímulo inflamatório, a PCT é produzida por 2 mecanismos alternativos: (1) por via directa induzida por lipopolissacáridos ou outros metabolitos tóxicos dos microorganismos; e (2) por via indirecta, induzida por vários mediadores inflamatórios com a IL-6 e o TNF- α . Após a instalação duma infecção bacteriana os níveis de PCT sobem rapidamente atingindo o pico às 6-24 horas e tem uma semi-vida de 22 a 35 horas [40].

A PCT tem demonstrado utilidade como biomarcador diagnóstico na sépsis. A semi-vida plasmática da PCT e a boa correlação com a intensidade do estímulo

inflamatório, fazem com que se correlacione bem com a gravidade da reacção inflamatória e que a sua medição diária seja adequada na monitorização destes processos. Pode fornecer informação sobre a evolução da doença, o sucesso da terapêutica ou o prognóstico. Nas infecções, especialmente bacterianas, a monitorização diária dos seus valores pode fornecer indicações importantes sobre a necessidade, a duração e a eficácia da terapêutica antibiótica.

Na tabela 3 resumem-se as características principais de ambos os biomarcadores.

Tabela 3: Principais Características, Vantagens e Limitações da Proteína C-reativa e da Procalcitonina

	PROCALCITONINA	PROTEÍNA C-REACTIVA
Propriedades	Hormonoquina	Proteína de fase aguda (pentraxina)
Valores normais	<1 ng/mL	0.08 mg/dL (mediana)
Pico	>100 ng/mL (>10.000x valor de referência)	>50mg/dL (>1000x valor de referência)
Biologia		
Local de produção	Todas as células	Fígado
Tempo de subida após estímulo	3-4 horas	4-6 horas
Tempo de duplicação		8 horas
Concentração pico	Cerca de 24 horas	36-50 horas
Semi-vida	22-35 horas	19 horas
Factores Modificadores		
Corticóides	Falsos negativos frequentes	Sem efeito
Imunossupressão	Falsos negativos frequentes	Sem efeito
Neutropenia	Falsos negativos frequentes	Sem efeito
Insuficiência renal	↑↑	Sem efeito
Terapêutica de substituição renal	↓↓	Sem efeito
Insuficiência hepática crónica	Sem efeito	↓ (70% do normal)
Insuficiência hepática aguda	Sem efeito	PCR não aumenta
Efeito do cancro e outras doenças	↑↑ no carcinoma medular da tiróide e no cancro do pulmão de pequenas células	Sem modificação no Lupus eritamatoso sistémico, esclerose sistémica, dermatomiosite, doença de Sjögren, colite ulcerosa, leucemia, doença enxerto vs hospedeiro
Infeção secundária (2º episódio)	↓↓ (10% do 1º episódio)	↓ (70% do 1º episódio)
Infeções bacterianas vs virais	Fraco	Fraco

3. PNEUMONIA ADQUIRIDA NA COMUNIDADE

3.1 Introdução

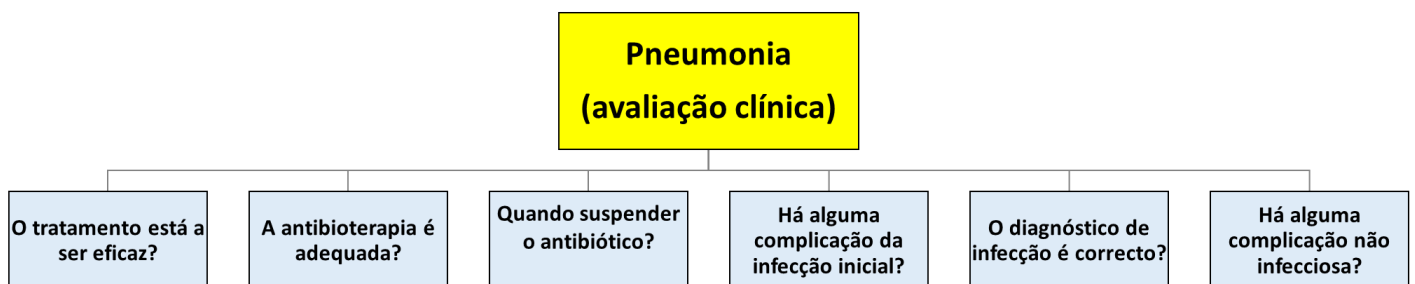
A PAC continua a ser uma doença frequente e grave, com uma incidência estimada na Europa e Estados Unidos da América em 2-12 casos/1,000 habitantes/ano [41, 42]. A maioria dos casos de pneumonia são tratados em regime ambulatorio. No entanto, cerca de 20% necessita de internamento hospitalar e aproximadamente 10% evolui para PAC grave [43], necessitando de tratamento numa unidade de cuidados intensivos (UCI). Nos doentes que necessitam de admissão em UCI, a morbilidade e mortalidade permanecem elevadas, apesar dos avanços nas medidas de suporte vital e na melhoria da adesão às normas de tratamento clinico da pneumonia e da sépsis [2, 44]. Nos doentes internados em UCI, mais de 50% necessitam de ventilação mecânica e apresentam choque séptico concomitante [8, 45, 46]. Estes doentes, em algumas casuísticas, podem apresentar uma taxa de mortalidade superior a 50% [41, 47]. O maior número de mortes ocorre nos primeiros dias de internamento hospitalar [48], de modo que a identificação precoce dos doentes com PAC grave é fundamental para a rápida instituição da terapêutica antibiótica e implementação das medidas de suporte de órgão adequadas.

No entanto, a necessidade do início precoce da antibioterapia, aumenta o risco de sobretratamento das pneumonias cuja etiologia não é bacteriana (viral ou fúngica). Também muitos doentes apresentam alterações na radiografia do tórax que imitam as alterações radiológicas observadas na pneumonia, mas correspondem a doenças pulmonares não infecciosas ou a doença pulmonar estrutural prévia. O início de antibioterapia nestes casos é desnecessária e tem sido um dos principais factores de aumento da pressão antibiótica sobre as bactérias, resultando no aparecimento de organismos multirresistentes como o *Staphylococcus aureus* resistente à metilina, as enterobactereaceas produtoras de beta lactamases de espectro alargado ou de carbapenemases, e de superinfecções fúngicas [2, 49].

Após o diagnóstico da pneumonia e o início da antibioterapia, habitualmente a partir do terceiro dia (D3) de tratamento, colocam-se várias questões na monitorização do doente com infecção grave (Figura 2):

1. Se o tratamento está a ser eficaz.
2. Quando é que a infecção está definitivamente tratada e é seguro suspender a antibioterapia.
3. Se a evolução clínica não é a esperada, se isso se deve a uma complicação da infecção inicial (ex: empiema, abscesso pulmonar), a uma nova infecção (noutro foco) ou a antibioterapia inicial inadequada.
4. Se o doente apresenta uma complicação não infecciosa (ex: insuficiência cardíaca, cardiopatia isquémica) que influencia negativamente a evolução clínica do doente e apresenta manifestações clínicas difíceis de distinguir da infecção inicial.
5. Se o diagnóstico de infecção estava errado.

Figura 2: Avaliação Clínica da Pneumonia



A maior parte dos parâmetros clínico-laboratoriais (temperatura, frequência respiratória, glóbulos brancos, radiografia do tórax) usados na avaliação da resposta clínica [13] tem uma evolução irregular ou lenta, e são muitas vezes influenciados por factores que não estão directamente relacionados com a infecção em curso. Por exemplo, a radiografia do tórax, fundamental no diagnóstico da PAC, tem um papel muito limitado na avaliação da resposta clínica à antibioterapia, uma vez que em muitos casos se observa uma deterioração inicial do padrão radiológico, e num grande número de casos a resolução das alterações imagiológicas é tardia [50]. Por outro lado, muitos dos fármacos utilizados no tratamento destes doentes, podem influenciar quase todos os parâmetros de avaliação da melhoria ou deterioração clínica. Os mais frequentemente utilizados são os corticóides, os antipiréticos e os bloqueadores beta adrenérgicos. Assim, a utilização destes marcadores clínico-laboratoriais torna a avaliação da resposta à antibioterapia pouco fiável [21].

3.2 Biomarcadores na Pneumonia Adquirida na Comunidade Grave

Estima-se que aproximadamente 10-25% dos doentes com PAC não têm uma evolução clínica adequada no tempo previsto. O insucesso terapêutico pode resultar de uma falta de resposta por parte do hospedeiro, ou do desenvolvimento de uma complicação infecciosa local, tais como um empiema ou um abscesso pulmonar [26, 51]. Além disso, a falência do tratamento pode ser erradamente presumida quando, por exemplo, as alterações radiológicas observadas têm uma resolução lenta, ou o doente tem um outro problema associado, tal como a febre associada a fármacos, um tumor maligno, outra patologia inflamatória, insuficiência cardíaca, ou uma infecção hospitalar a partir de outro foco. Nestes casos, é muito difícil identificar qual a causa da falência do tratamento, uma vez que a avaliação clínica e radiológica é insuficiente para diferenciar uma complicação infecciosa de uma complicação não infecciosa [47].

Nestas circunstâncias, a utilização de determinações sequenciais de biomarcadores para monitorizar a resposta ao tratamento antibiótico tem sido avaliada em vários estudos. Um dos biomarcadores mais frequentemente avaliado tem sido a PCR [13, 52, 53]. É hoje consensual que a monitorização diária da PCR pode

ajudar na diferenciação precoce dos doentes com má evolução clínica dos com boa evolução no decorrer do tratamento antibiótico.

Apesar de ser mais comum monitorizar a evolução dos valores absolutos da PCR, o nosso grupo mostrou que a monitorização diária da PCR-ratio, é mais informativa, uma vez que a PCR tem uma cinética de eliminação de primeira ordem [13, 27]. Este conceito é facilmente compreensível com o exemplo seguinte: uma diminuição absoluta de 5 mg/dL na concentração de PCR de um dia para outro tem uma interpretação diferente, se o nível anterior é de 50 mg/dL ou 10 mg/dL. No primeiro caso a concentração de PCR caiu 10%, enquanto no segundo diminuiu 50%. Uma diminuição acentuada e rápida da PCR-ratio é um marcador de resolução da infecção, enquanto que um valor persistentemente elevado ou um aumento secundário da PCR-ratio sugere que a infecção não está responder à antibioterapia [54].

Nos nossos estudos [14, 15], acompanhámos a resolução clínica da PAC grave após instituição da terapêutica antibiótica, avaliando de forma seriada a concentração plasmática da PCR, a temperatura corporal e a contagem de leucócitos, a fim de identificar os doentes com boa e má evolução clínica. A avaliação da PCR-ratio desde o dia 0 (D0), dia de início da terapêutica antibiótica, serviu não apenas para prever o prognóstico, mas para descrever a evolução clínica dos doentes com PAC. Do D0 ao D7, a PCR-ratio mostrou uma diminuição significativa e constante nos sobreviventes, enquanto que nos falecidos permaneceu elevada. Nos sobreviventes, ao D3 a PCR-ratio tinha diminuído quase 50% em relação à concentração inicial. Uma PCR-ratio $>0,5$ no D3 de antibioterapia está associada a má resposta à antibioterapia e má evolução clínica, com uma sensibilidade de 0,91 e uma especificidade de 0,55.

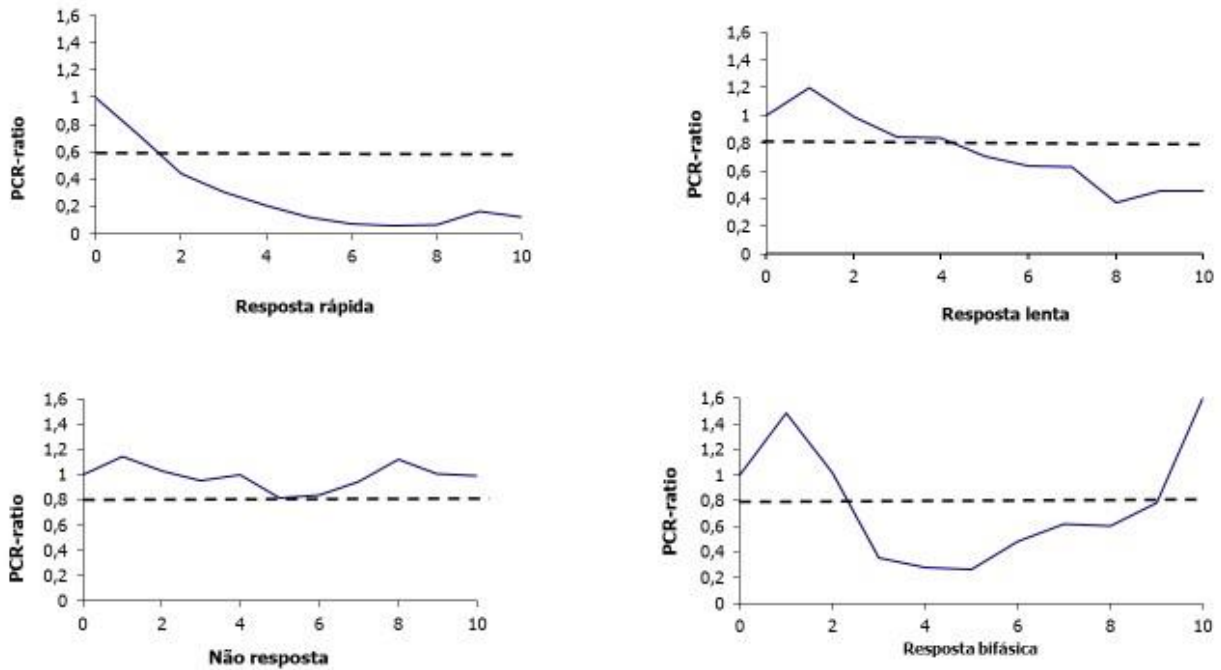
Em resumo, demonstrou-se que a medição diária de PCR, nomeadamente a avaliação da PCR-ratio, após a prescrição da terapêutica antibiótica na PAC grave, é útil na identificação precoce (entre D3-D4) dos doentes com boa e má evolução clínica.

3.3 Padrões de resposta da PCR-ratio na Pneumonia Adquirida na Comunidade Grave

As variações da PCR durante a evolução das infecções bacterianas, têm vindo a ser estudadas desde há vários anos por diferentes grupos, e em diferentes situações clínicas. Em 1986, Cox et al, descreveram 4 padrões básicos de resposta da PCR ao tratamento da infecção bacteriana [55]. As designações originais eram padrão de *infecção simples*, padrão de *infecção supurativa*, padrão de *infecção complicada* e padrão de *infecção recorrente*.

Baseados nesta classificação preliminar de Cox et al, e estudando as variações relativas da PCR, nós classificámos, de forma objectiva, quatro padrões de resposta individual da PCR-ratio à antibioterapia (Figura 3) [15, 54]:

1. Padrão de resposta rápida – quando a PCR no D4 de antibioterapia tem uma concentração inferior a 40% da concentração do D0;
2. Padrão de resposta lenta – caracterizada por uma diminuição contínua e lenta da PCR;
3. Padrão de não-resposta – quando a PCR permanece sempre igual ou superior a 80% da concentração da PCR do D0;
4. Padrão de resposta bifásica – caracterizada por uma diminuição inicial da PCR para níveis inferiores a 80% do valor de D0, seguido por um aumento secundário para valores iguais ou superiores a 80% da concentração do D0.

Figura 3: Padrões Individuais da PCR-ratio de Resposta à Antibioterapia

Na PAC grave, observámos que os doentes com os padrões de resposta rápida e lenta apresentaram mortalidade significativamente menor que a observada nos doentes com os padrões de não resposta e resposta bifásica [14, 15].

Assim, a identificação dos padrões de resposta da PCR-ratio, permite diferenciar precocemente os doentes com boa e má evolução, podendo na prática influenciar significativamente o processo de tomada de decisão clínica [37, 54]. Nos doentes com valores persistentemente elevados ou crescentes da PCR, ou seja, os padrões de não resposta ou resposta bifásica, podemos considerar que deve ser necessária uma reavaliação diagnóstica e da terapêutica, com o objectivo de evitar uma maior deterioração clínica. Devem procurar-se potenciais complicações infecciosas relacionadas ou não com a infecção primária, como por exemplo, um empiema, uma infecção intra-abdominal, uma bacteriémia associada a cateter ou uma antibioterapia inicial inadequada [26]. Por outro lado, os doentes com uma diminuição consistente da PCR-ratio, ou seja, os padrões de resposta rápida e lenta, têm

geralmente um tratamento antibiótico adequado, resolução da infecção e bom prognóstico.

Em conclusão, a identificação do padrão de resposta individual da PCR-ratio à antibioterapia reflecte a resposta da infecção, e além disso é útil na avaliação da adequação da terapêutica antibiótica, e pode servir como alerta para o aparecimento de complicações infecciosas [37, 54, 56].

4. CORTICOTERAPIA NA PNEUMONIA ADQUIRIDA NA COMUNIDADE GRAVE

A presença de comorbilidades e a intensidade da resposta inicial do sistema imunitário à infecção, parecem contribuir para aumentar a gravidade da pneumonia, com evolução em choque séptico e assim aumento da mortalidade [57, 58]. Vários mecanismos podem estar subjacentes a esta expressão da resposta imunitária do organismo, mediados por biomarcadores inflamatórios (IL-6) e anti-inflamatórios (IL-10) [59]. Esta resposta inflamatória intensa manifesta-se clinicamente sob a forma de disfunção orgânica, sendo o choque séptico e a síndrome de dificuldade respiratória aguda as suas expressões mais graves [60, 61].

Até agora, têm sido poucos os tratamentos adjuvantes que mostraram melhorar o prognóstico nos doentes com infecção grave. Na PAC, nos últimos anos, a utilização dos corticóides tem sido sugerida como sendo eficaz na melhoria da evolução clínica e do prognóstico dos doentes [62]. Em algumas infecções graves, como a pneumonia por *Pneumocystis jirovecii* em doentes com infecção pelo vírus da imunodeficiência humana, a corticoterapia sistémica demonstrou melhorar o prognóstico. No entanto, os resultados obtidos na PAC nem sempre têm sido concordantes. Entre os estudos randomizados [63-68], nenhum mostrou benefícios da corticoterapia na mortalidade. Apenas, as avaliações em meta-análises encontraram potencial benefício na mortalidade e apenas na PAC grave [69]. Os efeitos mais significativos foram a melhoria da relação da PaO_2/FiO_2 , resolução mais rápida da pneumonia e redução do tempo de internamento hospitalar.

No estudo apresentado na nossa tese [16], colocámos como hipóteses os corticóides sistémicos poderem reduzir a mortalidade dos doentes com PAC grave, e acelerarem a resolução da disfunção orgânica, do processo inflamatório sistémico e da insuficiência respiratória. Incluímos 111 doentes com PAC grave e necessidade de ventilação mecânica invasiva. No nosso estudo, a corticoterapia sistémica não apresentou qualquer impacto na mortalidade na UCI ou hospitalar, e não observámos diferenças na resolução das disfunções orgânicas avaliada pelo 'score' Sequential

Organ Failure Assessment (SOFA). Também a evolução da PCR na primeira semana de antibioterapia foi idêntica nos doentes com e sem corticoterapia. Esta observação é muito importante, pois demonstra que este biomarcador mantém a sua capacidade para monitorizar a resposta à antibioterapia mesmo nos doentes com PAC sob corticóides sistémicos.

5. PNEUMONIA ADQUIRIDA NO HOSPITAL

5.1 Introdução

A PAH é a segunda infecção nosocomial mais frequente e a principal causa de morte por infecção adquirida no hospital nos doentes críticos [3]. A sua incidência varia entre 5-20 casos por 1000 admissões hospitalares, sendo mais frequente nos doentes imunocomprometidos, nos doentes cirúrgicos e nos idosos. A mortalidade da PAH permanece elevada, podendo atingir os 70%, especialmente quando os doentes evoluem com um quadro clínico de sépsis ou choque séptico [3]. Em relação à PAV, estima-se que a sua incidência seja de 2-16 episódios por 1000 dias de ventilação [70]. A incidência da PAV tem vindo a diminuir ao longo dos últimos anos, provavelmente devido à implementação de medidas preventivas mais eficazes (ex: elevação da cabeceira do doente, redução do tempo de sedação, aspiração das secreções subglóticas) [71]. No entanto, a sua incidência é ainda elevada nos doentes politraumatizados e com traumatismo craneo-encefálico que, por serem causas de diminuição do nível de consciência, predis põem para a ocorrência de microaspirações na altura do traumatismo [4, 72]. A PAV tem um impacto económico muito importante por aumentar o tempo de internamento, e por isso também os custos associados aos cuidados de saúde [73]. As avaliações mais recentes da mortalidade atribuível estimam que atinja os 13% [74].

O risco de PAV não é constante durante todo o tempo da ventilação mecânica. Estima-se que o risco seja de 3% por dia durante os primeiros 5 dias de ventilação mecânica, 2% por dia entre o quinto e o décimo dia, e 1% por dia nos dias seguintes [75]. No entanto, estudos mais recentes têm mostrado que a maioria das PAV ocorre nos primeiros 7 dias de ventilação mecânica [4, 36]. Por outro lado, o tempo até ao aparecimento da PAV também afecta a etiologia microbiológica, o tratamento antimicrobiano empírico e o prognóstico [76]. A PAV pode, por isso, classificar-se quanto ao tempo de diagnóstico e de acordo com a duração da ventilação mecânica em precoce, quando se instala até ao 4º dia de ventilação, ou tardia, quando se instala

a partir do 5º dia de ventilação [77]. Na PAV precoce os microrganismos mais frequentemente envolvidos são o *Streptococcus pneumoniae*, o *Staphylococcus aureus* sensível à meticilina (SAMS), o *Haemophilus influenzae* e bacilos entéricos Gram negativo não pseudomônicos (*Enterobacter spp*, *E. coli*, *Klebsiella spp*, *Proteus spp*, *Serratia spp*). Na PAV tardia para além dos microrganismos isolados na PAV precoce, são também agentes frequentes microorganismos multirresistentes como a *Pseudomonas aeruginosa*, o *Acinetobacter spp* e o *Staphylococcus aureus* resistente à *meticilina* (SAMR).

5.2 Diagnóstico da Pneumonia Adquirida no Hospital

A suspeita de PAH ocorre quando um doente apresenta na radiografia do tórax um novo infiltrado pulmonar ou agravamento de infiltrados pulmonares prévios, associado a pelo menos duas das seguintes manifestações clínico-laboratoriais: febre, leucocitose ou leucopenia e alteração do volume/purulência das secreções brônquicas. A presença das manifestações clínicas sem as alterações na radiografia do tórax sugere a presença de traqueobronquite nosocomial [9, 10].

Nos doentes críticos, os sinais clínicos sugestivos de pneumonia são geralmente pouco específicos. Além disso, a radiografia do tórax é mais difícil de interpretar nestes doentes, porque a presença ou modificação dos infiltrados pulmonar pode ter múltiplas causas como o edema pulmonar, a contusão pulmonar, atelectasia ou sequelas de doença pulmonar prévia. Para melhorar a sensibilidade diagnóstica, podemos recorrer a escalas como o Clinical Pulmonary Infection Score (CPIS), baseados na avaliação clínica, manifestações radiológicas e avaliação do aspirado traqueal [78]. No entanto, a utilidade destas escalas permanece por definir, uma vez que não estão validadas para utilização generalizada, e apresentam limitações importantes, especialmente nos doentes que apresentam infiltrados pulmonares bilaterais.

Por outro lado, a presença de bactérias nas vias aéreas inferiores dos doentes intubados não é suficiente para diagnosticar infecção pulmonar, pois a sua árvore traqueobrônquica apresenta-se frequentemente colonizada por múltiplas estirpes bacterianas como, por exemplo, bacilos Gram negativo entéricos. As normas

publicadas mais recentemente recomendam a colheita de amostras das secreções respiratórias para culturas quantitativas ou qualitativas, de forma a poder usar-se os resultados microbiológicos para estreitar a antibioterapia sempre que possível [9, 10].

5.3 Biomarcadores na Pneumonia Adquirida no Hospital

A medição dos biomarcadores no dia do diagnóstico da PAH, ou nos dias que o antecedem, pode permitir um diagnóstico mais precoce e mais preciso desta infecção, em conjugação com a restante informação clínica, laboratorial, microbiológica e radiológica. Em vários estudos [34, 36, 79], tem-se demonstrado que as variações dos biomarcadores nos dias que antecedem o diagnóstico de infecção, podem ser úteis no diagnóstico precoce da PAH. A sua utilidade poderá não só limitar-se a fornecer informação sobre a existência de infecção, mas também a excluí-la de forma segura.

O papel dos biomarcadores no diagnóstico da PAH tem sido amplamente estudado, nomeadamente a PCR e a PCT. A PCT tem mostrado um desempenho bastante limitado [79-82], embora a sua utilização clínica seja crescente. Em vários estudos a PCR tem mostrado resultados muito promissores [36, 83] e ser segura mesmo nos doentes imunocomprometidos [84]. Outros biomarcadores como o soluble triggering receptors expressed on myeloid cells-1 (sTREM), a interleucina (IL)-1b, a IL-6, a IL-8, o granulocyte colony-stimulating factor (G-CSF) e a macrophage inflammatory protein (MIP)-1a [85-92], também foram avaliados em múltiplos estudos, mas nenhum deles mostrou sensibilidade ou especificidade suficientes para que a sua utilização isolada na prática clínica fosse considerada útil, não estando por isso recomendada pelas principais normas de tratamento da PAH das sociedades científicas europeias ou americanas [9, 10].

No nosso estudo [17], avaliámos pela primeira vez a capacidade de dois biomarcadores, a PCR e a PCT, para distinguir entre duas infecções nosocomiais com gravidade diferente, a PAV e a TAV. Incluímos 404 doentes com PAV e TAV microbiologicamente documentadas. Ambos os biomarcadores apresentaram valores significativamente mais elevados nos doentes com PAV, contudo com grande

sobreposição dos valores de ambos os biomarcadores nos dois tipos de infecção. Por isso, nem a PCR nem a PCT parecem ser úteis para distinguir estas duas infecções.

Também demonstrámos que os valores de PCR e PCT eram idênticos nos doentes com infecções por agentes Gram positivo e Gram negativo, por agentes multirresistentes e não multirresistentes e em diferentes agentes microbiológicos (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacteria spp* e *Acinetobacter baumannii*). Ou seja a avaliação destes biomarcadores não é útil na identificação do agente etiológico envolvido na TAV/PAV [17].

6. BIOMARCADORES DO AR EXPIRADO NO DIAGNÓSTICO DA PNEUMONIA

A principal função dos pulmões é realizar as trocas gasosas de oxigénio e dióxido de carbono. Estes dois gases, em conjunto com o azoto e o vapor de água, constituem a maior parte do volume de ar mobilizado no ciclo respiratório. No entanto, o ar expirado é constituído por muitas outras substâncias voláteis presentes em quantidades muito pequenas. Muitas destas moléculas são COV que podem resultar do metabolismo das células do pulmão, de bactérias presentes no pulmão, da resposta do hospedeiro à infecção ou, nos doentes com neoplasias do pulmão, resultado do metabolismo das células neoplásicas [93, 94].

A análise dos COV no ar expirado tem várias vantagens: o material de colheita é fácil de obter, o procedimento da colheita pode ser realizado as vezes que forem necessárias, a colheita pode ser feita facilmente à cabeceira do doente sem qualquer risco e os resultados das colheitas estão rapidamente disponíveis. Desta forma, a análise dos COV tem vindo a demonstrar utilidade no diagnóstico de várias doenças respiratórias [94].

No artigo 5 é realizada uma revisão sistemática para avaliar o valor potencial da análise dos COV no diagnóstico de pneumonia [18]. Foram analisados os estudos encontrados em pesquisa na *Medline* até 7 de Março de 2017 relacionados com os seguintes tópicos: 1) diagnóstico de pneumonia, 2) detecção do agente microbiológico específico causador da pneumonia, e 3) valor dos COV na monitorização da resposta ao tratamento antibiótico da pneumonia. Resultante desta pesquisa, foram identificados e analisados 18 artigos referentes a 13 estudos em humanos e 5 estudos em animais. A análise destes estudos mostrou que existe uma relação entre a presença de COV específicos no ar expirado e a existência de infecção respiratória, embora não se tenha encontrado ainda um teste suficientemente específico para afirmar o diagnóstico de pneumonia de forma inequívoca.

7. PUBLICAÇÕES

Neste capítulo apresentam-se os trabalhos científicos publicados propostos alternativos à tese (Artigo 20º, Regulamento n.º 519/2015 publicado em Diário da República, 2ª série, Nº 153, a 7 de agosto de 2015).

Artigo 1: Usefulness of C-reactive protein in monitoring the severe community-acquired pneumonia clinical course.

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Research

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Usefulness of C-reactive protein in monitoring the severe community-acquired pneumonia clinical course

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Abstract

Background The aim of the present study was to evaluate the C-reactive protein level, the body temperature and the white cell count in patients after prescription of antibiotics in order to describe the clinical resolution of severe community-acquired pneumonia.

Methods A cohort of 53 consecutive patients with severe community-acquired pneumonia was studied. The C-reactive protein levels, body temperature and white cell count were monitored daily.

Results By day 3 a C-reactive protein level 0.5 times the initial level was a marker of poor outcome (sensitivity, 0.91; specificity, 0.59). Patients were divided according to their C-reactive protein patterns of response to antibiotics, into fast response, slow response, nonresponse, and biphasic response. About 96% of patients with a C-reactive protein pattern of fast

response and 74% of patients with a slow response pattern survived, whereas those patients with the patterns of nonresponse and of biphasic response had a mortality rate of 100% and 33%, respectively ($P < 0.001$). On day 3 of antibiotic therapy, a decrease in C-reactive protein levels by 0.31 or more from the previous day's level was a marker of good prognosis (sensitivity, 0.75; specificity, 0.85).

Conclusion Daily C-reactive protein measurement after antibiotic prescription is useful in identification, as early as day 3, of severe community-acquired pneumonia patients with poor outcome. The identification of the C-reactive protein pattern of response to antibiotic therapy was useful in the recognition of the individual clinical course, either improving or worsening, as well as the rate of improvement, in patients with severe community-acquired pneumonia.

Introduction

Community-acquired pneumonia (CAP) remains a common and serious illness, with an estimated incidence of 2–12 cases/1,000 population per year [1]. The majority of cases are managed outside hospital, but approximately 20% require hospital admission. Out of this group of patients, around 10% develop severe CAP [2] requiring treatment in an intensive care unit (ICU) with a mortality rate exceeding 50% [1,3]. The largest numbers of deaths occur in the first few days of hospitalization [4], so the early recognition of patients with severe CAP not only aids in the early initiation of antibiotic therapy but also in adequate supportive care.

It has been estimated that approximately 10–25% of patients with CAP do not resolve within the anticipated time [5]. Treatment failure can result from a lack of response by the host or from the development of an infectious complication, such as postobstructive pneumonia, empyema, or lung abscess. In addition, treatment failure may be wrongly presumed when radiologic infiltrates are resolving slowly but the patient has a superimposed problem, such as drug fever, malignancy, inflammatory conditions, heart failure, or a hospital-acquired infection from another source [3]. In such clinical situations, it is very difficult to identify the cause of the presumed treatment failure, since clinical and radiological evaluation is insufficient to differentiate an infectious complication from a noninfectious complication. Some studies [6,7] evaluated the value of some

AUC = area under the curve; CAP = community-acquired pneumonia; CRP = C-reactive protein; FiO_2 = fractional inspired oxygen; ICU = intensive care unit; IL = interleukin; PaO_2 = arterial oxygen tension; SOFA = Sequential Organ Failure Assessment; WCC = white cell count.

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serum markers of infection, such as C-reactive protein (CRP) and interleukins, in monitoring the response to antibiotic treatment. In the present study we hypothesize that daily monitoring of plasma CRP can recognize patients with bad outcome and patients with good outcome early in the course of antibiotic treatment.

Plasma CRP is an acute phase-protein synthesized only by the liver largely under transcriptional control of IL-6 [8]. CRP levels rise rapidly in response to several inflammatory stimuli, bacterial infection being one of the most potent. The secretion of CRP begins within 4–6 hours of the stimulus, doubling every 8 hours, and peaking at 36–50 hours. After the disappearance of or removal of the stimulus, the CRP concentration decreases rapidly with a half-life of 19 hours [9].

The aim of the present study was to assess the value of serial CRP determinations after prescription of antibiotics in the evaluation of the resolution of severe CAP, in order to recognize, early in the clinical course, patients with good outcome and patients with bad outcome, as well as to identify the individual patterns of the CRP response to antibiotics.

Materials and methods

Study subjects

A prospective observational cohort study was conducted between November 2001 and December 2002 in the ICU of Garcia de Orta Hospital (Almada, Portugal). All patients who were aged ≥ 18 years and admitted for severe CAP were enrolled. The Ethics Committee of Garcia de Orta Hospital approved the study design; informed consent was waived as there was no need for additional blood samples.

Study design

The data collected included the admission diagnosis, the past medical history and vital signs. The CRP concentration, the body temperature, the white cell count (WCC), the Sequential Organ Failure Assessment (SOFA) score [10,11] and the PaO₂/FiO₂ ratio were recorded daily. After clinical CAP diagnosis, all patients received empirical antibiotic therapy according to the American Thoracic Society CAP guidelines [2].

For the purposes of time-dependent analysis, day 0 was defined as the day of CAP clinical diagnosis. The following days were successively defined as day 1, day 2, and so on.

Withdrawal of the inflammatory stimulus results in a sharp decrease in the serum CRP concentration, similar to first-order elimination kinetics [8]. As a result, time-dependent analysis of the relative CRP concentration (CRP ratio) was also performed. The CRP ratio was calculated in relation to the day 0 CRP concentration. The maximal relative CRP variation from the previous day's CRP level was also analysed.

Patients were followed-up until pneumonia was cured or until death. The progression of the CRP concentration, the CRP ratio, the body temperature and the WCC throughout the course of severe CAP was analysed, comparing survivors with nonsurvivors.

Definitions

Severe CAP was defined according American Thoracic Society guidelines [3]. Previous antibiotic treatment was defined as any antibiotic treatment in the week before ICU admission. Adequate antibiotic therapy was defined, in the empirical therapy prescribed by the onset of severe CAP, as at least one antibiotic covering all of the pathogens isolated, as determined by the sensitivity pattern in the antibiogram. In patients started with initially inadequate treatment, antibiotics were changed according to the pathogen isolated and according to antimicrobial susceptibility testing.

Patients were retrospectively classified according to previously defined CRP patterns of the response to antibiotic [12,13]: fast response occurred when the CRP ratio at day 4 was < 0.4 relative to the day 0 CRP; slow response was characterized by a continuous and slow decrease in the CRP ratio; nonresponse was when the CRP ratio remained ≥ 0.8 ; and biphasic response was characterized by an initial CRP ratio decrease to levels < 0.8 followed by a secondary rise to values ≥ 0.8 . CAP patients were retrospectively divided into four groups according to their pattern of CRP response.

Analysis

Continuous variables are presented as the mean \pm standard deviation, unless stated otherwise. The Shapiro–Wilk test was used for normality assessment. Comparisons between groups were performed using the parametric unpaired and paired *t*-test, or the nonparametric Mann–Whitney U-test and the Wilcoxon signed-rank test for continuous variables according to data distribution. The chi-squared test was used to carry out comparisons between categorical variables. Time-dependent analysis of different variables was performed via general linear model univariate repeated-measures analysis using a split-plot design approach.

Receiver-operating characteristic curves were drawn for the CRP ratio, the body temperature and the WCC on day 3 of antimicrobial therapy. The indicative accuracy of these variables at day 3 was assessed by calculation of the area under the curve (AUC), as described elsewhere [14]. In medical practice, a diagnostic test with an AUC < 0.75 is regarded as noncontributive [15]. Comparison of the AUC of two variables was performed using the method of Hanley and McNeil [16]. Results are reported with the 95% confidence interval. Significance was accepted at $P < 0.05$.

Table 1**Characteristics of the patient population with severe community-acquired pneumonia**

	Survivors (n = 40)	Nonsurvivors (n = 13)	P value
Age (years)	59.4 ± 14.8	61.1 ± 12.1	0.220
Sex (male/female)	31/9	8/5	0.257
C-reactive protein day 0 (mg/dl)	23.6 ± 18.4	23.9 ± 11.6	0.591
Acute Physiology, Age, and Chronic Health Evaluation II score	17.8 ± 5.7	26.1 ± 6	<0.001
Sequential Organ Failure Assessment score day 0	6.5 ± 2.5	9.7 ± 2.9	0.002

Data presented as the mean ± standard deviation.

Results

During the study period, 53 patients were admitted to the ICU with severe CAP. Of these 53 patients, 13 (24.5%) died in the ICU, all deaths occurring while patients were still on antibiotic treatment and were mechanically ventilated. Fourteen patients (26.4%) were already receiving empiric antibiotic treatment on ICU admission; all patients maintained the antibiotic treatment already prescribed. The microbiological diagnosis was established in 11 patients (21%). All patients with microbiological diagnosis had initial adequate antibiotic treatment; only one patient with initial adequate antibiotic therapy died. Five patients (9.4%) were on corticosteroid treatment on ICU admission for chronic obstructive pulmonary disease exacerbation. The demographic characteristics of the patients with severe CAP are presented in Table 1. On ICU admission, 91% of patients were already mechanically ventilated.

At day 0, the CRP concentration, the body temperature and the WCC of survivors and nonsurvivors were not significantly different: 23.6 ± 18.4 mg/dl versus 23.9 ± 11.6 mg/dl ($P = 0.591$) (Figure 1), 38.0 ± 0.75°C versus 37.9 ± 1.1°C ($P = 0.856$) and 16.2 ± 13.9 × 10³ cells/μl versus 13.9 ± 12.7 × 10³ cells/μl ($P = 0.227$), respectively. From day 0 to day 7 of antibiotic therapy, time-dependent analysis of the CRP ratio in survivors showed a more steady and significant decrease than that in nonsurvivors ($P = 0.039$) (Figure 2). Over the same time period, the body temperature decreased likewise in both groups ($P = 0.249$). Analysis of the WCC showed no differences between survivors and nonsurvivors ($P = 0.423$).

At day 3, the CRP ratio in survivors was 0.49 relative to the initial level ($P < 0.001$), whereas in nonsurvivors the CRP ratio remained elevated at 0.71 ($P = 0.002$). The AUC for the CRP ratio by day 3 was 0.76 (95% confidence interval = 0.61–0.87), whereas the AUCs of the WCC and the body temperature by day 3 were 0.45 (95% confidence interval = 0.25–0.65) and 0.44 (95% confidence interval = 0.24–0.64), respectively. The AUC of the CRP ratio by day 3 was significantly greater than that of the WCC and the body temperature ($P = 0.022$ and $P = 0.047$, respectively). A CRP ratio >0.5 of the day 0 concentration by day 3 was a marker of poor outcome, with a sensitivity of 0.91, a specificity of 0.55, a negative

predictive value of 0.95 and a positive predictive value of 0.4 (positive likelihood ratio, 6.05; negative likelihood ratio, 0.49).

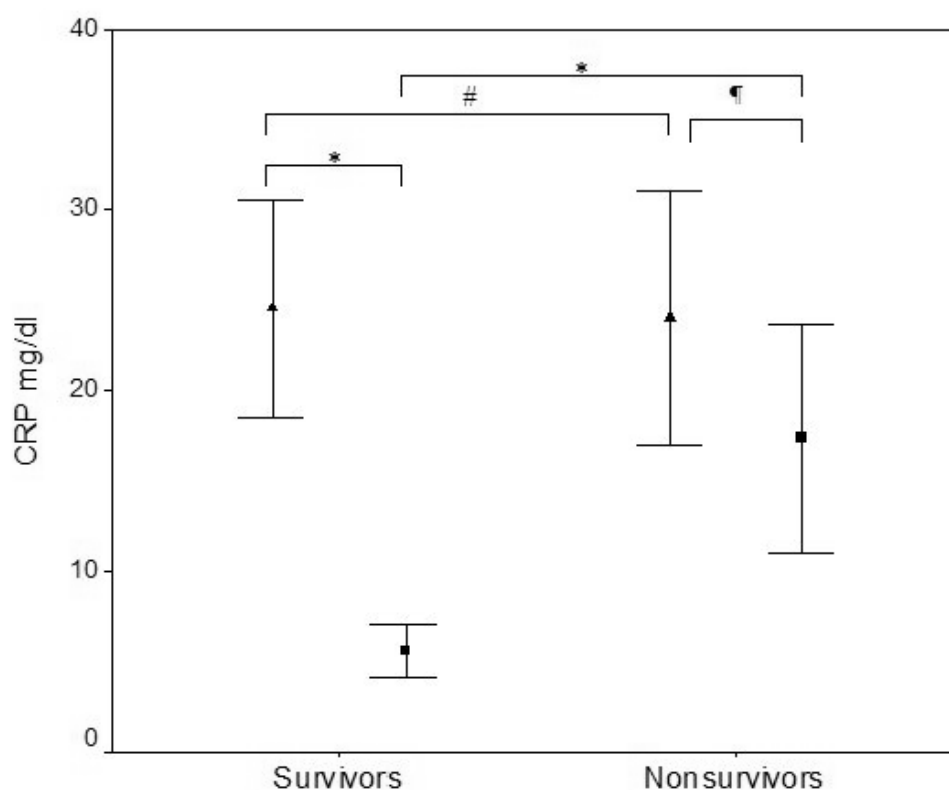
At the end of antibiotic therapy, the CRP concentration of survivors was 5.4 ± 4.2 mg/dl. In nonsurvivors, on the day of death the CRP concentration increased from the day 7 value, reaching 16.3 ± 8.8 mg/dl ($P < 0.001$). The body temperature at the end of antibiotic therapy in survivors was similar to that in nonsurvivors on the day of death (37.1 ± 0.9°C and 37.5 ± 0.7°C, respectively; $P = 0.60$) and the WCC was not significantly different (11.1 ± 5.0 × 10³ cells/μl versus 16.3 ± 9.8 × 10³ cells/μl, respectively; $P = 0.165$). Only survivors showed a significant decrease in body temperature ($P < 0.001$).

Patients with severe CAP were retrospectively divided according to four patterns of the CRP ratio course during antibiotic therapy. Twenty-two patients were classified as fast response, 23 patients as slow response, five patients as nonresponse and three patients as biphasic response. Time-dependent analysis of the CRP ratio of the four different patterns showed that these patterns of progression were significantly different ($P < 0.001$). By day 3, the CRP ratio was 0.31 ± 0.10, 1.30 ± 1.50, 0.90 ± 0.26 and 0.97 ± 0.27 in patients exhibiting a fast response, a slow response, nonresponse and a biphasic response pattern, respectively ($P < 0.001$). Conversely, during the same time period, no significant difference between the different patterns was found in the progression of the WCC and the body temperature ($P = 0.731$ and $P = 0.152$, respectively).

We then went on in our analysis to study the correlation between the CRP ratio patterns and the outcome. About 96% of patients with a CRP ratio pattern of fast response and 74% of patients with a slow response pattern survived, whereas those patients with the patterns of nonresponse and biphasic response exhibited overall mortality rates of 100% and 33%, respectively ($P < 0.001$). Together, the combined mortality rate of patients with these two latter patterns was 75%.

We analysed the maximal daily relative CRP concentration variation from the previous day's level between day 0 and the last day of antibiotic therapy. The receiver-operating characteristic curve AUC for maximal daily relative CRP variation was 0.76

Figure 1



C-reactive protein levels. C-reactive protein (CRP) levels on the day of antibiotic prescription (▲, day 0) and on the last day of antibiotic therapy in survivors or at death in nonsurvivors (■). Data presented as the mean \pm standard deviation. # $P = 0.591$. † $P = 0.021$. * $P < 0.001$.

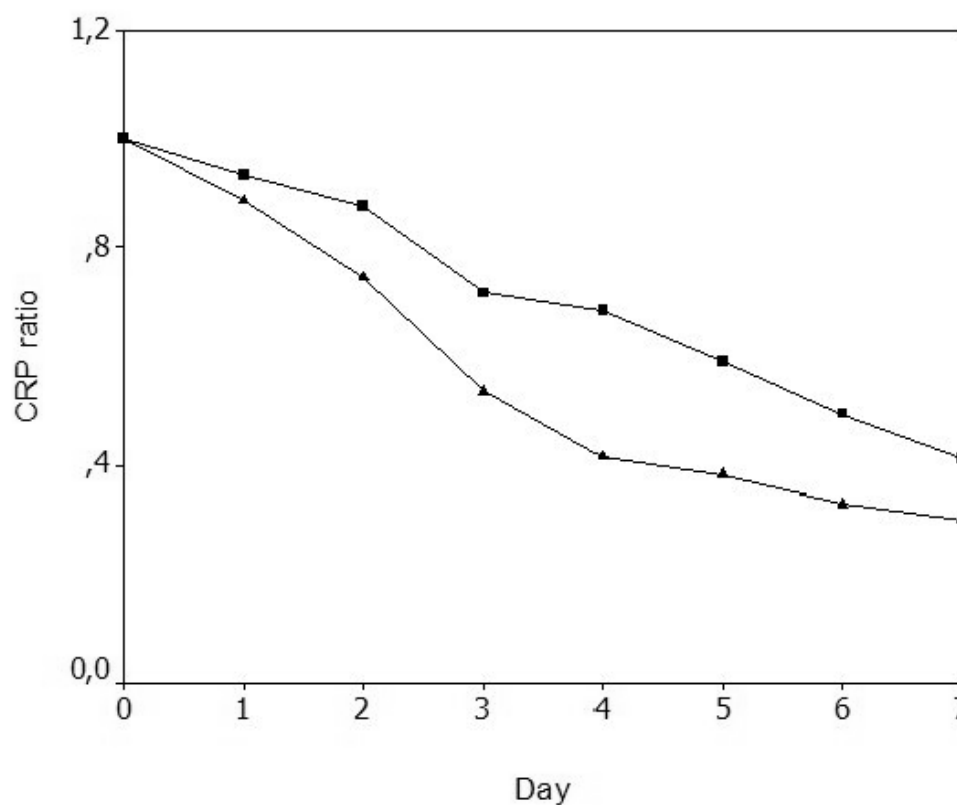
(95% confidence interval = 0.61–0.86) (Figure 3). A decrease in CRP levels by 0.31 or more from the previous day's concentration was a marker of good prognosis (sensitivity, 0.75; specificity, 0.85; positive likelihood ratio, 4.87; negative likelihood ratio, 0.30; negative predictive value, 0.92; positive predictive value, 0.61).

During antibiotic therapy, 29 out of 53 patients with severe CAP had, at least once, a relative CRP variation from the previous day's level ≥ 0.31 . Out of these 29 patients, 27 were survivors and two patients were nonsurvivors ($P = 0.001$); in

addition, in one-half of the patients this variation took place in the first 3 days of antibiotic therapy. By day 3, 90% of severe CAP patients with a fast response pattern had had at least one relative CRP variation from the previous day's level of 0.31 or more, whereas this was observed in only 60% of patients with a pattern of slow response.

Clinical progression during antibiotic therapy was monitored with daily measurement of the SOFA score and the $\text{PaO}_2/\text{FIO}_2$ ratio. The result of time-dependent analysis of the $\text{PaO}_2/\text{FIO}_2$ ratio from day 0 to day 7 of antibiotic therapy in survivors and

Figure 2



Time-dependent analysis of the C-reactive protein ratio during antibiotic therapy. Time-dependent analysis of the C-reactive protein (CRP) ratio during antibiotic therapy, from day 0 to day 7 of antibiotic therapy, was significantly different between survivors (▲) and nonsurvivors (■). $P = 0.039$.

nonsurvivors was not significantly different ($P = 0.339$). Moreover, the same analysis of the $\text{PaO}_2/\text{FiO}_2$ ratio for the four different CRP ratio patterns from day 0 to day 7 showed no significant differences between the patterns ($P = 0.229$).

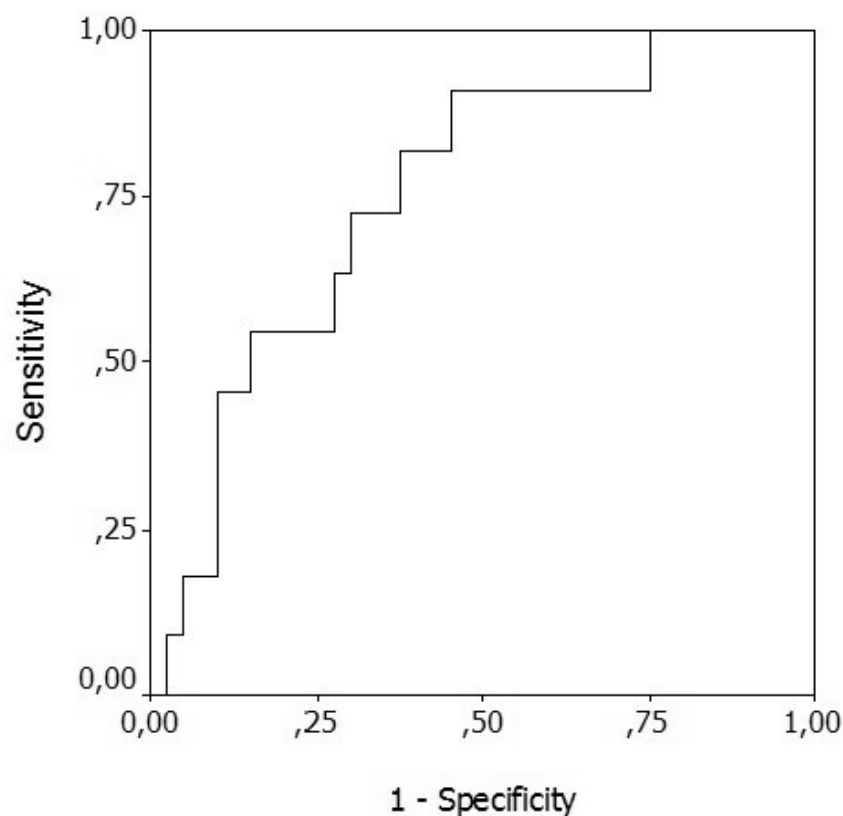
During the same period, the SOFA score progression between survivors and nonsurvivors was significantly different ($P = 0.013$). The assessment of the SOFA score progression according to the four different CRP ratio patterns, however, showed no differences ($P = 0.142$).

Discussion

In the present study, we monitored the clinical resolution of severe CAP after institution of antibiotic therapy assessed by serial measurements of the CRP concentration, the body temperature and the WCC, in order to identify, early in the clinical course, patients with good outcome and patients with bad outcome.

The evaluation of clinical resolution of CAP is presently based on the daily assessment of the same parameters used in diagnosis, namely X-ray scan, body temperature and WCC. Most of these parameters are unspecific, however, and can be

Figure 3



Maximal daily C-reactive protein variation. Receiver-operating characteristics curve of the maximal daily C-reactive protein variation from the level of the previous day. Area under the curve, 0.76 (95% confidence interval = 0.61–0.86).

influenced by factors not related to CAP itself. In addition, the radiological resolution often lags behind the clinical improvement from CAP, so it is not a useful tool to predict outcome [2,17,18].

The use of biomarkers to estimate the presence of an infection and its treatment response is not well studied in CAP patients. Several studies have shown that CRP is a good marker of CAP diagnosis, as well as useful for assessing its clinical severity [19-21]. Other markers, such as procalcitonin, have proved to be good predictors of complications and mortality [22].

Smith and colleagues studied 28 CAP patients after the prescription of antibiotics, from day 1 until day 5 of therapy, assessing the serial changes of the plasma CRP, tumour necrosis factor alpha and IL-6 [7]. In that study, on the day of CAP diagnosis all patients presented high CRP levels, >5 mg/dl. Another interesting finding was that the admission CRP concentration was significantly influenced by the antibiotic prescription prior to hospital admission in comparison with those patients without therapy (10.7 ± 4.2 versus 15.2 ± 4.4 , respectively; $P = 0.023$). The authors showed that in patients with a good outcome the CRP concentration fell sharply, whereas in patients who died of pneumonia there was a pro-

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gressive rise in the CRP level prior to death, to concentrations >10 mg/dl. We found in our study a similar CRP course in survivors and nonsurvivors. The other biomarkers studied by Smith and colleagues were not helpful in the assessment of the CAP clinical course. Tumour necrosis factor alpha was detectable in only six patients on the day of hospital admission, and only a further seven patients had detectable concentrations during the period of follow-up. Concerning IL-6, only six patients had detectable concentrations during some point of their hospital stay.

In a previous study, our group assessed the value of daily measurements of CRP, WCC and body temperature after the prescription of antibiotics in ventilator-associated pneumonia patients [12]. In that study, daily CRP measurements after antibiotic prescription were useful in the identification, as early as day 4, of ventilator-associated pneumonia patients with poor outcome. Moreover, both the WCC and the body temperature were not useful early markers of the ventilator-associated pneumonia course. Patients were also divided according to the pattern of CRP response to antibiotics; all patients with fast and slow response patterns survived, whereas those patients showing nonresponse and a biphasic response pattern exhibited a mortality of 78% and 75%, respectively. The influence of adequate initial antibiotic therapy on the outcome of ventilator-associated pneumonia patients was also studied. Patients with inadequate initial antibiotic therapy had a mortality rate of 66.7%, while patients with adequate therapy showed mortality of 18.4%.

In the present study, serial measurements of the CRP concentration, the body temperature and the WCC were performed in patients with severe CAP from the day of antibiotic prescription (day 0) to the day of death or to the end of antibiotic therapy, dividing patients into survivors and nonsurvivors. Daily CRP measurements were performed not to predict outcome but to describe the clinical course. From day 0 to day 7 the CRP ratio showed a significant and steady decrease in survivors, whereas in nonsurvivors it remained elevated. In survivors, by day 3 the CRP ratio had decreased by almost 50% from the admission concentration. Comparisons of receiver-operating characteristic curves showed that the prognostic performance of the CRP ratio by day 3 was significantly better than that of the body temperature and the WCC. A CRP ratio >0.5 by day 3, with a sensitivity of 0.91 and a specificity of 0.55, was associated with the diagnosis of nonresolving severe CAP.

We additionally performed the analysis of the maximal relative variation of CRP from the previous day's level. We found that a decrease higher than 0.31 from the previous day was a marker of good prognosis, with an AUC of 0.76, a sensitivity of 0.75 and a specificity of 0.85. Almost 80% of survivors showed a decrease higher than 0.31. In addition, the rate of the CRP decrease expressed by the maximal relative CRP var-

iation from the previous day's level had a good correlation with a good clinical course.

The CRP ratio patterns of patient response to antibiotics were found to be closely correlated with outcome. About 76% of patients with fast and slow response patterns survived, whereas the combined mortality rate of the patients showing the nonresponse and biphasic response patterns was 75%.

The optimal duration of antibiotic therapy in CAP is still unknown, and possibly should vary from patient to patient depending of the severity of the pneumonia as well as the clinical course. Current guidelines recommend antibiotic courses from 7 to 21 days, depending on the pneumonia severity and the type of pathogen [2,3]. In a recent published study, Christ-Crain and colleagues proposed procalcitonin to diagnose and guide the duration of antibiotic therapy in CAP patients. Patients in the procalcitonin guidance group reduced their antibiotic therapy duration to 5 days, compared with 12 days in patients treated according with guidelines [23]. Twenty-nine per cent of the patients included in this study, however, had an almost undetectable level of procalcitonin on the day of diagnosis. Consequently, in those patients it is virtually impossible to evaluate the rate of procalcitonin decline since it is already very low. As a result, procalcitonin can hardly be a valuable marker to guide the duration of antibiotic therapy or to predict outcome at least in patients that were diagnosed as CAP but had unexpectedly very low procalcitonin levels.

The evaluation of changes in clinical variables, such as the SOFA score and the $\text{PaO}_2/\text{FiO}_2$ ratio, can be helpful in the assessment of the effect of different therapeutic interventions [24]. In this study, the $\text{PaO}_2/\text{FiO}_2$ ratio did not discriminate between survivors and nonsurvivors during the first week of antibiotic therapy, confirming the data published previously for ventilator-associated pneumonia patients [12]. This ratio parameter depends profoundly on noninfectious factors and can be easily influenced, for example, by the FiO_2 administered or by the ventilator settings.

Conversely, a significant decrease in the SOFA score from day 0 to day 7 was found in survivors, whereas in nonsurvivors the values remain almost unchanged. Patients with good outcome had a progressive decrease in the CRP ratio, showing a good correlation with the resolution of organ failure measured by the SOFA score. Lobo and colleagues [24] found that increased CRP concentrations were associated with organ failure, prolonged ICU stay and high infection and mortality rates. Increasing or persistently high levels (suggesting ongoing inflammatory activity) indicated poor prognosis, while declining values (suggesting a diminishing inflammatory reaction) were associated with a more favourable prognosis. In our study, patients who maintained high levels of CRP, suggesting a persistent inflammatory response – namely those with nonresponse and biphasic response patterns of

response – had significantly higher SOFA scores as well as higher mortality rates. On the contrary, patients who presented progressively declining levels of CRP showed a SOFA score improvement and a better prognosis. The SOFA score is not a sepsis-related score as the authors initially thought, however, but just an organ failure/dysfunction score [10,11]. Consequently, the SOFA score can be influenced by several non-infectious conditions unrelated to the course of the primary infection.

We should note some limitations of the present investigation. The study is a cohort, single-centre, observational study using variables collected daily at the bedside to evaluate the clinical course of severe CAP. We should note that this issue was only fully addressed in a very limited number of studies, however – and the CRP concentration used in only one other study [7] – so it is very difficult to compare results.

Conclusion

In summary, it has been demonstrated that daily CRP measurements after prescription of antibiotic therapy are useful in the identification, as early as day 3, of severe CAP patients with poor outcome, and the measurement performs better than the commonly used markers of infection, such as body temperature and WCC. In addition, recognition of the pattern of the CRP ratio response to therapy could provide more information about the individual clinical course improving or worsening, as well as the rate of improvement. In addition, our data suggest that, in patients with severe CAP with a rapid CRP ratio decline, a shorter duration of antibiotic therapy could be equally effective, reducing toxicity, reducing the risks of emergence of resistant strains and reducing costs. Conversely, for patients showing the patterns of nonresponse and biphasic response, we should perform an aggressive diagnostic and therapeutic approach to prevent further clinical worsening. If these findings are confirmed, the duration of antibiotic therapy could be tailored to each patient's clinical response, and CRP can be an important marker in daily monitoring for the efficacy of antibiotic therapy of patients with severe CAP. Further studies to assess the clinical impact of daily monitoring should be performed.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LC and PP conceived the study. All authors participated in the original design and in writing the original protocol. LC and PP collected and analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

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Key messages

- Daily CRP measurement is useful in monitoring the clinical course of severe CAP and is a good early marker of favourable outcome.
- The rate of CRP decrease expressed by the maximal relative CRP variation from the previous day's level has a good correlation with a good clinical course.
- The identification of the pattern of the CRP response to antibiotic therapy might be useful in the recognition of the individual clinical course either improving or worsening in patients with severe CAP, as well as the rate of improvement.
- Daily CRP ratio measurements and the patterns of the CRP response to antibiotics have a good correlation with the clinical course assessed by the SOFA score in patients with severe CAP.

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Artigo 2: Patterns of C-reactive protein RATIO response in severe community-acquired pneumonia: a cohort study.

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Patterns of c-reactive protein RATIO response in severe community-acquired pneumonia: a cohort study

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Abstract

Introduction: Community-acquired pneumonia (CAP) requiring intensive care unit (ICU) admission remains a severe medical condition, presenting ICU mortality rates reaching 30%. The aim of this study was to assess the value of different patterns of C-reactive protein (CRP)-ratio response to antibiotic therapy in patients with severe CAP requiring ICU admission as an early maker of outcome.

Methods: In total, 191 patients with severe CAP were prospectively included and CRP was sampled every other day from D1 to D7 of antibiotic prescription. CRP-ratio was calculated in relation to D1 CRP concentration. Patients were classified according to an individual pattern of CRP-ratio response with the following criteria: fast response - when D5 CRP was less than or equal to 0.4 of D1 CRP concentration; slow response - when D5 CRP was > 0.4 and D7 less than or equal to 0.8 of D1 CRP concentration; nonresponse - when D7 CRP was > 0.8 of D1 CRP concentration. Comparison between ICU survivors and non-survivors was performed.

Results: CRP-ratio from D1 to D7 decreased faster in survivors than in non-survivors ($p = 0.01$). The ability of CRP-ratio by D5 to predict ICU outcome assessed by the area under the ROC curve was 0.73 (95% Confidence Interval, 0.64 - 0.82). By D5, a CRP concentration above 0.5 of the initial level was a marker of poor outcome (sensitivity 0.81, specificity 0.58, positive likelihood ratio 1.93, negative likelihood ratio 0.33). The time-dependent analysis of CRP-ratio of the three patterns (fast response $n = 66$; slow response $n = 81$; nonresponse $n = 44$) was significantly different between groups ($p < 0.001$). The ICU mortality rate was considerably different according to the patterns of CRP-ratio response: fast response 4.8%, slow response 17.3% and nonresponse 36.4% ($p < 0.001$).

Conclusions: In severe CAP, sequential evaluation of CRP-ratio was useful in the early identification of patients with poor outcome. The evaluation of CRP-ratio pattern of response to antibiotics during the first week of therapy was useful in the recognition of the individual clinical evolution.

Introduction

Community-acquired pneumonia (CAP) is a major cause of death due to infectious diseases in the Western world [1]. Among hospitalized patients, up to 36% require admission to the intensive care unit (ICU), and despite advances in antimicrobial therapy, more than one third of the patients will die during hospital stay [2-6].

After prescription of antibiotics, the evaluation of the individual clinical response and the assessment of resolution of pneumonia usually rely on the monitoring of the same criteria used for clinical diagnosis. Commonly used variables, such as temperature and white blood cell count, have a limited value in the assessment of clinical response to antibiotics [7,8] since both present significant improvements late in the course and can be influenced by drugs frequently used in ICU, such as steroids, antipyretics, or beta blockers.

To overcome these limitations, physicians frequently use serum biomarkers to assist in the clinical decision-

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making process, namely in the assessment of clinical response to antibiotic therapy. C-reactive protein (CRP) is one of these biomarkers and probably the most widely used [9]. In different infections and clinical settings, CRP discriminates, early in the clinical course, survivors from non-survivors. In addition, the course of relative CRP variations, the CRP ratio, after prescription of antibiotic therapy, can be classified in different patterns as fast response, slow response, and non-response [7,8]. Previous studies have demonstrated that the identification of the individual pattern of CRP ratio response to antibiotic therapy appears to be a reflection of the clinical course of infection independently of other possible confounders [7,8,10].

The aims of the present study were to evaluate the course of CRP ratio and identify the patterns CRP ratio response to antibiotic therapy during the first week in patients with severe CAP in order to differentiate between patients with good and poor outcome early in the clinical course and potentially provide a useful and easy-to-use tool for antibiotic stewardship.

Materials and methods

Study subjects

We conducted a prospective observational cohort study in two medical-surgical ICUs at tertiary hospitals (Barra D'Or Hospital, Rio de Janeiro, Brazil, and Garcia de Orta Hospital, Almada, Portugal). Patients with severe CAP that required ICU admission were consecutively included between November 2001 and December 2002 at Garcia de Orta Hospital and between August 2003 and June 2007 at Barra D'Or Hospital. To perform the present analysis, the independent databases were merged. The institutional review boards approved the study design and waived the need for informed consent. The present study was strictly observational and did not interfere in the decision-making process or clinical management. Patients were treated according to best standard ICU practice without any reference to the response patterns of CRP in their daily evaluation.

Study design and definitions

Demographic, clinical, laboratory, and outcome data were prospectively collected. The Acute Physiology and Chronic Health Evaluation II (APACHE II) score was calculated after 24 hours of ICU admission [11]. Severe CAP was diagnosed according to the American Thoracic Society (ATS) criteria, and the CURB-65 scale - confusion of new onset (defined as an abbreviated mental test score of 8 or less), urea of greater than 7 mmol/L (19 mg/dL), respiratory rate of 30 breaths per minute or greater, systolic blood pressure of less than 90 mm Hg or diastolic blood pressure of 60 mm Hg or less, and age of at least 65 years - was

used to evaluate its severity [12]. Patients were followed until death or hospital discharge. Antimicrobial therapy was prescribed in accordance with the ATS guidelines to all patients [12]. Patients with severe immunosuppression (for example, from solid organ or bone marrow transplant, HIV infection, or immunosuppressive treatment) and tuberculosis were excluded from the present study.

CRP, arterial oxygen tension/inspiratory oxygen fraction ratio (PaO₂/FiO₂ ratio), and Sequential Organ Failure Assessment (SOFA) [13] score were routinely measured during the first week of ICU stay at day 1 (D1), D3, D5, and D7. The CRP ratio was calculated in relation to the D1 CRP concentration. Patients were retrospectively classified with a modified version of previously defined CRP ratio patterns of the response to antibiotics [7,14]: fast response - when D5 CRP was not more than 0.4 of D1 CRP concentration; slow response - when D5 CRP was greater than 0.4 and D7 CRP was not more than 0.8 of D1 CRP concentration; or non-response - when D7 CRP was greater than 0.8 of D1 CRP concentration. Comparison between survivors and non-survivors was performed.

Statistical analysis

Continuous variables are presented as the mean \pm standard deviation unless stated otherwise. Comparisons between groups were performed by using the parametric unpaired and paired *t* test or the non-parametric Mann-Whitney *U* test and the Wilcoxon signed-rank test for continuous variables according to data distribution. The chi-squared test was used to carry out comparisons between categorical variables. Time-dependent analysis of different variables was performed via general linear model univariate repeated-measures analysis using a split-plot design approach. Receiver-operating characteristic curves were drawn for the D5 CRP ratio. The optimal CRP ratio cutoff was defined as the value associated with the highest sum of sensitivity and specificity (Youden's index).

To identify the variables (independent variables) predicting ICU outcome of severe CAP (dependent variable) during antibiotic therapy, a multivariable logistic regression model was constructed. Age, sex, APACHE II score, D1 PaO₂/FiO₂ ratio, mechanical ventilation, ICU-acquired infection, septic shock, D5 CRP ratio of greater than 0.5, and D1 SOFA score were included in the initial model to control for potential confounding factors. Backward stepwise variable elimination was then performed in order to develop the final model, and a *P* value of less than 0.05 was a requirement for acceptance. Model calibration and discrimination were assessed by using the Hosmer-Lemeshow goodness-of-fit test and the *c* statistic, respectively. Results are

reported as adjusted odds ratio (AOR) with 95% confidence interval (CI). Data were analyzed by using PASW version 18.0 for MAC (SPSS, Inc., Chicago, IL, USA). All statistics were two-tailed, and the significance level was set at 0.05.

Results

A total of 191 patients with severe CAP requiring ICU admission were included. The main characteristics of the study population are presented in Table 1. The ICU and hospital mortality rates were 21.9% and 24.6%, respectively. A microbiological diagnosis was established in 33 patients (17.2%) (Table 2). All patients with microbiological diagnosis had initial adequate antibiotic treatment. On ICU admission, 111 patients (58.1%) required invasive mechanical ventilation.

D1 CRP and CRP ratio course from D1 to D7

At D1, CRP concentration was not significantly different between survivors and non-survivors (15.5 versus 14.3 mg/dL, $P = 0.91$). The course of CRP ratio during the first week of antibiotic therapy showed a steady and significant decrease in survivors ($P = 0.01$). As early as D3, the CRP ratio of survivors decreased by an average of 24% but by only 11% in non-survivors ($P = 0.106$). By D5 of antibiotic therapy, this divergent evolution of CRP ratio was markedly different; the CRP ratio was 0.46 ($P < 0.001$) in survivors but remained elevated in non-survivors, at 0.79 ($P = 0.313$). The ability of the CRP ratio by D5 to predict outcome assessed

Table 2 Microorganisms isolated from the 33 (17.2%) patients with severe community-acquired pneumonia

Microorganism	n = 33
Gram-positive organisms	
<i>Streptococcus pneumoniae</i>	19
Methicillin-sensitive <i>Staphylococcus aureus</i>	
Group A <i>Streptococcus</i>	1
<i>Streptococcus agalactiae</i>	1
Gram-negative organisms	
<i>Haemophilus influenzae</i>	3
<i>Pseudomonas aeruginosa</i>	3
<i>Neisseria pneumoniae</i>	2
Atypical	
<i>Mycoplasma pneumoniae</i>	2

by the area under the receiver operating characteristic curve was 0.73 (95% CI = 0.64 to 0.82). By D5, a CRP concentration of above 0.5 of the initial level was a marker of poor outcome (sensitivity of 0.81, specificity of 0.58, positive likelihood ratio of 1.93, and negative likelihood ratio of 0.33).

In the multivariable analysis, only three variables - D1 SOFA score (per 1-point increment AOR = 1.20, 95% CI = 1.06 to 1.37; $P = 0.006$), D5 CRP ratio of greater than 0.5 (AOR = 4.47, 95% CI = 1.64 to 12.20; $P = 0.003$), and mechanical ventilation (AOR = 9.39, 95% CI = 2.63 to 33.60; $P = 0.001$) - were independently associated with ICU mortality (model $n = 175$ and goodness of fit = 0.447).

Table 1 Comparisons between survivors and non-survivors of severe community-acquired pneumonia

	All patients (n = 191)	Non-survivors (n = 42)	Survivors (n = 149)	P value*
Age in years	70 (54-81)	77 (64-83)	69 (53-79)	0.03
Male gender, number (percentage)	102 (53.4%)	19 (45.2%)	83 (55.7)	0.29
APACHE II score in points	15 (12-19)	18 (14-23)	15 (11-19)	0.004
CURB-65 in points	3 (3-4)	4 (3-5)	3 (2-4)	0.008
PaO ₂ /FiO ₂ ratio in mm Hg	244 (172-295)	210 (157-266)	250 (177-300)	0.052
SOFA score in points	5 (3-8)	7 (3.7-10)	4 (3-7)	0.004
Positive blood cultures, number (percentage)	22 (11.5%)	7 (16.9%)	15 (10.0%)	0.99
Invasive mechanical ventilation, number (percentage)	111 (58.1%)	34 (80.9%)	77 (51.6%)	0.0007
Septic shock at ICU admission, number (percentage)	79 (41.3%)	26 (61.9%)	53 (35.5%)	0.0007
COPD, number (percentage)	41 (21.4%)	8 (19%)	33 (22.1%)	0.83
Diabetes, number (percentage)	36 (18.8%)	7 (16.6%)	29 (19.4%)	0.86
Asthma, number (percentage)	10 (5.2%)	2 (4.7%)	8 (5.3%)	0.81
Cardiac failure, number (percentage)	9 (4.7%)	1 (2.3%)	8 (5.3%)	0.69
C-reactive protein in mg/dL	15.3 (7.8-24.2)	14.3 (7.7-24.6)	15.5 (7.9-24.2)	0.91
ICU LOS in days	7 (4-15)	11.5 (5-22.7)	7 (4-13)	0.01
Hospital LOS in days	13 (9-26)	16 (6-36.7)	13 (9-23.5)	0.57

Results are expressed as median (25% to 75% interquartile range) unless stated otherwise. *For comparisons among survivors and non-survivors. APACHE II, Acute Physiology and Chronic Health Evaluation II; COPD, chronic obstructive pulmonary disease; CURB-65 scale, confusion of new onset (defined as an abbreviated mental test score of 8 or less), urea of greater than 7 mmol/L (19 mg/dL), respiratory rate of 30 breaths per minute or greater, systolic blood pressure of less than 90 mm Hg or diastolic blood pressure of 60 mm Hg or less, and age of at least 65 years; ICU, intensive care unit; LOS, length of stay; PaO₂/FiO₂ ratio, arterial oxygen tension/inspiratory oxygen fraction ratio; SOFA, Sequential Organ Failure Assessment.

CRP ratio patterns of response to antibiotic therapy

Patients with severe CAP were retrospectively divided according to three patterns of the CRP ratio course during antibiotic therapy. Sixty-six (35%) patients were classified as fast response pattern, 81 (42%) as slow response, and 44 (23%) as non-response. The time-dependent analysis of CRP ratio (Figure 1) variations of the three different patterns was statistically different ($P < 0.001$). By D5, the CRP ratios were 0.23, 0.74, and 1.47 in patients exhibiting a fast response, a slow response, and a non-response pattern, respectively ($P < 0.001$).

When we analyzed the correlation between the CRP ratio patterns and outcome, we found marked differences in ICU mortality rate. Patients with the fast response, slow response, and non-response patterns presented ICU mortality rates of 4.6%, 17.3%, and 36.4%, respectively ($P < 0.001$) (Figure 1). Similarly, the hospital mortality was significantly different according to the

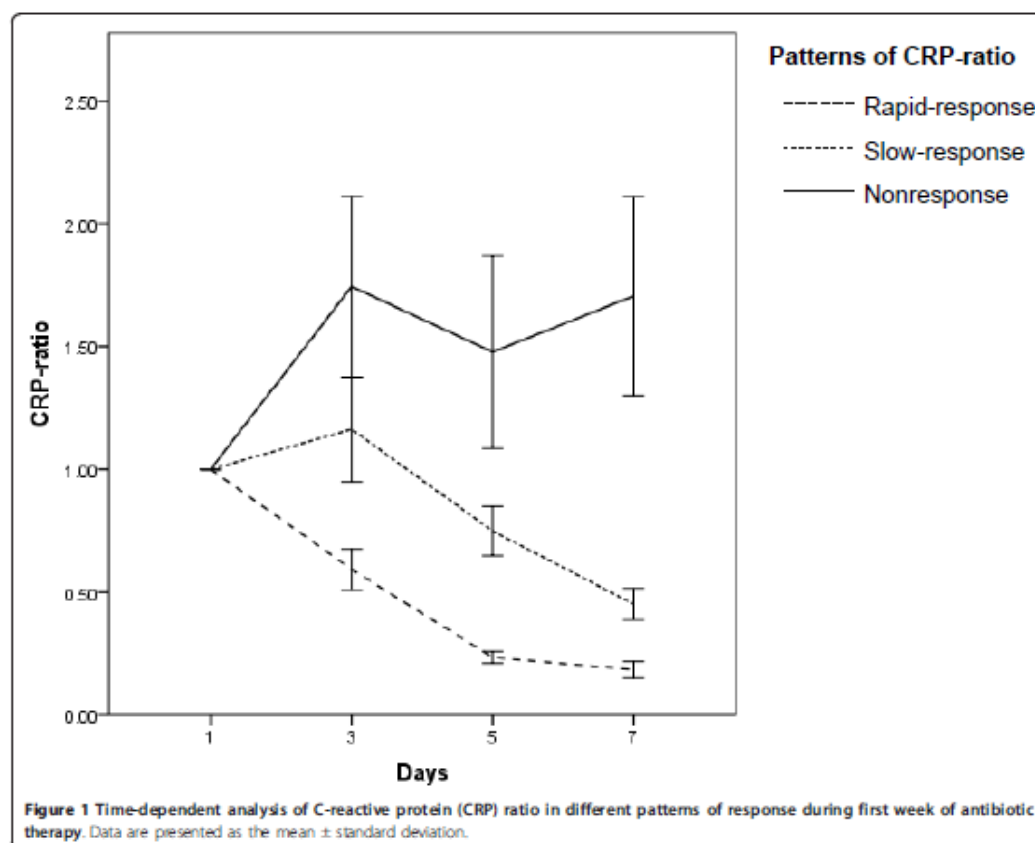
CRP ratio pattern: fast response, 9.5%; slow response, 25.9%; and non-response, 43.2% ($P = 0.001$).

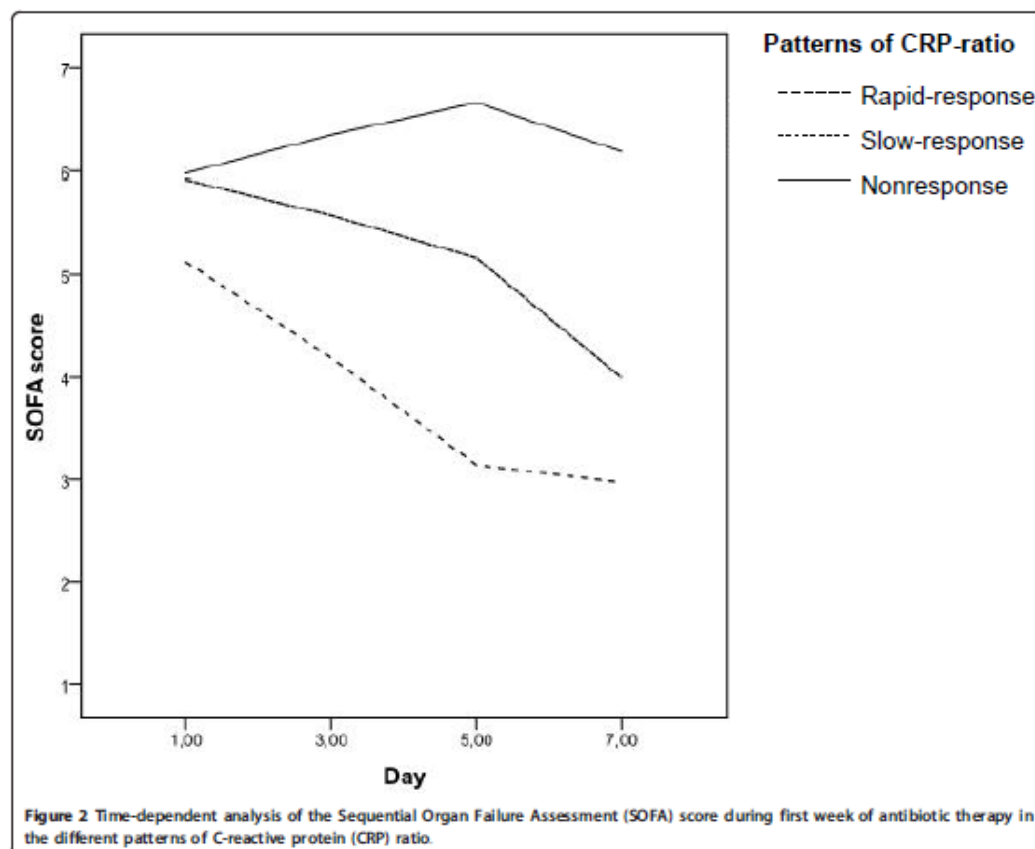
CRP ratio patterns of response and clinical course

The clinical course during antibiotic therapy was monitored with SOFA score and the PaO₂/FiO₂ ratio. The time-dependent analysis of the PaO₂/FiO₂ ratio from D1 to D7 of antibiotic therapy in survivors and non-survivors was significantly different ($P < 0.001$) but was not different between the different patterns of CRP ratio ($P = 0.210$). However, the SOFA score from D1 to D7 of antibiotic therapy in survivors and non-survivors was significantly different ($P < 0.001$) as well as between the different patterns of CRP ratio response ($P < 0.001$) (Figure 2).

Mechanically ventilated patients with CAP

In the subpopulation of mechanically ventilated patients ($n = 111$, ICU mortality of 26.1%), the time-dependent





analysis of CRP ratio was also significantly different between survivors and non-survivors as well as between the different patterns of CRP ratio response to antibiotics ($P = 0.002$ and $P < 0.001$, respectively). In addition, in this subgroup of patients, the course of $\text{PaO}_2/\text{FiO}_2$ ratio during the first week of antibiotic therapy in the different patterns of CRP ratio response was not significantly different ($P = 0.437$). However, again, the SOFA score according to the different patterns of CRP ratio response during the same time period was significantly different ($P = 0.001$).

Discussion

In the present study, we described the patterns of serial measurements of CRP ratio and its relation with clinical resolution of severe CAP in a large cohort of patients requiring intensive care admission. With the resolution of infection, the concentration of CRP decreases at a rate that is dependent on its half-life, since this marker

exhibits a first-order elimination kinetics [15]. So the assessment of relative variations is more informative about the course of infection than absolute variations are [9,16]. In our study, survivors showed a continuous and significant decrease of CRP ratio during the first week of antibiotic therapy. Conversely, in non-survivors, CRP ratio remained elevated, and at D5, a CRP ratio of higher than 0.5 was associated with a fivefold increase in the risk of death in the ICU. Interestingly, in our study, patients with the non-response CRP ratio pattern presented a significantly higher ICU mortality than patients with fast or slow response patterns. Additionally, the identification of CRP ratio pattern of response to antibiotics, during the first week of therapy, was useful in the recognition of the individual clinical evolution of patients with severe CAP.

These data suggest that persistently elevated CRP values are indicative of poor response to antibiotic therapy. This could be the result of inadequate initial

antibiotic therapy [7,8], the presence of other infectious complications [17], or a newly developed infection in another location [9,16]. Several studies have confirmed that serial measurements of CRP are useful in the monitoring of clinical course as well as assessment of patient outcome in different severe infections. In all of these studies, survivors by D3 to D4 presented values of CRP ratio of between 0.5 and 0.8 of the initial value [7,8,18-23]. This means that, after 72 to 96 hours of antibiotic therapy, the CRP of survivors decreases by 30% to 50% of the initial concentration. Besides, serial measurement of CRP allows the identification of various patterns of response to antibiotic therapy, as previously described by our group [7].

This concept of patterns of CRP ratio response to antibiotics has been tested in different clinical settings and reproduced by different research groups. In a recent study, Moreno and colleagues [20] studied the value of daily measurements of CRP in a cohort of 64 patients with nosocomial pneumonia. Patients were classified according to the CRP ratio in two groups: 'good' response (CRP ratios of lower than 0.67 at D10) and 'poor' response (non-response or biphasic response). The poor-response group ($n = 34$) had a mortality rate of 53% in comparison with 20% in the good-response group ($n = 30$) (relative risk = 2.65, 95% CI = 1.21 to 5.79; $P = 0.01$). Significant differences between the two groups were found on CRP ratios at D4 ($P = 0.01$). Also, in a cohort of 891 patients who had community-acquired sepsis and who were admitted to the ICU, Pova and colleagues [19] found that patterns of CRP ratio response to antibiotics presented a marked correlation with hospital mortality. Patients with a non-response pattern had a 2.5 times higher probability of dying in comparison with patients with fast response (AOR = 2.5, 95% CI = 1.6 to 4.0; $P < 0.001$). Slow responders showed a non-significant increase on the odds of mortality in comparison with the fast responders (AOR = 1.5, 95% CI = 0.9 to 2.5; $P = 0.124$). The results of the present study are in agreement with this concept in the population of patients with severe CAP.

In all of these studies, the patterns of CRP ratio response allowed the early identification (between D3 and D4) of patients with poor response to antibiotics and consequently with poor prognosis. In our study, by D5, a CRP concentration of above 0.5 of the initial level was a marker of poor outcome.

The evaluation of changes in organ dysfunction/failure, assessed by the PaO₂/FiO₂ ratio and the SOFA score, could be helpful in the assessment of the effect of different therapeutic interventions. However, the PaO₂/FiO₂ ratio was not helpful in distinguishing between the different patterns of CRP ratio response during the first

week of antibiotic therapy. The same was true in the subgroup of mechanically ventilated patients. Similar results were found in other studies in patients with ventilator-associated pneumonia and bloodstream infection [7,8].

When we studied the SOFA score from D1 to D7, we found a significant decrease in survivors in comparison with non-survivors. Also, patients with CRP ratio patterns of fast or slow response presented a significant decrease of SOFA score, whereas in patients with non-response, SOFA score remained almost unchanged. Similar findings were observed in the subgroup of mechanically ventilated patients. In our study, we show a good correlation between CRP ratio course and organ failure evolution measured by the SOFA score, either improving or not as well as the rate of improvement, respectively. In fact, the decrease of CRP ratio by D3 anticipates the decrease in SOFA score, and this could suggest that CRP is a better marker of resolution of severe CAP. Similar results were observed in patients with ventilator-associated pneumonia and bloodstream infections [7,8,24]. Additionally, the clinical application of the SOFA score is not as easy and straightforward as CRP interpretation, since SOFA score calculation implies the collection of data of several clinical and laboratorial parameters. So SOFA score determination is more time-consuming and difficult to perform routinely at the bedside. Consequently, the identification of the CRP ratio patterns of response in combination with the clinical evaluation could become a useful tool with good potential to reduce the length of antibiotic therapy as well as to reduce the risks of emergence of resistant strains and costs of medication, a hypothesis to be tested in future trials of CRP-guided therapy.

Our study has important strengths. It contained a reasonably large sample collected in two ICUs and evaluated the CRP ratio patterns of response to antibiotics in patients with severe CAP. However, there are limitations to the study; namely, it was an observational study. Also, the data came from two independent databases and were collected in different periods. As a result, we cannot exclude the possibility of time-dependent effect on the results. Most of the diagnoses were made on the basis of clinical and radiological criteria according to the ATS criteria, although only 17.2% of the patients were found to have microbiologically documented pneumonia. In addition, we have no information concerning antibiotic therapy previous to ICU admission. Besides, the attending physicians were not blinded to the CRP results. Finally, in this study, we evaluated only one biomarker, namely CRP. So we cannot exclude the possibility that similar results could be obtained with other biomarkers.

Conclusions

Serial evaluation of CRP ratio was useful in the early identification of patients with severe CAP with a poor outcome. Besides, the recognition of the patterns of CRP ratio in patients with severe CAP provided additional information about the individual clinical course and this information could significantly influence the clinical decision-making process at the bedside. In patients with persistently elevated or rising CRP ratio (that is, a non-response pattern), an aggressive diagnostic and therapeutic approach should be attempted in order to prevent further clinical deterioration in an effort to change the poor associated prognosis. In contrast, patients with consistent CRP ratio decrease (that is, patterns of fast or slow response) usually have an adequate antibiotic therapy, rapid resolution of infection, and good prognosis.

Key messages

- Serial evaluation of C-reactive protein (CRP) ratio is useful in the early identification of patients with severe community-acquired pneumonia (CAP) with a poor outcome.
- Recognition of the patterns of CRP ratio in patients with severe CAP provides information about the individual clinical course.
- In patients with a non-response pattern, an aggressive diagnostic and therapeutic approach should be attempted in order to change the poor associated prognosis.

Abbreviations

AOR: adjusted odds ratio; APACHE II: Acute Physiology and Chronic Health Evaluation II; ATS: American Thoracic Society; CAP: community-acquired pneumonia; CI: confidence interval; CRP: C-reactive protein; D: day; ICU: intensive care unit; PaO₂/FIO₂: oxygen tension/inspiratory oxygen fraction; SOFA: Sequential Organ Failure Assessment.

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Authors' contributions

LMC, JFS, and PP conceived the study, analyzed the data, and drafted the manuscript. All authors participated in the original design and in writing the

original protocol and collected the data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Artigo 3: Impact of systemic corticosteroids on the clinical course and outcomes of patients with severe community-acquired pneumonia: a cohort study.

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Impact of systemic corticosteroids on the clinical course and outcomes of patients with severe community-acquired pneumonia: A cohort study[☆]

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Mechanical ventilation;
Multiple organ failure;
Severe sepsis

Abstract

Introduction: Our aim was to evaluate the impact of corticosteroids on clinical course and outcomes of patients with severe community-acquired pneumonia (CAP) requiring invasive mechanical ventilation.

Methods: This was a cohort study of patients with severe CAP from 2 intensive care units in tertiary hospitals in Brazil and Portugal.

Results: A total of 111 patients were included (median age, 69 years; 56% men; 34% hospital mortality). Corticosteroids were prescribed in 61 (55%) patients. Main indications for their use were bronchospasm (52.5%) and septic shock (36%). Mortality rate of patients treated with and without corticosteroids was comparable (29.5% vs 32%, $P = .837$). No significant differences were observed on clinical course from day 1 to day 7 as assessed by the Sequential Organ Failure Assessment score ($P = .95$). Furthermore, C-reactive protein declined similarly in both groups ($P = .147$). In a multivariate analysis, mortality was associated with older age and higher Acute Physiology and Chronic Health Evaluation II score.

[☆] Authors' contributions: JIFS, PP, MS, and LMC contributed to the study conception and design, carried out and participated in data analysis, and drafted the manuscript. JRIS, FAB, PTB, JCRV, and HCFN conceived the study, participated in its design and coordination, supervised data analysis, and helped to draft the manuscript. All authors read and approved the final manuscript.

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Conclusions: In patients with severe CAP requiring invasive mechanical ventilation, adjunctive therapy with corticosteroids did not influence intensive care unit and hospital mortality. In addition, no changes were observed on weaning from vasopressors, on recovery from organ failure/dysfunction as assessed by the Sequential Organ Failure Assessment score, as well as on C-reactive protein course.
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1. Introduction

Community-acquired pneumonia (CAP) is the most common cause of death associated with infectious disease and a major cause worldwide [1,2]. More than 1 million patients with CAP require hospitalization annually, 10% of whom will be admitted to an intensive care unit (ICU) [1]. Despite improvements in critical care support [1,3] and antimicrobial therapy [1,4], mortality rates remain exceedingly high. Among ICU patients, it can be as high as 50% in those requiring mechanical ventilation (MV) or vasopressors [5]. To date, few adjunctive therapies are associated with improved outcomes in patients with severe infections [3]. In recent years, the use of corticosteroids has been suggested to be effective in selected patients with CAP [6-8]. Nevertheless, recent reviews concluded that available studies cannot support recommendation for the use of corticosteroids as standard of care for all patients with severe CAP [9,10]. Finally, data from a recent prospective randomized controlled trial (RCT) showed absence of corticosteroids impact on outcome in a cohort of hospitalized non-ICU CAP patients [11]. Therefore, further studies evaluating a large number of severe CAP patients are needed. We hypothesized that the use of corticosteroids could reduce mortality and hasten the resolution of systemic inflammation, organ dysfunction, and gas exchange in patients with severe CAP requiring invasive MV.

2. Patients and methods

2.1. Design and setting

This cohort prospective observational study was performed in 2 medical-surgical ICUs at tertiary hospitals (Barra D'Or Hospital, Rio de Janeiro, Brazil, and Garcia de Orta Hospital, Almada, Portugal). The institutional review boards approved the study design and waived the need for informed consent. There were no patient interventions performed for the study.

2.2. Selection of participants, data collection, and definitions

Patients with CAP that required ICU admission were consecutively included between November 2001 and December 2002 at Garcia de Orta Hospital [12] and between August 2003 and June 2007 at Barra D'Or Hospital [13]. To

perform the present analysis, these independent databases were joined together, segregating CAP patients requiring invasive MV.

Patients with severe immunosuppression (eg, from solid organ or bone marrow transplant, HIV infection, or immunosuppressive treatment) and tuberculosis were excluded from the present study.

Demographic, clinical, laboratory, and outcome data were prospectively collected. The Acute Physiology and Chronic Health Evaluation (APACHE) II score [14] was calculated after 24 hours of ICU admission. Severe CAP was diagnosed according to the American Thoracic Society (ATS) criteria [15], and the CURB-65 scale was used to evaluate its severity [16]. Sepsis was stratified according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference criteria [17]. Patients were followed until death or hospital discharge. Intensive care unit-acquired infections were defined according to the Centers for Disease Control and Prevention definitions [18]. Antimicrobial therapy was prescribed in accordance with the ATS guidelines [15] to all patients. In patients with microbiologic documentation, the appropriateness of empiric antibiotic therapy was monitored. Appropriate antibiotic therapy was considered if, in the empiric therapy prescribed by the onset of CAP, at least one antibiotic covers all pathogens isolated as determined by the sensitivity pattern in the antibiogram. In patients started with initial appropriate treatment, antibiotics were changed according to the isolated pathogen and the antimicrobial susceptibility testing.

C-reactive protein (CRP), arterial oxygen tension/inspiratory oxygen fraction ratio (P/F ratio), and Sequential Organ Failure Assessment (SOFA) score [19] were routinely measured during the first week of ICU stay at day (D) 1, D3, D5, and D7. The decision to treat patients with corticosteroids, as well as the steroid weaning, was at the discretion of the attending physician. Reasons for the use of corticosteroids were routinely recorded. To homogenize data, corticosteroid doses are presented as equivalent doses of methylprednisolone. Etomidate, ketoconazole, and rifampicin were not used in any of the screened patients. The primary end point was in-hospital death from any cause.

2.3. Data presentation and statistical analysis

Standard descriptive statistics were used. Continuous variables were reported as median (interquartile range [IQR]). Univariate analysis was used to identify factors

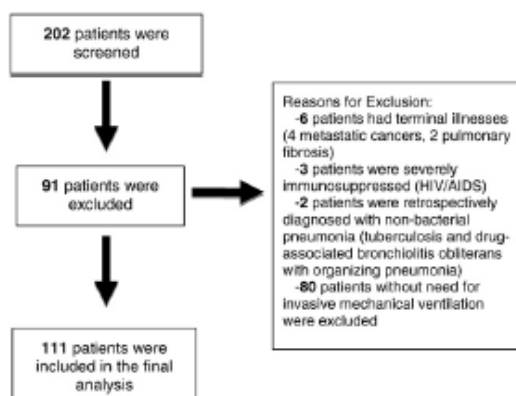


Fig. 1 Study flowchart.

associated with hospital mortality. Univariate and multivariate logistic regressions were used to identify factors associated with hospital mortality. Linearity between continuous variables and the dependent variable was demonstrated using locally weighted scatterplot smoothing [20]. Variables yielding P values $< .2$ by univariate analysis were entered into a forward multivariate logistic regression analysis. Multivariate analysis results were summarized by estimating odds ratios (ORs) and respective 95% confidence intervals (CIs). Possible interactions were tested. To adjust for the influence of potential selection bias regarding the

patient's likelihood of having received corticosteroids, a propensity score was built and forced into the final models. Variables associated with a P value $< .2$ in univariate analysis between patients who have received or not corticosteroids were used to build the propensity score. The following variables were retained for propensity score: CURB-65 score and chronic obstructive pulmonary disease (COPD). This propensity score was entered as a continuous variable into the models [21,22]. The area under the receiver operating characteristic curve was used to assess the models' discrimination [23]. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate agreement between the observed and expected results across all strata of probabilities of the outcome of interest (calibration) [20]. P values $> .05$ indicate a good fit for the model. Time-dependent analysis of different variables was performed via general linear model univariate repeated-measures analysis using a split-plot design approach.

Two-tailed P values $< .05$ were considered statistically significant. The PASW Statistics 18.0 software package (Chicago, IL) was used for statistical analysis.

3. Results

3.1. Characteristics of the study population

A total of 111 patients who fulfilled the entry criteria, severe CAP and invasive MV, were enrolled in the study

Table 1 Demographic and clinical variables of patients with severe CAP requiring invasive MV and according to corticosteroid treatment

Variables	All patients (N = 111)	Treated with corticosteroids		P value ^a
		Yes (n = 61, 55%)	No (n = 50, 45%)	
Age, y	69 (25)	73 (20)	59 (25)	<.001
Male sex, n (%)	62 (55.9)	32 (52.5)	30 (60.0)	.129
APACHE II score	17 (8)	18 (8)	16.5 (9)	.717
CURB-65	4 (2)	4 (2)	3 (2)	<.001
P/F ratio, mm Hg	190 (123)	202 (114)	189 (140)	.196
SOFA score	7 (5)	7 (6)	7 (5)	.95
Positive blood cultures, n (%)	14 (12.6)	10 (16.4)	4 (8.0)	.254
Duration of MV, d	9 (11)	9.5 (15)	8 (9)	.093
Septic shock at ICU admission, n (%)	69 (62.2)	39 (63.9)	30 (60.0)	.698
Weaned from vasopressors on D5, ^b n (%)	30 (43.5)	13 (33.3)	17 (56.7)	.053
COPD, n (%)	28 (25.2)	23 (37.7)	5 (10.0)	.001
CRP, mg/dL	16.3 (15)	15.7 (12.9)	17.8 (16.2)	.014
ICU-acquired infection, n (%)	36 (32.4)	23 (37.7)	13 (26.0)	.225
ICU LOS, d	13 (15)	15 (15)	11 (10)	.003
Hospital LOS, d	18 (24)	20 (28)	14 (21)	.023
ICU mortality, n (%)	29 (26.1)	15 (24.6)	14 (28.0)	.828
Hospital mortality, n (%)	34 (30.6)	18 (29.5)	16 (32.0)	.837

Results are expressed as median (25%-75% IQR).

^a For comparisons among patients treated with and without systemic corticosteroids.

^b Excluding those who died before D5, a total of 68 patients were on vasopressors at D1.

(Fig. 1). The main characteristics of the study population are presented in Table 1. Overall, 86 (78%) patients had relevant comorbidities; and 28 (25%) had a diagnosis of COPD. All patients were directly admitted to the ICU from the emergency department. Antimicrobial therapy was prescribed in accordance with the ATS guidelines [15] to all patients. In our group of CAP patients, we identified 25 microbial agents in respiratory samples and/or blood cultures from a total of 23 patients. All patients received appropriate empiric antibiotic therapy.

3.2. Outcome analysis

Overall, the ICU and hospital mortality of CAP patients requiring invasive MV was 26% and 34%, respectively. Table 2 shows detailed data of survivors and nonsurvivors. As expected, age and APACHE II were significantly higher in nonsurvivors. However, there were no significant differences between the 2 groups for baseline CURB-65, SOFA scores, CRP levels, frequency of positive blood cultures, or the presence of COPD.

Age, vasopressor use, septic shock, ICU-acquired infection, CURB-65, SOFA, and APACHE II scores were entered in multivariate analyses. As expected, potential collinearity between the SOFA and APACHE II scores (Pearson correlation coefficient, $r = .422$) was observed. Therefore, 2 models were fitted containing either the APACHE II or the SOFA score. Older age was selected in both models; APACHE II was retained, but SOFA score was excluded from the respective final model (Table 3). Both models had reasonable discrimination and calibration.

Table 3 Multivariate analyses of factors associated with increased hospital mortality in patients with severe CAP requiring invasive ventilation (N = 111)

Variables	Coefficient	OR (95% CI)	P value
<i>Model containing the APACHE II score</i>			
Age, y	0.037	1.038 (1.007-1.07)	.015
APACHE II score, points	0.078	1.081 (1.006-1.16)	.033
Constant	-4.817		
<i>Model containing the SOFA score</i>			
Age, y	0.039	1.039 (1.01-1.07)	.009
Constant	-3.434		
Model containing the APACHE II score: area under receiver operating characteristic curve (c statistics) = 0.714 (95% CI, 0.618-0.809); Hosmer-Lemeshow goodness-of-fit ($\chi^2 = 7.248$, $P = .51$). Model containing the SOFA score: area under receiver operating characteristic curve (c statistics) = 0.649 (95% CI, 0.546-0.752); Hosmer-Lemeshow goodness-of-fit ($\chi^2 = 5.663$, $P = .685$).			

3.3. Use of corticosteroids and mortality

Corticosteroids were always prescribed by parenteral route in the first 24 hours of ICU admission. A total of 61 (55%) patients received corticosteroids at an equivalent of methylprednisolone dose (median) of 60 mg/d for a median duration of 7 days. The main reasons for their use were bronchospasm ($n = 32$, 52.5%) and septic shock ($n = 22$, 36%).

No significant differences in mortality were observed in patients treated with and without corticosteroids (29.5% vs

Table 2 Comparisons between survivors and nonsurvivors of severe CAP requiring invasive MV

	All patients (N = 111)	Nonsurvivors (n = 34, 30.6%)	Survivors (n = 77, 69.4%)	P value ^a
Age, y	69 (25)	71.5 (18)	64 (25)	.002
Male sex, n (%)	62 (55.9)	19 (55.9)	43 (55.8)	1.0
APACHE II score	17 (8)	20 (8)	17 (9)	.013
CURB-65	4 (2)	4 (1)	4 (2)	.375
P/F ratio, mm Hg	190 (123)	207 (138)	188 (117)	.678
SOFA score	7 (5)	8 (6)	7 (5)	.177
Positive blood cultures, n (%)	14 (12.6)	5 (14.7)	9 (11.7)	.758
Septic shock at ICU admission, n (%)	69 (62.2)	24 (70.6)	45 (58.4)	.290
COPD, n (%)	28 (25.2)	9 (26.5)	19 (24.7)	.818
CRP, mg/dL	16.3 (15)	15.2 (18.8)	16.3 (14.1)	.460
Use of corticosteroids, n (%)	61 (55.0)	18 (52.9)	43 (55.8)	.837
Median daily dose of corticosteroids, equivalent to methylprednisolone in mg	60 (16)	60 (20)	60 (14)	.539
Duration of corticosteroids, d	7 (6)	6 (6)	7 (6)	.787
ICU LOS, d	13 (15)	13 (23)	13 (10)	.505
Hospital LOS, d	18 (24)	19 (19)	15.5 (36)	.307
ICU-acquired infection, n (%)	36 (32.4)	24 (31.2)	12 (35.3)	.667

Results expressed as median (25%-75% IQR).

^a For comparisons among survivors and nonsurvivors.

Table 4 Propensity score for the use of systemic corticosteroids in patients with severe CAP requiring invasive MV (N = 111)

Variables	Coefficient	OR (95% CI)	P value
COPD	1.933	6.91 (2.034-23.47)	.002
CURB-65	0.927	2.527 (1.679-3.802)	<.001
Constant	-2.239		

32%, $P = .837$). In addition, corticosteroids therapy had no significant impact on the weaning from vasopressors of septic shock patients ($n = 69$) by D5 (33% vs 57%, respectively; $P = .053$).

Furthermore, patients treated with and without corticosteroids developed similar rates of ICU-acquired infections (38% vs 26%, respectively; $P = .225$). Finally, no differences in mortality were observed when subgroup analysis was performed for the presence of septic shock and SOFA score.

However, significant imbalances were present when the 2 populations were compared. Patients treated with corticosteroids were older (73 [20] vs 59 [25] years, $P < .001$), had higher CURB-65 score (4 [2] vs 3 [2], $P < .001$), had more frequent diagnosis of COPD (37.7% vs 10.0%, $P = .001$), and presented a lower CRP level at admission (15.7 [12.9] vs 17.9 [16.2] mg/dL, $P = .014$). Nevertheless, we performed a logistic regression analysis and did not observe an independent association between the use of corticosteroids and hospital mortality. Finally, the propensity score for the use of corticosteroids was forced into the final models and was not selected (OR = 1.140 [0.998-1.303], $P = .053$ for the model containing the SOFA score and OR = .266 [0.36-1.974], $P = .195$ for the model containing the APACHE II score, respectively) (Table 4); and again, no association with improved mortality could be demonstrated.

In addition, the use of corticosteroids was not independently associated with an increased risk of ICU-acquired infection (OR = 1.723 [0.761-3.899], $P = .192$).

Finally, both the ICU and hospital lengths of stay (LOSs) were significantly higher in corticosteroid-treated patients (15 [15] vs 11 [10] days, $P = .003$; 20 [28] vs 14 [21] days, $P = .023$, respectively).

We also analyzed patients treated with corticosteroids according to the clinical indication, namely, for septic shock ($n = 22$) and bronchospasm ($n = 32$), to examine possible

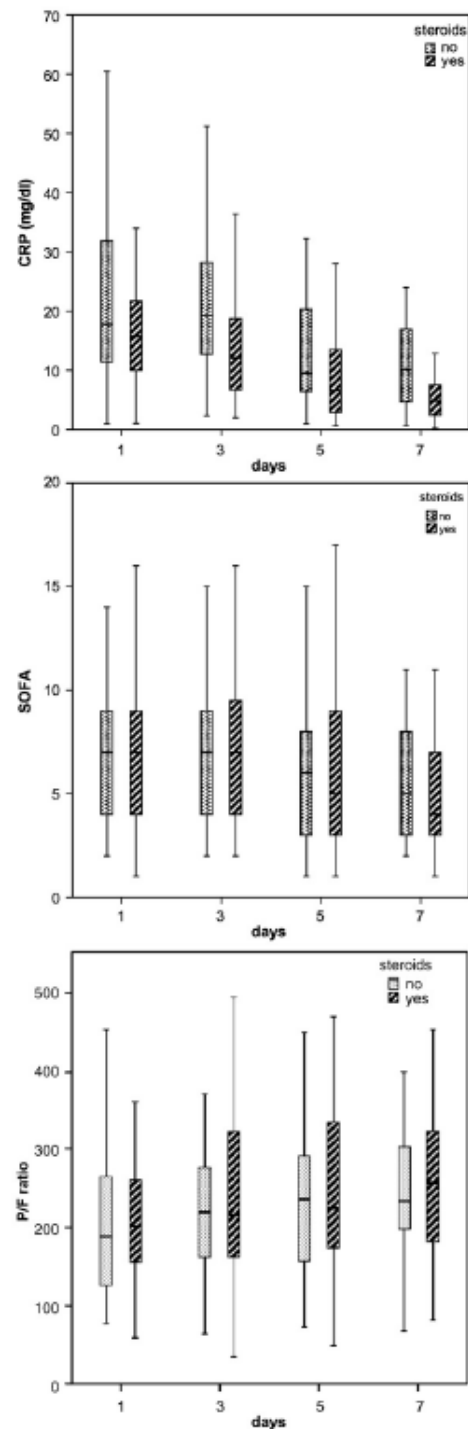


Fig. 2 Time-dependent analysis of CRP, SOFA score, and P/F ratio during antibiotic therapy, according to corticosteroid therapy. Data are presented as box plots. From D1 to D7, CRP, SOFA, and P/F ratio courses were not statistically different in severe CAP patients requiring invasive MV treated with and without corticosteroids ($P = .147$, $P = .952$, and $P = .737$, respectively).

differences in these subgroups. Concerning age (75 [23] vs 73 (20) years, $P = .58$), sex distribution (male, n [%]: 7 [31.8] vs 19 [59.4]; $P = .071$), D1 SOFA score (7.5 [8] vs 6 [5], $P = .205$), ICU LOS (14 [18] vs 15 [11] days, $P = 1.0$), hospital LOS (22.5 [36] vs 20.5 [18] days, $P = .839$), ICU mortality (22.7% vs 18.8%, $P = .103$), and hospital mortality (36.6% vs 18.8%, $P = .105$), no significant differences were found between patients with septic shock and bronchospasm treated with corticosteroids, respectively.

3.4. Impact of corticosteroids in the course of CRP, SOFA score, and oxygenation

At D1, before steroid therapy was started, CRP levels were somewhat lower in patients treated with corticosteroids (15.7 [12.9] vs 17.8 [16.2] mg/dL, $P = .014$). Even so, the time-dependent analysis of CRP (Fig. 2), from D1 to D7 of antibiotic therapy, showed that CRP decreased similarly in both groups ($P = .147$). At D1, SOFA score (7 [6] vs 7 [5], $P = .95$) and P/F ratio (202 [114] vs 189 [140] mm Hg, $P = .196$) were comparable in patients treated with and without corticosteroids, respectively. Likewise, the time-dependent analysis of SOFA score and P/F ratio showed an almost parallel course in both groups ($P = .952$ and $P = .737$, respectively), decreasing in the SOFA score analysis and increasing in the P/F ratio analysis.

4. Discussion

To date, only a few studies evaluated the impact of systemic corticosteroid administration on the outcomes of patients with severe CAP [7,8,24-26], most involving small samples and only one evaluated exclusively critically ill patients [7]. Our study is the largest cohort of severe CAP patients requiring invasive MV ($N = 111$) where the impact of systemic corticosteroids on mortality, organ failures, and the evolution of systemic inflammation, assessed by serial measurements of CRP, could be evaluated.

In the 61 (55%) patients who received corticosteroids, we were unable to find any positive impact in survival rate, in resolution of organ failures, or in the course of CRP, even after adjusting for other relevant clinical characteristics and for the probability of having received corticosteroids.

The potential benefits of corticosteroids in severe CAP are usually attributed to their immunomodulatory and hemodynamic effects [6,9,27]. In addition, in recent guidelines, their use is recommended for patients with severe CAP [4]. Confalonieri et al [7], in a small ($N = 46$) RCT, evaluated 23 patients treated with parenteral corticosteroids, with a dose equivalent to 48 mg/d of methylprednisolone, for 7 days. The authors observed significant improvements in mortality, systemic inflammation, and oxygenation [7]. These results were not subsequently reproduced [11]. In fact, neither a

recent systematic review of the literature [10] nor an RCT involving non-ICU patients hospitalized for CAP [11] could demonstrate significant survival benefits from corticosteroid therapy. In addition, subgroup analysis of mechanically ventilated CAP patients also showed no differences in outcomes [11]. In addition, in this study, Snijders et al [11] found a significant increase in the recurrence of signs and symptoms of pneumonia after 72 hours of treatment, despite an initially beneficial response to treatment in patients treated with systemic corticosteroids.

Besides mortality, the resolution of systemic inflammation is usually considered a relevant end point for studies assessing the efficacy of adjuvant therapies in severe CAP [28]. C-reactive protein is the prototype of an acute phase reactant [29]; and treatments and/or interventions that resolve or control the primary inflammatory insult, responsible for the acute phase reaction, should change its concentration [12]. In a previous study, we demonstrated that persistently elevated CRP levels are associated with an ongoing bacterial infection refractory to antibiotics [30,31]. However, the available data concerning the effect of corticosteroid therapy on CRP course are still a matter of debate. One study that evaluated patients with severe CAP reported that corticosteroid therapy could blunt CRP response to infection [7]. In contrast, Perren et al [24] were unable to demonstrate any difference in the time course of CRP in a small group of CAP patients ($N = 20$) that received systemic corticosteroids, as compared with controls that received only antimicrobials. Also in the present cohort of patients with severe CAP requiring invasive MV ($N = 111$), we observed that the CRP time course over the first week of antibiotic therapy showed similar rates of decline in patients treated with and without corticosteroid therapy. These results are consistent with a recent RCT involving non-ICU patients with CAP, where CRP concentrations at the end of the second week were comparable in patients treated with and without prednisolone [11].

We also assessed the time courses of the resolution of extrapulmonary organ failures and of oxygenation recovery and observed that they were not affected by the use of corticosteroids. These findings were consistently demonstrated by the monitoring of SOFA score and the P/F ratio and during the first week of ICU stay.

Altogether, we were unable to demonstrate any beneficial effect from the use of corticosteroids in our cohort of severe CAP patients requiring invasive MV, namely, ICU and hospital mortality. However, corticosteroid therapy was not associated with a higher incidence of ICU-acquired infection. Therefore, in severe CAP patients requiring steroids for clinical conditions such as bronchospasm or in patients on chronic steroid treatment, their administration seems to be safe and not associated with an increase risk of death. Finally, we found that patients treated with corticosteroids had a longer length of ICU and hospital stay ($P = .003$ and $P = .023$, respectively) and that these differences could not be attributed to differences in

clinical severity at admission, as both APACHE II and SOFA scores were well matched. Because myopathy and other steroid-related adverse events were not systematically evaluated, we cannot exclude that the findings in the present study could be associated with these factors. However, the higher rate of ICU-acquired infections in patients treated with corticosteroids could by itself prolong the LOS. Nevertheless, in a recent RCT involving non-ICU patients with CAP, with a low number of patients on MV, LOS was not significantly different in patients treated with and without prednisolone [11].

Our study has important strengths. If this is the largest multiple-center epidemiologic study assessing the impact of parenteral corticosteroids in severe CAP patients requiring invasive MV, the study also has limitations. Furthermore, the data in the present study came from 2 independent databases collected in different periods. As a result, we cannot exclude a time-dependent effect on the results. Because the study was not randomized, the use of corticosteroids could not be controlled. The indications, doses, and weaning of steroids varied among patients. As previously mentioned, adverse events such as hyperglycemia and myopathy were not systematically addressed. Nevertheless, despite adjusting for severity of illness and confounding factors, no beneficial effects of corticosteroids on the outcomes could be observed. Moreover, adjustment for potential bias of indication and use of steroids was performed using propensity analysis; and the results remained unchanged.

5. Conclusions

In the present study involving a large cohort of patients with severe CAP who required invasive MV, adjunctive corticosteroid therapy had no influence on mortality; on clinical course, evaluated both by SOFA score and P/F ratio; or on the resolution of the systemic inflammatory process, assessed by serial CRP measurements.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcrc.2010.07.014.

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Artigo 4: C-reactive protein and procalcitonin profile in ventilator-associated lower respiratory infections.

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C-reactive protein and procalcitonin profile in ventilator-associated lower respiratory infections



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ABSTRACT

Purpose: Ventilator-associated tracheobronchitis (VAT) has been suggested as an intermediate process between tracheobronchial colonization and ventilator-associated pneumonia (VAP) in patients receiving mechanical ventilation. The aim of this study was to evaluate the ability of C-reactive protein (CRP) and procalcitonin (PCT) to differentiate between VAT and VAP.

Methods: Pre-planned analysis of the prospective multinational TAVeM database, performed on 2960 patients receiving mechanical ventilation for >48 h, including 689 patients with VA-LRTI. Patients with the diagnosis of VAT or VAP microbiologically documented and with one measurement of CRP and/or PCT on the day of diagnosis were included.

Results: Four hundred and four patients (mean age 63 years, 298 men, ICU mortality 40%) were studied, 207 with VAT and 197 with VAP. On the day of infection diagnosis, the median CRP was elevated in both groups but significantly higher in VAP (18 mg/dL vs. 14 mg/dL, $p = .001$). Median PCT was also significantly higher in VAP (2.1 ng/dL vs. 0.64 ng/dL, $p < .001$). Both biomarkers could not help distinguish between VAT and VAP.

Conclusion: Although PCT and CRP presented lower values in VAT as compared to VAP, there was a marked overlap of both biomarkers values in both VA-LRTI not allowing adequate discrimination.

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1. Introduction

Ventilator-associated lower respiratory tract infections (VA-LRTI) are the most common nosocomial infections in the intensive care unit (ICU), affecting up to half of the mechanically ventilated patients [1,2]. These infections comprise ventilator-associated pneumonia (VAP) and ventilator-associated tracheobronchitis (VAT). VAP is associated with increased duration of MV, intensive care unit (ICU) and hospital length of stay (LOS), morbidity, mortality, antibiotic consumption and costs [3,4]. On the other hand, VAT is considered an intermediate process between colonization of the lower respiratory tract and VAP [5]. We recently published the TAVeM study [2] showing that VAT is a frequent

complication of MV and it is also associated with increases in the duration of MV and LOS in the ICU but presents lower mortality than VAP. Although it remains a controversial issue, an appropriate antibiotic treatment for both VAT and VAP was associated with improved outcomes and on the other hand inadequate treatment of VAT was associated with a higher risk of progression to VAP [5]. Thus, the ability to distinguish between these two infections is critical of a successful treatment.

Several biomarkers have been studied with potential use in the diagnosis of respiratory infection. The two of the most studied biomarkers are C-reactive protein (CRP) and procalcitonin (PCT) [6]. Both biomarkers have been assessed in several infectious clinical settings and also have strengths and limitations that should be acknowledge. Several factors, such as immunosuppression, renal or hepatic failure can influence their behavior [7–9].

In the present study, we aimed to compare CRP and PCT concentrations and evaluate its ability to distinguish between VAP and VAT.

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2. Materials and methods

The TAVEM study [2] was a prospective international multicenter observational study in 114 ICUs across eight countries in Europe and South America (Spain, France, Portugal, Brazil, Argentina, Ecuador, Bolivia, and Colombia). Data were prospectively collected from patients older than 18 years admitted to their ICUs who received MV for >48 h between the preplanned dates of Sept 1, 2013, and July 31, 2014. All participating centers received ethics approval from their institutions. Informed consent was waived because of the observational nature of the study.

Patients were prospectively followed up for outcome until death or ICU discharge. Demographical data were obtained along with clinical data including comorbidities, severity of illness scores, antibiotic use, and diagnostic procedures for VAP and VAT. Patients with severe hepatic failure were excluded from data analysis. The diagnosis of VA-LRTI was based on the presence of at least two of the following criteria: body temperature > 38.5 °C or < 36.5 °C, leucocyte count >12,000 cells/ μ L or < 4000 cells/ μ L, and purulent endotracheal aspirate (ETA). Additionally, all episodes of infection had to have a positive microbiological isolation in the ETA of at least 10^5 colony-forming units (CFU)/mL, or with bronchoalveolar lavage (BAL) of at least 10^4 CFU/mL, to be included in the final analysis.

VAT was defined with the aforementioned criteria with no radiographical signs of new infiltrate; VAP was defined by the presence of new or progressive infiltrate on chest radiograph. VAP was deemed as occurring subsequently to VAT if it was diagnosed in the 96 h period after diagnosis of tracheobronchitis and the same microorganism. VAP was considered as early-onset when it was diagnosed <5d, and late-onset when it was diagnosed \geq 5d, after starting MV [10]. Empirical antibiotic therapy was defined as that given before microbiological documentation of infection. Antibiotic treatment was considered appropriate when at least one antibiotic, active in vitro on all microorganisms causing VA-LRTI, was administered to treat these infections [11]. Microbiological identification and susceptibility tests were performed using standard methods. Multidrug-resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [12]. Circulating levels of CRP were measured using an immunoturbidimetric method and PCT was measured in serum by immunoluminometry. For purposes of infection diagnosis it were considered a cut-off of 4 mg/dL for CRP and of 0.25 ng/dL for PCT [6]. Biomarkers were measured in each hospital at the day of microbiological sampling. Only the first episode of VA-LRTI was considered per

patient in the final analysis. More details on methods are available elsewhere [2].

2.1. Statistical analysis

Data are expressed as medians [with interquartile range (IQR)] unless specified otherwise and were compared as follows: comparisons between groups were performed with unpaired Student's t-test, one-way ANOVA, Mann-Whitney U or Kruskal-Wallis H-tests for continuous variables according to data distribution. Chi-square tests were used to carry out comparisons between categorical variables. Multiple receiver-operating characteristics (ROC) curves were used to compare each biomarker for the ability to differentiate between VAT and VAP. This analysis was performed with SPSS Statistics 21. A two-sided *p* value <.05 was considered statistically significant.

3. Results

Among the 2960 mechanically ventilated patients, 689 patients developed VA-LRTI with microbiological documentation, being 320 VAT and 369 VAP. On the day of infection diagnosis, a measurement of PCT and/or CRP was performed in 404 patients (207 with VAT and 197 with VAP) whose fulfilled eligibility criteria and were evaluated (Fig. 1).

Patients with VAT and VAP presented overall similar characteristics as age, Simplified Acute Physiology Score (SAPS) II score, Sequential Organ Failure Assessment (SOFA) score, McCabe's score, duration of MV, acute respiratory distress syndrome (ARDS), ICU and hospital length of stay (Table 1). The therapy with systemic corticosteroids had no influence on the CRP levels ($n = 31$ vs. $n = 373$, median [IQR] 13.9 [14.3] mg/dL vs. 15.6 [17.2] mg/dL, $p = .346$, respectively). However, PCT was significantly lower in patients under systemic corticosteroids therapy ($n = 31$ vs. $n = 373$, median [IQR] 0.4 [2.2] mg/dL vs. 1.4 [7.2] mg/dL, $p = .003$, respectively). Patients with VAP had a higher incidence of septic shock and ICU mortality ($p < .001$).

On the day of infection diagnosis, plasma CRP concentration in VAP patients was significantly higher than in VAT (median [IQR] 18 [18.9] mg/dL vs. 14 [16.6] mg/dL, respectively, $p = .001$). Similarly, plasma PCT was also significantly higher in VAP than in VAT (median [IQR] 2.1 [12.9] ng/dL vs 0.64 [2.9] ng/dL, respectively, $p < .001$) (Fig. 2). CRP and PCT concentrations presented a marked overlap between both groups. On the day of diagnosis of VA-LRTI the area under the ROC curve (AUC) for the ability of CRP to differentiate between VAT and

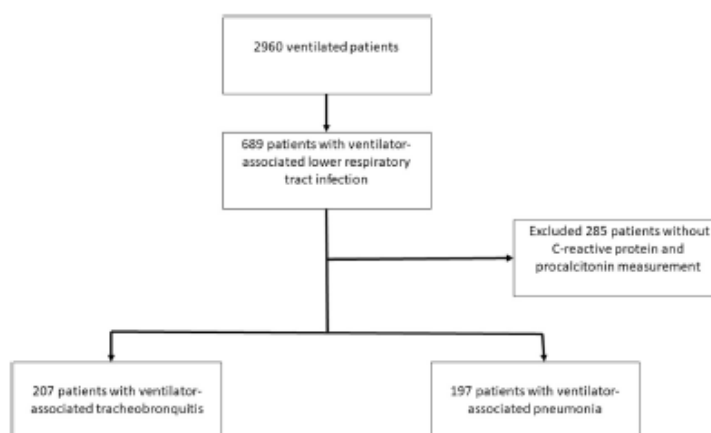


Fig. 1. Study flowchart.

Table 1
Baseline characteristics of patients.

	All patients with VA-LRTI (N = 404)	VAT (n = 207)	VAP (n = 197)	p-value
Age (years)	63 [25]	65 [24]	62 [27]	0.103
Male gender, n (%)	298 (74%)	139 (67%)	159 (80%)	0.112
SAPS2 (points)	50 [26]	51 [26]	48 [26]	0.134
SOFA (points)	8 [6]	8 [5]	8 [5]	0.600
Mechanical ventilation (days)	16 [17]	14 [14]	15 [18]	0.570
ARDS at diagnosis, n (%)	42 (10%)	18 (8%)	24 (12%)	0.254
Septic shock at diagnosis, n (%)	127 (31%)	33 (16%)	94 (48%)	< 0.001
ICU LOS (days)	22 [22]	22 [20]	22 [23]	0.350
Hospital LOS (days)	37 [40]	39 [34]	34 [37]	0.700
ICU mortality, n (%)	162 (40%)	69 (33%)	93 (47%)	0.029

VA-LRTI: ventilator-associated lower respiratory tract infections; VAT: ventilator-associated tracheobronchitis; VAP: ventilator-associated pneumonia; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment; ARDS: acute respiratory distress syndrome; ICU: intensive care unit; LOS: length of stay.

VAP was 0.6 (95% confidence interval (CI), 0.54–0.65) and that of PCT was 0.63 (95% CI 0.57–0.70) (Fig. 3).

We assessed both biomarkers in early-onset and late-onset VAT and VAP and did not find any differences (CRP in early-onset vs late-onset VAT median [IQR] 15.4 [15] mg/dL vs 12.5 [15.2] mg/dL, respectively, $p = .122$; PCT in early-onset vs late-onset VAT median [IQR] 1 [5.6] ng/dL vs 0.5 [1.1] ng/dL, respectively, $p = .584$; CRP in early-onset vs late-onset VAP median [IQR] 19.3 [19.4] mg/dL vs 17 [17.4] mg/dL, respectively, $p = .156$; PCT in early-onset vs late-onset VAP median [IQR] 3.6 [20.3] ng/dL vs 1.1 [11.9] ng/dL, respectively, $p = .074$).

We also found that roughly 15% of VAT patients and 12% of VAP patients had PCT values below 0.25 ng/dL ($p = .32$), and 24% of VAT patients and 23% of VAP patients had PCT values below 0.5 ng/dL ($p = .75$), while 14% of VAT patients and 7% of VAP patients had CRP values below 4.0 mg/dL ($p < .05$).

Patients with VAT and VAP were divided into 3 clinical groups according to the ACCP/SCCM Consensus Conference criteria [13], namely those with sepsis, severe sepsis, and septic shock. The levels of PCT were significantly different according to the clinical severity ($p = .008$), whereas, with CRP that was not the case ($p = .097$). In patients with sepsis ($n = 211$), severe sepsis ($n = 80$) and septic shock ($n = 113$), PCT concentrations were 0.69 [5.1], 1.09 [8.1] and 2.5 [5.4] ng/dL, respectively whereas CRP levels were 15 [16], 16 [13.9] and 20 [20.9] mg/dL, respectively. Comparing patients with ($N = 113$) and without ($N = 291$) septic shock PCT was again significantly higher (PCT 2.5 vs 0.8 ng/dL, $p = .005$; CRP 20 mg/dL vs 13.3 mg/dL, $p = .052$).

3.1. Microbiological characteristics

Assessing the infections caused by Gram-positive and Gram-negative pathogens, we observed similar plasma CRP and PCT concentrations (15.9 [17.5] mg/dL vs 15.5 [16.8] mg/dL, $p = .81$; 0.55 [3.5] ng/dL vs 1.4 [7.1] ng/dL, $p = .10$, respectively).

The concentrations of both biomarkers in VAT and VAP caused by MDR ($n = 275$) and non-MDR ($n = 129$) microorganisms were not significantly different (CRP 16.3 [17.1] mg/dL vs. 14.1 [17.8] mg/dL, respectively, $p = .21$; PCT 1.17 [5.2] ng/dL vs. 0.98 [8] ng/dL, respectively, $p = .53$). Also, in patients with VA-LRTI, the comparison of both biomarkers between different microorganisms showed no significant differences. Median plasma CRP values in *Staphylococcus aureus* ($n = 84$), *Pseudomonas aeruginosa* ($n = 109$), *Enterobacteria spp* ($n = 180$) and *Acinetobacter baumannii* ($n = 31$) were 16 [16.3] mg/dL, 12.5 [16.2] mg/dL, 15.8 [17.5] mg/dL and 20.7 [14.5] mg/dL ($p = .72$), respectively. Median plasma PCT values of the same microorganisms described above were respectively 0.61 [3.14] ng/dL, 0.8 [4.4] ng/dL, 1.8 [6.9] ng/dL and 5.2 [13.3] ng/dL, respectively ($p = .13$).

4. Discussion

Our study showed that PCT and CRP presented lower values in VAT in comparison with VAP, however, as there was a large overlap of both biomarkers they are not useful in distinguishing these infections, VAT from VAP. Also, none of the biomarkers were able to distinguish between the different microbiological etiologies of VAT and VAP.

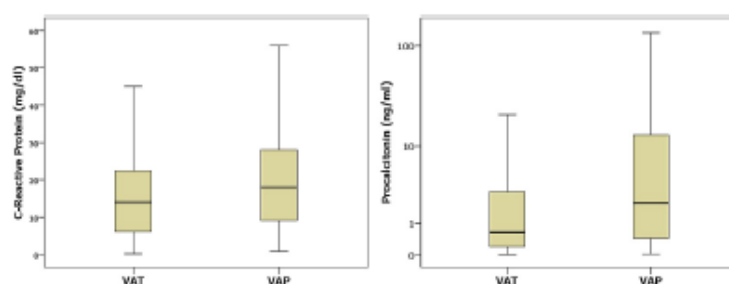


Fig. 2. Box-plot of C-reactive protein and procalcitonin levels on the ventilator-associated tracheobronchitis and ventilator-associated pneumonia at day of diagnosis. CRP: C-reactive protein; PCT: procalcitonin; VAT: ventilator-associated tracheobronchitis; VAP: Ventilator-associated pneumonia. Note: PCT presented with logarithmic scale.

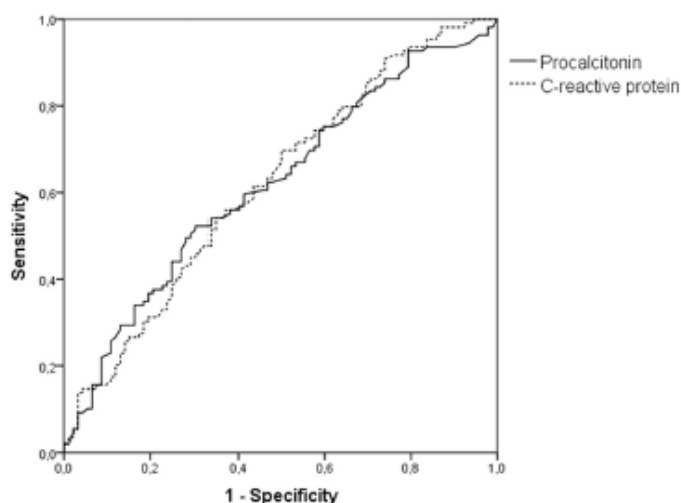


Fig. 3. Receiver operating characteristics (ROC) curves of C-reactive protein (CRP) (dashed line) and procalcitonin (PCT) (solid line) on the day of diagnosis of VA-LRTI for the ability of biomarkers to differentiate between ventilator-associated tracheobronchitis and ventilator-associated pneumonia.

Several studies had already demonstrated that CRP and PCT could be helpful in the diagnosis of VAP [14,15], but data in VAT are scarce. Our study showed that in VAT both CRP and PCT rise but less than in VAP. A significant number of patients with VA-LRTI presented very low levels of both biomarkers despite the microbiological confirmation. This has already been observed in other studies, especially with PCT, being partially explained by the relatively low virulence of many of the causative microorganisms found in ventilator-associated pneumonia [9,16].

In a previous study [17], involving 34 patients with documented VAP, the PCT values of patients infected with Gram-positive and Gram-negative microorganisms were not significantly different. In our study, with a much larger sample size ($N = 404$), both biomarkers did not show different values according to the isolated microorganism as well as according to the Gram stain. More recently, Thomas-Ruddel et al. found significant higher PCT concentrations in patients with Gram-negative bacteremia than in patients with Gram-positive bacteremia or candidemia [18]. In other study, patients with *Legionella pneumophila* community-acquired pneumonia had higher CRP levels than those with pneumonia of any other aetiology [19].

Likewise, biomarkers values, CRP and PCT, were not significantly different between infections caused by non-MDR and MDR microorganisms. Therefore, these biomarkers at baseline were not useful to distinguish VAT from VAP nor to guide in the presumption of the causative microorganism, namely Gram-positive/Gram-negative or the presence of MDR agents.

Patients with septic shock had significantly higher PCT values than patients with sepsis and severe sepsis. As in other studies, PCT showed to be useful in stratifying patients' severity [17,20–22]. In the study by Hillas et al. [23], which included 45 patients with microbiologically documented VAP, patients who developed septic shock had significantly higher PCT levels on D1 and D4. On the other hand, CRP was not associated with clinical severity as we have already showed [24]. Overall, CRP has a better performance as a diagnostic marker of infection than as prognostic marker [15].

In the present study we came to similar results since systemic steroids at the day of LRTI diagnosis (either VAT or VAP) had no impact on CRP levels. Our group have already showed that in severe community-acquired pneumonia (SCAP) patients the CRP course was not influenced by the adjuvant therapy with systemic steroids [25]. In other words, it seems that in infected patients CRP levels are not

dependent of the underlying pathology and not modified by any treatment or intervention (steroids/neutropenia/renal replacement therapy) unless interventions related to the source control and/or antibiotic therapy [8,25,26]. In contrast, we found that PCT levels were significantly lower in patients under systemic corticosteroid therapy. Similar results have been previously found by al-Nawas [27] et al., in a cohort of immunosuppressed septic patients.

The main limitation of our study is that it was not designed specifically to assess the diagnostic value of biomarkers in VAP and VAT. Second, we could only evaluate one measurement of the biomarkers, not being able to assess the relative variations of both biomarkers before the day of infection diagnosis or in response to antimicrobial therapy which could have been helpful to identify cases of inadequate antimicrobial therapy such as in MDR pathogen infection. Third, we could not evaluate the diagnostic value of CRP and PCT, since our study didn't have a control group with patients without infection as in other studies [14,28–30]. Finally, we did not evaluate the influence of several factors such as immunosuppression, corticosteroids or dialysis in the results [31].

5. Conclusion

In our study, we evaluated, for the first time, the ability of biomarkers, CRP and PCT, to distinguish VAT from VAP. Due to the overlap of the values of both biomarkers, CRP as well as PCT cannot help distinguishing VAT from VAP.

Moreover, we clearly show that in VA-LRTI CRP and PCT levels are not dependent of the isolated microorganism, nor the presence of Gram-positive/Gram-negative microorganisms nor the presence of MDR.

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Artigo 5: The potential role of exhaled breath analysis in the diagnostic process of pneumonia - a systematic review.

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TOPICAL REVIEW

The potential role of exhaled breath analysis in the diagnostic process of pneumonia—a systematic review

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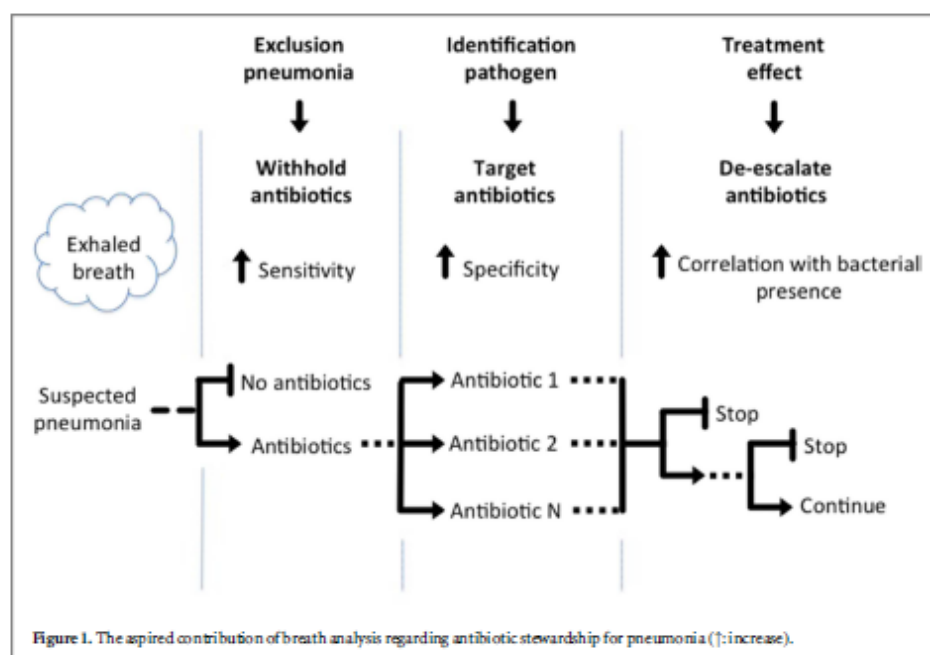
Abstract

Diagnostic strategies currently used for pneumonia are time-consuming, lack accuracy and suffer from large inter-observer variability. Exhaled breath contains thousands of volatile organic compounds (VOCs), which include products of host and pathogen metabolism. In this systematic review we investigated the use of so-called 'breathomics' for diagnosing pneumonia. A Medline search yielded 18 manuscripts reporting on animal and human studies using organic and inorganic molecules in exhaled breath, that all could be used to answer whether analysis of VOC profiles could potentially improve the diagnostic process of pneumonia. Papers were categorised based on their specific aims; the exclusion of pneumonia; the detection of specific respiratory pathogens; and whether targeted or untargeted VOC analysis was used. Ten studies reported on the association between VOCs and presence of pneumonia. Eight studies demonstrated a difference in exhaled VOCs between pneumonia and controls; in the individual studies this discrimination was based on unique sets of VOCs. Eight studies reported on the accuracy of a breath test for a specific respiratory pathogen: five of these concerned pre-clinical studies in animals. All studies were valued as having a high risk of bias, except for one study that used an external validation cohort. The findings in the identified studies are promising. However, as yet no breath test has been shown to have sufficient diagnostic accuracy for pneumonia. We are in need of studies that further translate the knowledge from discovery studies to clinical practice.

Introduction

The prevalence of community-acquired pneumonia (CAP) [1] and nosocomial pneumonia [2, 3] is high with substantial impact on morbidity and mortality [4–10]. Treatment of pneumonia is a balance between optimal antibiotic therapy for the patient (e.g. a combination of antibiotics that effectively target the

causative pathogen) and for the community (e.g. minimising the duration of exposure to broad spectrum antibiotics to limit antimicrobial resistance) [11]. In the ideal world, this balance would be met through a diagnostic test that is not only quick, non-invasive, reliable and available in real-time at the bedside, but most of all (1) excludes pneumonia in order to withhold antibiotic treatment from patients



without an infection; (2) enables targeting of antibiotic treatment of the causative pathogen; and (3) facilitates evaluation of the treatment response aiming to refine antibiotic de-escalation and duration of antibiotic treatment.

'Breathomics' refers to the analysis of volatile compounds in exhaled breath that resulted from, or are affected by metabolism [12]. The complete human breathome consists of thousands of compounds [13–17]. The volatile organic compounds (VOCs) that are present in the exhaled breath have various origins. Exogenous VOCs are derived from the environment and are taken in through inhalation or ingestion (e.g. via food or drugs). VOCs that are produced within the body can emerge as products of physiological metabolic processes from the host, as products of metabolic processes from microbial pathogens, or results from a host response to pathological processes such as infection or inflammation [18–20]. Changes, therefore, in host or microbial metabolism might lead to an impact on the composition of the exhaled breath profile.

In this systematic review we aim to investigate the potential role of exhaled breath analysis for diagnosing pneumonia, by providing (1) sensitive detection of pneumonia; (2) specific detection of the causative organism(s); and (3) a tool to monitor the treatment response after the initiation of antibiotics (see figure 1). We hypothesize that changed concentrations of VOCs in exhaled breath can be used to accurately discriminate patients with pneumonia from patients without pneumonia and may be used for specific identification of the causative pathogen.

Methods

Search

This is a systematic review following preferred reporting items for systematic reviews and meta-analyses guidelines, performed by two independent researchers. We searched Medline for potentially relevant articles up to 7 March 2017, using the following search terms: '(((Chromatography OR Spectrometry OR MS OR (Volatile AND Organic) OR Metabol*) AND breath) OR (volatile fingerprint*) OR (breathprin*) OR (electronic AND nose)) AND (pneumonia OR (lung infection*) OR (respiratoryinfection*) OR (lung bacteria*) OR (respirator* bacteria*))'. There was no restriction with respect to human or animal studies; but articles written in a language other than English and studies performed *in vitro* were excluded. Two authors (PvO and LB) reviewed the abstracts and/or full-text manuscripts independently and selected those that were regarded to be relevant. No disagreement on selection of articles was seen between the two reviewers.

Selection criteria

Inclusion criteria were (1) human or animal studies that (2) studied volatiles in exhaled breath to (3) diagnose bacterial pneumonia or identify the causative organism of pneumonia. Objective 3 as mentioned in the introduction (the evaluation of the treatment effect in patients with pneumonia) was let go, due to a lack of studies specifically investigating this. We excluded *in vitro* studies and studies that focused on very specific atypical causative organisms (such as *Aspergillus*).

Reference test

The diagnosis of pneumonia could be based on clinical symptoms alone, or could be supported by chest radiography and/or microbiology testing (cultures of endotracheal aspirate (ETA), non-directed bronchial lavage (NBL or mini-BAL) or bronchoalveolar lavage (BAL)). For CAP, the combination of clinical signs and symptoms with an evident infiltrate on the chest radiograph was considered a good reference test, while anything less was considered too non-specific. For ventilator-associated pneumonia, clinical signs, laboratory parameters, an infiltrate on chest radiography and quantitative cultures of BAL or NBL were considered an appropriate reference standard.

Index test

Advances in chemical analytics have enabled the measurement of inorganic [21, 22] and organic compounds [23–25] in biological matrices such as exhaled breath. Volatile molecules in breath can be studied via a targeted and an untargeted approach [12]. With the targeted approach the researcher identifies the molecules of interest beforehand and uses analytical assays to measure those compounds quantitatively. The untargeted approach entails analytical techniques that measure multiple molecules present in the breath. Untargeted analysis can be performed with mass-spectrometry-based techniques aimed to identify a variety of VOCs [26] or with so-called electronic nose technology that is based on pattern recognition [14, 27, 28]. The analytical details of these techniques are discussed in detail in previous publications [27, 29]. Figure 2 summarises the analytical methods that will be referred to in this systematic review. No single method is superior to the others, they provide different types of information, therefore the quality of the index test was assessed based on the use of an independent validation cohort, which has been shown to limit bias [27].

Methodological assessment and categorisation

The methodological quality of each selected full manuscript was evaluated using the QUADAS-2 tool by the same authors as describes above [30]. Risk of bias was assessed concerning patient selection, the interpretation or conduct of the index test, the interpretation or conduct of the reference standard and the patient flow. The papers were classified as either (1) studies concerning sensitive detection of the presence of pathogenic bacteria, either concerning studies investigating inorganic compounds, untargeted analysis of VOCs or eNose technology for discrimination between pneumonia and no pneumonia; or (2) studies investigating the use of VOC analysis for specific detection of pathogenic bacteria, in animals or in humans.

Results

The search was last updated on 7 March 2017 and yielded 321 articles, of which 18 were selected after screening on title/abstract and full text (figure 3). Of these, 13 studies were in humans and five were performed in murine models. Eight studies dealt with the detection of specific pathogenic bacteria, the others focused on discrimination between patients with and without pneumonia. One of the studies discussed treatment response. Table 1 demonstrates the areas of interest for each study and summarises the methodology used.

The studies were critically appraised and risk of bias was assessed regarding patient selection, index test, reference standard and flow and timing (table 2). The domain 'patient selection' was considered not applicable in the five animal studies. For most studies the risk of bias was valued as high, except for one that used a validation cohort [31], resulting in a low risk of bias regarding the index test.

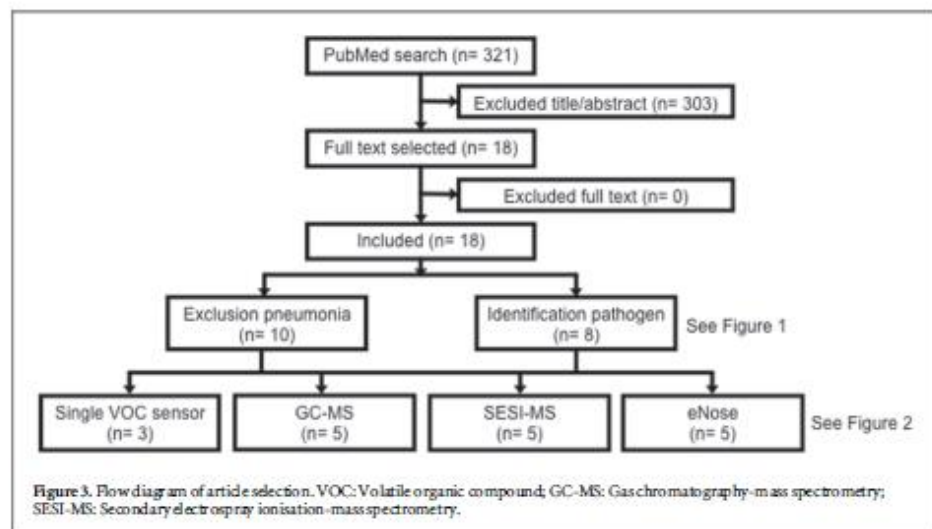
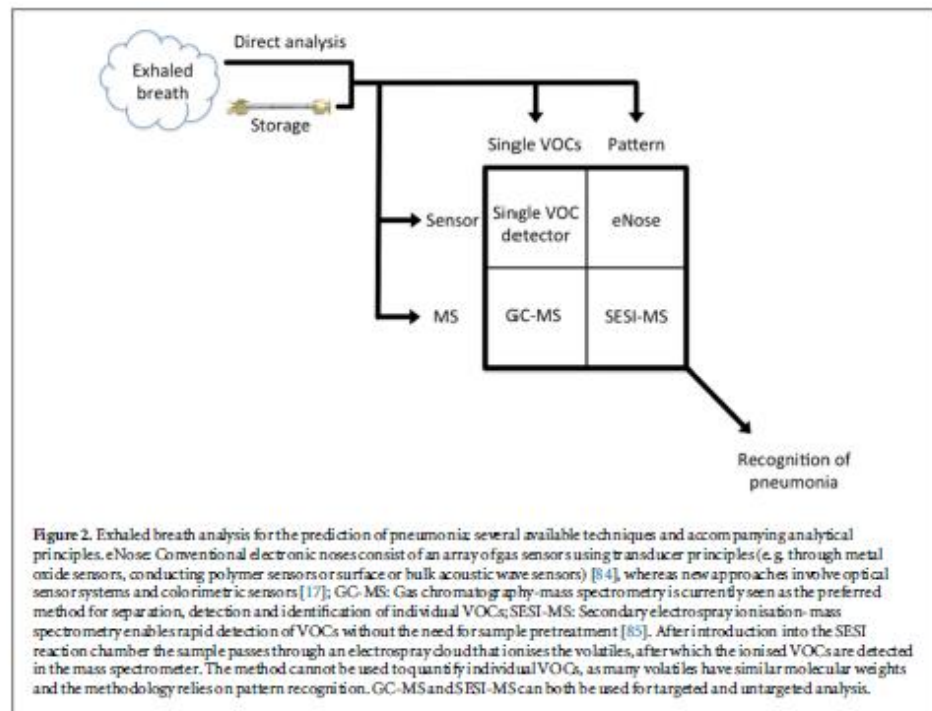
Discrimination between patients with and without pneumonia

Detection of volatile inorganic compounds

NO was not increased in the breath of a small group of patients admitted with pneumonia, when compared with control patients [32]. As expected it was elevated in patients with an exacerbation of asthma. This result was in contrast to the results of a larger study at less risk of bias (table 2) in which exhaled NO was measured in tracheal and nasal gas in patients ventilated within 72 h of ICU admission [31]. Some of these patients were later diagnosed with VAP and this was used as the reference standard. A validation cohort consisting of similar patients to the first group was used to determine sensitivity and specificity of the NO threshold that was calculated in the preceding group. NO concentrations were measured at multiple sampling points in the airway as well as in the nasal cavity, and significantly higher NO levels were found at all points in patients with pneumonia. Of these, the maximum (end-expiratory) tracheal NO values resulted in the highest sensitivity and specificity for the diagnosis of pneumonia: 88% and 76% respectively (see table 1). Results from one study with an imperfect reference test, namely subjective symptoms of lower respiratory infection, suggested a possible relationship between elevated exhaled CO levels and the clinical presence of pneumonia [33]. Notably, the exhaled CO concentration followed similar trends as the patients' symptoms after antimicrobial treatment.

Untargeted analysis of VOCs

The abundance of particular VOCs seems to be different in the breath of mechanically ventilated patients with pneumonia compared with those without pneumonia [34–36]. The results of studies using



gas chromatography and mass spectrometry (GC-MS, see figure 2), however, were not uniform. The described VOCs differed between studies and two compounds that were identified as being associated with VAP (ethanol and heptane) showed conflicting results in two studies (as shown in table 3). Differences between studies regarding investigated cohorts, reference standards and outcome measures (sensitivity, specificity and/or accuracy) can be found in table 1. Nevertheless, breath tests showed promising

discrimination between patients with and without pneumonia in the included clinical studies. The most frequently isolated pathogens in these studies were *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* [34–36].

Electronic nose technology

Preliminary results indicated a potential correlation between chest CT scans [37] or clinical pulmonary

Table 1. Full text selected articles.

Type	Study	Population	Sample size	Index test	Disease under study; reference test	Sens	Spec	Accuracy	Comments
C	31	ICU patients	49	NO analyser	VAP: clinical signs + culture of unknown specimen	88%	76%		
C	32	General ward	42	NO analyser	CAP: clinical signs + x-ray				Quantitative; lower NO concentration
C	47	Outpatient clinic	72	CO analyser	CAP: clinical signs			~90%	Accuracy estimated from figure
C	80	ICU patients	76	eNose	VAP: clinical signs			~90%–100%	
C	45	ICU patients	28	GC-MS	VAP: culture of BAL/ETA			Unknown	
C*	34	ICU patients	46	GC-MS	VAP: culture of ETA	98%	98%		Quantitative
C	66	ICU patients	60	GC-MS	VAP: clinical signs + radiology + culture of BAL / FSB / ETA			88%–89%	Inotely ventilated patients at risk for VAP
C	36	ICU patients	25	eNose	VAP: chest CT scan			91%–100%	
C	37	ICU patients	44	eNose	VAP: CPIS > 6			70%	
C*	33	ICU patients	100	GC-MS	VAP: clinical signs + radiology + culture of BAL	76%	73%		Only patients that were suspected of VAP
C	39	ICU patients	125	eNose	VAP: clinical signs + radiology + culture of BAL	76%	56%		Only patients that were suspected of VAP
C	38	COPD patients	43	eNose	CAP: sputum cultures	73%	76%		
C*	35	ICU patients	93	GC-MS	VAP: clinical signs + radiology + culture of ETA	72%	72%	73%	
P	43	Mix	12	SESI-MS	Instillation of MRSA or MSSA			100%	Experimental model with <i>Staphylococcus</i>
P	44	Mix	87	SESI-MS	Instillation of alive or lysated bacteria			100%	Experimental model with alive or dead bacteria
P	41	Mix	?	SESI-MS	Instillation of <i>Pseudomonas</i> or <i>Staphylococcus</i>			Unknown	Quantification of difference between <i>in vitro</i> and <i>in vivo</i>
P	40	Mix	42	SESI-MS	Instillation of seven different bacterial strains			Unknown	Quantification of difference between pathogens
P	42	Mix	?	SESI-MS	Instillation of <i>Pseudomonas</i> or <i>Staphylococcus</i>			Unknown	Quantification of dynamic changes in breath VOCs

C: Clinical; P: Pre-clinical; NO: Nitric oxide; COHD: Chronic obstructive pulmonary disease; FSB: Protected specimen brush; VAP: Ventilator-associated pneumonia; SESI-MS: Secondary electrospray ionisation-mass spectrometry; CPIS: Clinical Pulmonary Infection Score; GC-MS: Gas chromatography-mass spectrometry; BAL: Bronchoalveolar lavage; ETA: Endotracheal aspirate; Sens: sensitivity; Spec: specificity; VOC: Volatile organic compound.

* For details see table 3.

Table 2. QUADAS-2 (adapted version).

Study	Risk of bias				
	Year	Patient selection	Index test	Reference standard	Flow and timing
Adrie [31]	2001	No	No	No	No
Ali-Ali [32]	2001	Yes	Yes	Yes	No
Bean [44]	2014	N/A	Yes	No	No
Bean [45]	2015	N/A	Yes	No	No
Biernacki [33]	2001	Yes	Yes	Yes	No
Chiu [83]	2014	Yes	Yes	Yes	Unclear
Filipiak [46]	2015	Yes	Yes	No	No
Fowler [35]	2015	Unclear	Yes	No	No
Gao [47]	2016	Yes	Yes	No	No
Hockstein [37]	2004	Yes	Yes	Yes	No
Hockstein [38]	2005	Yes	Yes	Yes	No
Schnabel [34]	2015	Unclear	Yes	No	No
Schnabel [40]	2015	No	Yes	No	No
Van Geffen [39]	2016	Yes	Yes	No	No
Van Oort [36]	2017	No	Yes	No	No
Zhu [42]	2013	N/A	Yes	No	No
Zhu [41]	2013	N/A	Yes	No	No
Zhu [43]	2013	N/A	Yes	No	No

infection score (CPIS) [38] and the subsequent eNose sensor responses in mechanically ventilated patients. The eNose (see figure 2) seemed to distinguish patients with and without bacterial infection [39] (table 1). When specifically focusing on diagnosis of VAP, the eNose appeared to have good accuracy, moderate sensitivity and a rather poor specificity [40].

Specific detection of pathogens by VOC analysis

Secondary electrospray ionisation-mass spectrometry (SESI-MS, see figure 2) breathprint analysis was used to investigate the ability to identify respiratory infection caused by strains of *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Streptococcus pneumoniae* in mice [41–44]. Overall SESI-MS breathprints seemed to be able to distinguish between respiratory infection and no infection in mice and enabled differentiation between strains of aforementioned pathogens. A high degree of variation was seen when translating *in vitro* experiments to the *in vivo* VOC fingerprints [42]. The relative contribution of bacterial metabolism and host response on the exhaled breath profile could be inferred through an experiment in which mice were exposed to bacterial cell lysates [45]. This experimental set-up, using these bacterial cell lysates, allows for host and pathogen derived metabolites to be differentiated. The obtained SESI-MS breathprints changed over time after lysate exposure and appeared to (1) correlate to the host immune response; and (2) distinguish active infections of *P. aeruginosa* or *S. aureus* from cell lysate exposure.

Also using GC-MS specific VOCs in the exhaled breath seemed to reflect the presence of particular microorganisms in the respiratory tract and, in line

with the use of SESI-MS, direct translation of biomarkers from the *in vitro* to the *in vivo* setting proved difficult [46, 47]. In clinical studies, the evidence for specific detection of particular causative pathogens seemed speculative; as the published papers did not provide data on the accuracy of such measurements [46]. The only study that reported a high diagnostic accuracy for the identification of a causative pathogen focused on *Acinetobacter baumannii*. A set of eight compounds resulted in excellent separation of patients with *A. baumannii* pneumonia, colonisation with the same bacterium and controls. The major limitation of the described studies was that they did not evaluate the diagnostic accuracy of a breath test in the clinical scenario where such a test would be used, e.g. in patients with a clinical suspicion of VAP.

Discussion

Based on our systematic review, the presence of certain profiles or patterns of volatile molecules in the exhaled breath appeared to be associated with pneumonia. However, the precise identity of these volatile biomarkers remains largely unknown. Furthermore, none of the studied breath tests delivered results with sufficient clinical diagnostic accuracy that would likely impact on clinical decisions. Most of the available studies provided feasibility or proof of concept data with a substantial risk of bias and did not test a clear, pre-defined hypothesis.

There are two leads to follow in the diagnosis of pneumonia: measurement of the host response or direct identification of the pathogen [48, 49], both important establishing the ideal diagnostic test. *In vitro* results suggested that different pathogenic bacteria produced different volatile molecules, which

Table 3. VOCs identified by GC-MS: increased (↑) or decreased (↓) in breath of pneumonia versus no pneumonia patients.

Classification	Volatile organic compound	Reporting article		
		Schnabel [34]	Fowler [35]	Van Oort [36]
Ketone	Acetone	↓		↓
	2-methyl cyclopentanone		↓	
	Methylisobutylketone			↓
Aldehyde	Acrolein	↓		
	Nonanal		↑	
	Tetradecanal	↑		
Ether	Sevoflurane			↓
	Tetrahydrofuran	↓		
Alkane	2-methyl butane	↑		
	2-ethoxy-2-methylpropane			↓
	Carane	↑		
	Dodecane	↓		
	Heptane	↑	↓	
	Tetradecane	↑		
Alkene	2,6,11,15-tetramethyl-hexadecane		↑	
	Cyclohexene			↓
Terpene	3-carene		↑	
Alcohol	Ethanol	↑	↓	
	Isopropyl Alcohol	↓		
	Hexafluoroisopropanol			↓
	1-propanol			↓
Arene	Ethylbenzene	↑		
Ester	N-butyric acid 2-ethylhexyl ester		↑	
Sulfide	Carbon disulfide			↓
Amide	N-cyclohexyl-N(2-hydroxyethyl)thio-urea		↓	

might be used for identification [20]. One of the major challenges is that bacterial growth and metabolism are influenced by the chosen culture media, timing and the selection of particular strains and, therefore, may not be representative of growth *in vivo* [20]. A sterile inflammatory response altered the VOC release in several animal models of lung injury [21]. Thus, pneumonia may be recognised through exhaled breath analysis by detection of molecules produced either directly by the pathogen or through an altered host metabolism associated with the host response. Animal studies might offer advantages enabling the investigation of (1) a single bacterial infection, (2) the influence of timing on disease progression and (3) post-mortem histology for the gold-standard diagnosis of respiratory infection.

This systematic literature review demonstrates that certain volatile molecules could be useful as possible biomarkers for the diagnosis of pneumonia. One of them is nitric oxide (NO), a compound that has a bronchodilating and vasodilating effect in the respiratory tract and plays a key role in local inflammatory response [22]. NO is relatively easy to measure and thus forms an attractive candidate for diagnostic purposes [50, 51]. In the airways NO is produced by endothelial, epithelial and inflammatory cells. Generation of NO involves the oxidation of the amino acid L-arginine, a process that is catalysed by the enzyme NO synthase [52]. An increased concentration of exhaled NO is seen in asthma, bronchiectasis and

sepsis [53], and has also been associated with rhinitis, active pulmonary sarcoidosis and viral respiratory illnesses [52]. Table 3 shows other biomarkers of potential interest regarding the discrimination of patients with and without pneumonia. However, hardly any overlap is seen between the different VOCs reported in separate studies and they also show conflicting results for heptane and ethanol. Two studies found an association between pneumonia and a decrease in exhaled breath acetone. Generally, acetone is present in large quantities in the exhaled breath. Its decrease in the breath of pneumonia patients might be explained by a reduced ketogenesis that is seen during inflammation or infection [34].

As soon as a breath test fulfils the requirements for a diagnostic test for pneumonia, it shall be able to fulfil a role alongside the currently available and frequently used alternatives [54, 55] and can either compete with them, or complement them. The diagnosis of pneumonia relies on a combination of physical examination and chest radiography [56], potentially accompanied by measurement of inflammatory markers in plasma, urinary antigen testing [54], repeated determination of C-reactive protein [57] and collecting airway samples for microbiology cultures [58]. Current diagnostics lack clinical accuracy [59] and have high inter-observer variability [60]. Microbiology results take 48–72 h to become positive. The unnecessary prescription of antimicrobial treatment increases antimicrobial resistance [61–63], whereas applying the

wrong antibiotics is likely to increase mortality [64–66]. In order to withhold antibiotics, the CPIS [67] combines clinical and physiological data, pulmonary radiography and microbiology results into a numeric score that can be used to exclude pneumonia with moderate accuracy due to substantial inter-observer variability [68, 69]. Additionally, biomarkers like pulmonary interleukin-1 β (IL-1 β) and interleukin-8 (IL-8) measured in BAL fluid have shown promising results as discriminators for VAP [70, 71]. In the near future polymerase chain reaction of respiratory samples might be used to identify the causative pathogen rapidly and specifically [72–74] and serum procalcitonin has been proposed as an attractive candidate for determining antibiotic duration [75, 76]. How would exhaled breath analysis compete with these alternatives? In contrast to blood or BAL samples, breath can be collected completely non-invasive and it is continuously available. A breath test could also provide results rapidly and cost-effectively, which is important in the setting of pneumonia. A breath test with the right test characteristics could thus provide real opportunities for improved real-time diagnostic utility, patient acceptability and cost effectiveness.

Many different methods for breath sampling have been described in the literature, including but not limited to glass syringes, needle traps [77] steel or glass tubes filled with sorbent material and/or breath gas bags (e.g. Tedlar bags). Pre-concentration of the breath sample could be established through the absorption of the VOCs using for instance organic polymers (e.g. Tenax TA), graphitized carbon, activated charcoal or carbon molecular sieves [78]. A challenge in the process of breath sampling is the humidity of exhaled breath—especially true for mechanically ventilated patients—which possibly affects pre-concentration, separation and detection of individual compounds [16]. The use of storage containers such as Tedlar bags has been linked to loss of analytes or contamination of samples [77]. The lack of standardisation of analytical methods leads to a wide variation of results among studies. Application of a standardised method of exhaled breath analysis would lead to comparable results, thereby facilitating the potential use of breath biomarkers in the future [79].

Based on the results from the studies included in this review, we can conclude that the VOCs that are measurable in exhaled breath are altered during pneumonia and can derive from the bacterial metabolism as well as the host response. However, these results do not yet allow us to link specific compounds to particular pathogens or disease states, nor does it allow us to pool data from different experiments or studies due to bias and heterogeneity in experimental procedures. Future studies should utilise this understanding and not only focus on VOCs produced by bacteria or the host, but should also combine these two for optimal diagnostic accuracy. Additionally, a more stringent

approach towards the methodological design of the studies is recommended. This includes following the STARD guidelines for reporting studies on diagnostic accuracy to limit the amount of bias [80, 81]. Previous reviews [27, 82] properly summarised the necessary steps to validate preliminary results in breath research. Importantly, future studies should focus more on the clinical application of a breath test. As advocated in this review such a test would (1) exclude pneumonia in order to withhold antibiotic treatment from patients without an infection; (2) enable targeting of antibiotic treatment to the causative pathogen; and/or (3) facilitate evaluation of the treatment response aiming to refine or stopping antibiotics. To date, most focus has been on VAP rather than on CAP, implicating that currently most evidence is available for this particular respiratory infection aetiology. Therefore, this might also be the clinical problem that might require direct focus in the forthcoming years of breath research.

This systematic review of the literature has several strengths and weaknesses. We chose to apply wide inclusion criteria in order to fully cover the literature in this relatively nascent field of research. Naturally, this resulted in a wide diversity of selected articles and made it impossible to pool data due to the underlying heterogeneity, which can be seen as a limitation of our review. In general one can also wonder to what extent the results provided by animal experiments can be translated to the human situation. This study also has several strengths: clinical and pre-clinical studies with multiple analytical devices were included and the results were clustered into the clinical perspective of three scenarios where a biomarker could alter clinical decision-making.

This review demonstrates that a relationship exists between respiratory infection and the presence of particular VOCs in the exhaled breath. Presently, no available breath test is accurate enough to qualify for a role within the diagnostic process of pneumonia. Future studies should focus on clinical scenarios in which a breath test could impact on antimicrobial stewardship and should limit bias by strictly adhering to the latest guidelines.

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Author contributions

PvO and LB conceptually designed the manuscript and performed the literature search. PvO and LB prepared the initial version of the paper and PP, RS, PD, AA, DB, TF, LC, MS and SF advised on the composition of the subsequent final manuscript. All authors approved the submitted version of this article. PvO can be regarded the guarantor of the paper, taking responsibility for the integrity of the work as a whole, from inception to published article.

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8. PERSPECTIVAS PARA O FUTURO

Apesar de toda a investigação básica e clínica, a mortalidade e morbidade associada a infecções graves como a pneumonia permanecem elevadas. Os biomarcadores, em alguns casos, têm melhorado a nossa capacidade diagnóstica e a identificação dos doentes com má evolução clínica, estando a sua utilização na prática clínica muito generalizada. Por outro lado, a resistência aos antibióticos representa uma ameaça crescente para a saúde global, sendo por isso necessárias abordagens alternativas para o controlo da infecção e da emergência das resistências antibióticas [49]. Desta forma, conhecer melhor os mecanismos imunológicos envolvidos na defesa da infecção, compreender como é que a imunidade do hospedeiro contribui para a eficácia do tratamento com os antibióticos e como o tratamento antibiótico modula a resposta imunitária durante a infecção, são aspectos fundamentais para conseguir um avanço no tratamento da pneumonia e das outras infecções de uma forma geral.

Os biomarcadores clássicos estudados representam apenas uma pequena parte dos processos inflamatórios sistémicos que acontecem nas infecções respiratórias graves. A natureza complexa destas infecções e a interação com o sistema imunitário do hospedeiro necessitam de uma avaliação mais ampla. A título de exemplo, o LBA contém dezenas de proteínas, muitas das quais envolvidas nos processos inflamatórios do pulmão. O estudo dessas proteínas pode vir a ser importante na diferenciação da lesão pulmonar aguda causada ou não por infecção [95].

No seguimento dos trabalhos apresentados nesta tese, seria interessante avaliar no futuro próximo, num estudo multicêntrico, a utilidade e segurança da utilização dos padrões da PCR-ratio para reduzir o tempo de antibioterapia nos doentes com PAC e PAH, de forma a diminuir a pressão antibiótica e eventualmente diminuir a emergência de estirpes bacterianas resistentes.

Nos últimos anos, a análise dos COV no ar expirado e da sua relação com as infecções respiratórias tem sido investigada mais exaustivamente. Estes COVs medidos no ar expirado, podem derivar do metabolismo bacteriano ou da resposta do hospedeiro à infecção. A sua medição ainda não está padronizada, mas os estudos realizados têm demonstrado o seu potencial para um diagnóstico mais precoce e não

invasivo das infecções pulmonares [18]. No futuro, os estudos a realizar devem focar-se na avaliação da aplicação clínica da medição dos COVs, especialmente em três aspectos importantes: (1) capacidade para excluir a existência de pneumonia de forma a decidir não iniciar ou interromper a antibioterapia nos doentes que não apresentem infecção, (2) identificar o provável agente bacteriano envolvido de forma a instituir antibioterapia mais dirigida, e (3) permitir a avaliação da resposta ao tratamento e eventualmente definir quando é seguro suspender o tratamento antibiótico.

9. CONCLUSÃO

Esta Tese estudou a utilidade de dois biomarcadores, a PCR e a PCT, no diagnóstico das infecções respiratórias das vias aéreas inferiores associadas ao ventilador e da PCR/PCR-ratio no tratamento da PAC. Nos dois primeiros trabalhos, demonstrou-se que a monitorização diária da PCR e da PCR-ratio pode ser útil na identificação precoce dos doentes com boa e má evolução clínica. A identificação precoce destes doentes pode levar a modificações da abordagem terapêutica que potencialmente melhorem o seu prognóstico.

Para além disso, o reconhecimento dos padrões de resposta da PCR-ratio após o início da antibioterapia, pode diferenciar individualmente os doentes com boa e má evolução clínica. Nos doentes com os padrões de não resposta e resposta bifásica, justifica-se uma atitude de reavaliação clínica mais agressiva de forma a procurar eventuais complicações da infecção inicial ou considerar a possibilidade da antibioterapia inicial não ser adequada. Por outro lado, nos doentes com o padrão de resposta rápida pode considerar-se a suspensão precoce da antibioterapia se o doente apresentar também uma boa evolução clínica.

No terceiro trabalho, demonstrou-se que a terapêutica adjuvante da PAC grave com corticóides sistémicos não contribui para a melhoria do prognóstico destes doentes. Além disso, ficou ainda demonstrado que a corticoterapia não influencia significativamente a evolução da PCR no decurso do tratamento antibiótico nos doentes com PAC. Este biomarcador mantém por isso a sua utilidade na monitorização do tratamento desta infecção mesmo nos doentes imunossuprimidos pela corticoterapia sistémica.

No artigo 4, mostrou-se que a PCR e a PCT não são uteis na distinção entre duas infecções com gravidade clínica diferente como a PAV e a TAV, devido à sobreposição dos valores de ambos os biomarcadores, nem permitem a distinção entre as diferentes etiologias microbiológicas destas infecções.

No artigo 5, mostrou-se que a análise dos COV no ar expirado, pode vir a ter um papel importante no diagnóstico de pneumonia e na possível análise do agente microbiológico envolvido na infecção.

Estes trabalhos contribuíram para um melhor conhecimento do comportamento dos biomarcadores na pneumonia, e abriram caminho a uma melhor utilização dos mesmos na prática clínica diária na abordagem diagnóstica e terapêutica dos doentes críticos.

A importância dos resultados destes estudos reflecte-se, entre outros, no facto do artigo nº1 se encontrar citado nas duas principais normas de tratamento da PAC europeias [96, 97].

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