UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS PNEUMOLÓGICAS

O USO DE PROTEÍNA C-REATIVA COMO BIOMARCADOR NA EVOLUÇÃO DE PACIENTES COM PNEUMONIA ASSOCIADA À VENTILAÇÃO MECÂNICA

Thiago Costa Lisboa

PORTO ALEGRE

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Tese apresentada ao programa de Pós-graduação em Ciências Pneumológicas, Universidade Federal do Rio Grande do Sul, como requisito parcial para o título de doutor.

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4

Ao meu orientador pela paciência, estimulo e suporte durante todo processo.

Aos meus pais e irmãs por todo o suporte desde os mais tenros anos. Ao meu pai por ter inspirado e servido de exemplo na Medicina e na vida.

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A minha esposa pelo carinho, compreensão e companheirismo em todos os momentos.

A Rita por me lembrar de tudo que realmente importa.

SUMÁRIO

LISTA DE ABREVIATURAS

ANCOVA – Analysis of covariance

CCI – Chronic critical illness

COPD – Chronic obstructive pulmonar disease

CPIS – Clinical pulmonar infection score

CRP – C-reactive protein

DAMP – Danger associated molecular patterns

FiO2 – Fração inspirada de oxigenio

GNB – Gram negative bacilli

HR – Hazard ratio

ICU – Intensive Care unit

IFN – Interferon

IL – Interleucina

IQR – Interquartile range

LOS – Lenght of stay

MDR – Multidrug resistant

NYHA – New York Heart association

OR – odds ratio

PAMP – Pathogen associated molecular patterns

PAV – Pneumonia associada a Ventilação

PCR – Proteina C-reativa

PCT – Procalcitonina

s-TREM – soluble Triggering receptor expressed on myeloid cells

TNF – Fator de necrose tecidual

UFC – Unidades formadoras de colônia

UTI – Unidade de Terapia Intensiva

VAP – Ventilator-associated pneumonia

VARI – Ventilator-associated respiratory infection

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Resumo

A pneumonia nosocomial é uma infecção prevalente e associada com elevados custos e morbi-mortalidade. A maioria destes episódios ocorre em pacientes criticamente doentes em ventilação mecânica. Os biomarcadores, como a proteína C-reativa, tem se mostrado uteis na avaliação da evolução dos pacientes, podendo se descrever padrões de resposta associados ao sucesso da terapia antimicrobiana e ao prognostico de paciente com pneumonia associada a ventilação mecânica.

Nesta tese, apresenta-se uma revisão da literatura abordando aspectos da fisiopatologia, diagnostico e manejo da pneumonia associada a ventilação mecânica. Além disso, é feita a análise do uso de biomarcadores em duas populações especificas de pacientes criticamente doentes (pacientes com doença critica cronica e pacientes idosos). Foram avaliados 405 pacientes com diagnostico clínico de pneumonia associada a ventilação mecânica.

Descreve-se que pacientes com doença critica crônica apresentam episódios de pneumonia associada a ventilação mecânica com pior prognostico do que pacientes que não apresentam doença critica crônica. Entretanto, esses achados não parecem associados a um comprometimento da resposta inflamatória, uma vez que nao houve diferença significativa nem nos níveis basais, nem na evolução dos níveis de proteína C-reativa comparando episódios de pacientes com doença critica crônica com aqueles sem esta condição, sugerindo que seu uso é válido nessa população de pacientes.

Ainda, descreve-se a evolução dos pacientes com pneumonia associada a ventilação mecânica de acordo com a idade. A partir dos 65 anos, parece haver um efeito da idade na mortalidade dos pacientes com PAV. No entanto, não houve alteração na resposta da PCR ou na sua cinética nas primeiras 96h quando comparamos pacientes com diferentes faixas etárias a partir de um ponto de corte de 65 anos, também sugerindo a validade do uso deste biomarcador nesta população de pacientes.

Estes achados originais permitem que estudos futuros avaliem intervenções baseadas em biomarcadores em pacientes com pneumonia nosocomial levando em consideração estas populações especificas de pacientes não avaliadas previamente na literatura.

Palavras chave: Medicina Intensiva; Pneumonia, Biomarcador; Proteina C-reativa, Ventilação Mecanica.

ABSTRACT

Nosocomial pneumonia is a prevalent infection associated with higher costs and worse outcomes. Most episodes occur in mechanically ventilated critically ill patients. Biomarkers, such as C-reactive protein, are useful to assess patients evolution, allowing identification of patterns associated with antimicrobial treatment success and prognosis in ventilator-associated pneumonia patients.

In this thesis, a literature review is presented evaluating aspects of pathopsysiology, diagnosis and management of ventilator-associated pneumonia patients. In addition, biomarker use in two specific populations (chronic critical illness and elderly) was assessed. Four hundred and five patients with ventilator associated pneumonia clinical diagnosis were evaluated.

Patients with chronic critical illness presented ventilator-associated pneumonia episodes associated with worse prognosis. However, these findings were not associated with a compromise of inflamatory response, assessed by comparison of C-reactive protein basal levels and kinetis evolution in patients with and wihtout chronic critical illness, suggesting its use remains valid in this specific population.

Still, evolution of ventilator-associated pneumonia according to age is described. After 65 years old, our data suggest an effect of age on mortality in ventilator-associated pneumonia patients. However, no change in C-reactive protein basal levels, response or kinetics within 96h was found when comparing patients younger and older than 65 years old, also suggesting this biomarker usefulness in this specific population.

These original findings allow that future studies assessing intervention based on biomarkers evolution in patients with nosocomial pneumonia consider these specific populations, never assessed before in literature.

Key words: Critical Care; Pneumonia; Biomarker; C-reactive protein; Mechanical ventilation

INTRODUÇÃO

A pneumonia nosocomial é a segunda infecção mais frequente no ambiente hospitalar, correspondendo a aproximadamente 15% destas e afetando de 0,5 a 2% dos pacientes hospitalizados (1). Cerca de 60% destes episódios ocorrem dentro de um ambiente de cuidados intensivos, onde é a infecção nosocomial mais comum, usualmente associada a ventilação mecânica. Tem uma prevalência variável, com taxas desde 6 até 50 casos por 100 admissões na UTI (2,3). Tal variabilidade se deve principalmente a dois aspectos: a presença de diferentes *case-mix* em diferentes unidades e a inexistência de critérios diagnósticos precisos que permitam um diagnóstico operacional acurado, tornando a subjetividade um aspecto importante na definição dos casos e nas decisões terapêuticas. Esta complexidade diagnóstica dificulta a comparação entre diferentes estudos e até mesmo estratégias de *benchmarking* baseadas na utilização das taxas de pneumonia associada a ventilação mecânica (PAV) como um marcador de qualidade assistencial (4,5).

O desenvolvimento de pneumonia nosocomial, e no ambiente de cuidados intensivos especificamente da PAV, tem morbidade significativa associada, prolongando o tempo de ventilação mecânica, bem como o tempo de permanência na UTI, com todos os custos associados a este prolongamento (6,7). A mortalidade atribuída a PAV ainda é um aspecto controverso na literatura. A mortalidade global nos episódios de PAV variam de 20 a 60%, refletindo em grande parte a gravidade da doença de base destes pacientes, a disfunção orgânica pré-existente ou instalada e especificidades da população estudada e do agente etiológico envolvido. Embora estudos mais antigos com uma metodologia mais simples (ex. caso-controle) (8,9) sugerissem um aumento de até 30% em média na mortalidade com o desenvolvimento

de PAV, estudos mais recentes, utilizando análise de desfechos concorrentes e análise causal, levando em consideração o tempo de aquisição da PAV bem como as relações complexas entre a gravidade da doença de base e o risco de desenvolver PAV, sugerem que tal impacto é superestimado e que a mortalidade atribuível estaria abaixo de 2% (10). Novamente, é provável que algumas características de populações específicas, bem como de agentes etiológicos específicos estejam sub-representadas nestas estimativas.

O diagnóstico de Pneumonia nosocomial à beira do leito leva em consideração uma combinação de achados clínicos, radiológicos e laboratoriais (1,11). Dados microbiológicos são utilizados como uma tentativa de refinar a acurácia diagnóstica, dada a baixa especificidade dos critérios clínicos isoladamente. Esses critérios incluem:

- presença de infiltrado persistente novo ou progressivo OU consolidação OU cavitação;

E

- pelo menos dois desses critérios: febre (temperatura axilar acima de 38ºC), sem outra causa OU leucopenia (<4.000 cel/mm^3) ou leucocitose (>12.000 cel/mm^3) OU surgimento de secreção purulenta ou mudança das características da secreção ou aumento da secreção.

Ainda podem ser considerados fatores importantes a presença de comprometimento funcional (hipoxemia, com piora da relação pressão parcial de oxigênio/fração inspirada de oxigênio - PO_2/FiO_2), o aumento nos níveis de biomarcadores, confusão mental ou surgimento de sepse grave/choque séptico.

A PAV é considerada com confirmação microbiológica se está presente pelo menos um dos critérios laboratoriais: hemocultura positiva, sem outro foco de

infecção aparente OU cultura positiva do líquido pleural OU cultura do lavado broncoalveolar $\geq 10^4$ UFC/mL ou do aspirado traqueal $\geq 10^6$ UFC/mL OU exame histopatológico com evidência de infecção pulmonar OU antígeno urinário ou cultura para *Legionella spp*. OU outros testes laboratoriais positivos para patógenos respiratórios (sorologia, pesquisa direta e cultura). Na ausência, de uma dos critérios microbiológicos, é feito o diagnóstico de PAV clinicamente definida (1,11).

Uma tentativa de tornar o diagnóstico mais objetivo inclui o uso de um escore clínico - CPIS, entretanto não há um claro benefício na literatura no uso sistemático deste escore, como confirmação, mas seu valor preditivo negativo foi usado em um ensaio clinico para suspensão precoce do tratamento antimicrobiano em pacientes com suspeita de PAV sem piora no desfecho clinico. Escore acima de 6 pontos é sugestivo de pneumonia(12).

USO DE BIOMARCADORES

Diversos biomarcadores foram avaliados como ferramentas para auxiliar no diagnóstico de pneumonia. Inclui-se entre eles proteína C-reativa (PCR), procalcitonina (PCT), *soluble-triggering receptor expressed on myeloid cells* (s-TREM), interleucinas 8, 9 e 10 (IL8, IL6, IL10), fator de necrose tumoral (TNF-alfa), entre outros. Na ausência de um padrão-ouro seria mais prudente o uso integrado de todas as variáveis clínicas disponíveis ao invés de limitar a uma única variável a definição do diagnóstico.

O uso de biomarcadores séricos como PCR ou PCT como fatores determinantes do inicio de tratamento empírico e, portanto, do diagnóstico foi avaliado em diversos estudos e não foi possível determinar um ponto de corte   adequado, nem uma

estratégia segura que pudesse ser incorporada a prática clínica(13,14).

A PCR foi identificada em 1930 pela primeira vez no soro de pacientes com pneumonia pela capacidade de precipitar frações de polisacarídeo, chamadas de fração C, do *Streptococcus pneumoniae (15)*. Pertence a família das pentraxinas, proteínas que se mantiveram preservadas ao longo da evolução dos vertebrados, sugerindo seu papel na resposta imunológica inata. Juntamente com o complemento tem ação na opsonização promovendo fagocitose bem como representa estímulo a ação citotóxica das células NK e ativação neutrofílica(16). Além disso parece ter ação antibacteriana direta através da ligação a parede celular bacteriana (16). A PCR é sintetizada predominantemente no fígado e apresenta boa correlação com outros marcadores como IL6 e TNF-alfa, que tem ação reguladora de sua secreção.

Os níveis de PCR se elevam sempre que houver um processo inflamatório em evolução e sua concentração depende da intensidade do estímulo. Os níveis não são alterados por terapia de substituição renal, sendo influenciados apenas por intervenções que interfiram no processo inflamatório que gerou a alteração (17). Os níveis de PCR encontram-se elevados na maioria dos quadros infecciosos. Infecções bacterianas, fúngicas invasivas e alguns quadros virais estão associados com aumentos significativos no nível sérico de PCR, mesmo em pacientes com deficiência imunológica(18). A secreção de PCR costuma iniciar em 4-6h após o estímulo inicial, dobrando em 8h e atingindo o pico de concentração em 36-48h. Uma vez cessado o estímulo a PCR cai rapidamente e sua meia-vida estimada é de cerca de 18-20h (16,17).

O uso de PCR foi avaliado em diversos estudos em pacientes com sepse e pneumonia. Tentativas de identificar pontos de corte que auxiliem no diagnostico de pacientes com sepse não permitiram estabelecer uma abordagem definitiva, não sendo

recomendado o uso destes biomarcadores no processo de tomada de decisão para o inicio de tratamento antimicrobiano. A variabilidade individual e diferenças nas respostas sugeriu o uso da PCR como uma marcador de evolução de pacientes com sepse.

A avaliação da evolução dos pacientes com sepse inclui o uso de parâmetros de resposta clínica, como resolução da febre, leucocitose e uso de biomarcadores, como proteína C-reativa e procalcitonina, que permitem avaliar a evolução clinica. No mínimo 48 a 72 horas são necessárias para que os parâmetros de melhora clínica sejam avaliados. A diminuição da febre, a redução na quantidade e purulência da secreção brônquica e a redução na contagem de leucócitos são critérios importantes de resposta clínica. A melhora da oxigenação possibilitando a redução da fração inspirada de oxigênio (FiO$_2$), a redução das pressões e a estabilidade hemodinâmica são fortes indícios de resposta terapêutica. Além disso, o padrão de resposta clínica, avaliado a partir da variação de biomarcadores como PCR e procalcitonina parecem se correlacionar com adequação da antibioticoterapia empírica, bem como com o prognóstico destes pacientes (19-23).

Em pneumonia nosocomial, Povoa et al. (19) avaliou 47 pacientes com VAP e descreveu que queda >0,4 vezes no quarto dia de evolução está associado com melhor prognóstico, com melhor performance comparado com evolução de febre e leucograma, sem influência da presença de infecção prévia, presença de síndrome de distresse respiratório do adulto ou motivo da ventilação mecânica. Além disso, uma associação entre a queda nos niveis de PCR com antibioticoterapia empírica adequada, sugerindo o uso deste biomarcador para avaliação da evolução e resolução clinica de um quadro infeccioso pulmonar grave. Este achado foi confirmado em estudos posteriores (20-23). Algumas populações especificas foram avaliadas como

pacientes HIV, hepatopatas, neutropenicos ou com câncer (18, 23-25). Entretanto, em algumas populações especificas, cuja prevalência tem aumentado nas unidades de terapia intensiva como pacientes doença critica crônica ou persistente e idosos, o uso de PCR ainda não foi avaliado.

Nos estudos apresentados nesta tese, procuramos explorar algumas das lacunas na literatura, apresentando uma revisão extensa da literatura sobre a pneumonia nosocomial no paciente ventilado e avaliando o uso de biomarcadores em duas populações especificas de pacientes criticamente doentes (pacientes com doença critica crônica e pacientes idosos).

JUSTIFICATIVA

O uso de biomarcadores como a PCR para auxiliar na avaliação da evolução de pacientes com PAV esta demonstrado na literatura. Quedas significativas nos níveis deste biomarcador estão associados com melhor prognóstico, assim como com a adequação do tratamento antimicrobiano. Entretanto, a heterogeneidade dos pacientes criticamente doentes, torna muitas vezes necessária a avaliação das estratégias utilizadas em alguns subgrupos, pois eventualmente o comportamento dos diferentes marcadores biológicos pode variar de maneira significativa.

Neste documento, a estratégia de usar biomarcadores para estudar a evolução de pacientes com PAV é avaliada em duas populações especificas: pacientes idosos e pacientes com doença critica crônica. Esta informação é relevante cientificamente pois permitirá que intervenções desenhadas baseadas na variação dos biomarcadores possam ser avaliadas também nestas populações. Potenciais diferenças no comportamento dos biomarcadores nestas populações que forem identificados neste estudo podem comprometer a utilidade do uso de biomarcadores ou indicar ajustes na avaliação nos pacientes com doença critica crônica e idosos.

OBJETIVOS

Geral

1) Descrever a epidemiologia, aspectos microbiológicos e resposta clinica, baseada na evolução de proteína C-reativa nos pacientes com pneumonia nosocomial admitidos a unidade de terapia intensiva, com ênfase em populações especificas pre-definidas.

Específicos

1) Avaliar o uso da proteína C-reativa como marcador de evolução em pacientes com doença crítica crônica e descrever a epidemiologia dos episódios de PAV nesta população.

2) Avaliar proteína C-reativa como marcador de evolução em pacientes idosos, bem como descrever a epidemiologia dos episódios de PAV nesta população específica.

ASPECTOS ÉTICOS

O autor garante confidencialidade quanto aos dados obtidos, assegurando que foram usados com fim único e exclusivo da pesquisa clínica e foram analisados de modo agregado, preservando a identidade dos participantes, garantindo a anonimização. Foram observadas as recomendações da Resolução número 196 de 10/10/1996 – Conselho Nacional de Saúde para Pesquisa Científica em Seres Humanos. Zelou-se pela *beneficência*, comprometendo-se com o máximo de benefícios e o mínimo de danos e riscos, e pela *não-maleficência*, garantindo que danos previsíveis serão evitados. Os procedimentos foram realizados rotineiramente na UTI e faziam parte do cuidado habitual dos pacientes.

Referencias Bibliograficas

1. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171: 388–416.

2. Torres A, Ewig S, Lode H, et al. Defining, treating and preventing hospital acquired pneumonia: European perspective. Intensive Care Med 2009; 35: 9–29.

3. Rello J, Lisboa T, Koulenti D. Respiratory infections in patients undergoing mechanical ventilation. Lancet Respir Med 2014; 2:764-774

4. Lisboa T, Craven D, Rello J. Should Ventilator-associated pneumonia be a quality indicator for patient safety. Clin Pulm Med 2009; 16: 28-32

5. Klompas M, Platt R. Ventilator-associated pneumonia–the wrong quality measure for benchmarking. Ann Intern Med. 2007; 147: 803– 805.

6. Heyland DK, Cook DJ, Griffith L, et al. The attributable morbidity and mortality of ventilator-associated pneumonia in the critically ill patient. Am J Respir Crit Care Med 1999; 159: 1249–1256.

7. Rello J, Ollendorf DA, Oster G, et al. Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. Chest 2002; 122: 2115–2121.

8. Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS. Epidemiology and outcomes of health-care-associated pneumonia: Results from a large US database of culture-positive pneumonia. Chest 2005; 3854 - 3862.

9. Timsit JF, Zahar JR, Chevret S. Attributable mortality of ventilator-associated pneumonia. Curr Opin Crit Care. 2011; 17: 464- 471

10. Bekaert M, Timsit JF, Vansteelandt S, et al. Attributable mortality of ventilator associated pneumonia: a reappraisal using causal analysis. Am J Respir Crit Care Med 2011; 184: 1133- 1139.

11. Lisboa T, Rello J. Diagnosis of ventilator associated pneumonia: Is there a gold standard and a simple approach?. Curr Opin Infect Dis 2008; 21: 174- 178.

12. Luna CM, Blanzaco D, Niederman MS et al. Resolution of ventilator-associated pneumonia: prospective evaluation of the clinical pulmonary infection score as an early clinical predictor of outcome. Crit Care Med 2003; 31: 676-682.

13. Póvoa P, Salluh JIF. Biomarker-guided antibiotic therapy in adult critically ill patients: a critical review. Ann Intensive Care 2012; Jul 23;2(1):32.

14. Oliveira CF, Botoni FA, Oliveira CRA, Silva CB, Pereira HA, Serufo JC, et al. Procalcitonin versus C-reactive protein for guiding antibiotic therapy in sepsis: a randomized trial. Crit Care Med 2013 Oct;41(10):2336–2343.

15. Tillett W, Francis T. Serological reactions in pneumonia with a non-protein somatic fraction of Pneumococcus. J Exp Med 1930:52:561.

16. Szalai A. The antimicrobial activity of C-reactive protein. Microbes Infect 2002; 4:201-205.

17. Povoa P. C-reactive protein: a valuable marker of sepsis. Intensive Care Med 2002; 28:235-243.

18. Jabs WJ, Logering BA, Gerke P, et al. The kidney as a second site of human C-reactive protein formation in vivo. Eur J Immunol 2003; 33:152-161.

19. Povoa P, Coelho L, Almeida E, et al. C-reactive protein as a marker of ventilator-associated pneumonia resolution: a pilot study. Eur Respir J 2005; 25: 804–812.

20. Seligman R, Meisner M, Lisboa TC, et al. Decreases in procalcitonin and C-reactive protein are strong predictors of survival in ventilator-associated pneumonia. Crit Care 2006; 10: R125.

21. Lisboa T, Seligman R, Diaz E, Rodriguez A, Teixeira PJ, Rello J. C-reactive protein correlates with bacterial load and appropriate antibiotic therapy in suspected ventilator-associated pneumonia. Crit Care Med. 2008;36(1):166-171.

22. Moreno MS, Nietmann H, Matias CM, Lobo SM. C-reactive protein: a tool in the follow-up of nosocomial pneumonia. Journal of Infection 2010 Sep;61(3):205–211.

23. Póvoa P, Souza-Dantas VC, Soares M, Salluh JF. C-reactive protein in critically ill cancer patients with sepsis: influence of neutropenia. Crit Care 2011; May 19;15(3):R129.

24. Schleicher GK, Herbert V, Brink A, et al. Procalcitonin and C-reactive protein levels in HIV-positive subjects with tuberculosis and pneumonia. Eur Respir J 2005; 25: 688-692.

25. Park WB, Lee KD, Lee CS, et al. Production of C-reactive protein in Escherichia coli-infected patients with liver dysfunction due to liver cirrhosis. Diagn Microbiol Infect Dis 2005, Apr; 51(4): 227-230.

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RESPIRATORY INFECTIONS IN PATIENTS UNDERGOING MECHANICAL VENTILATION

A Review article for Lancet Respiratory Diseases.

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ABSTRACT

Lower airway infections in mechanically ventilated patients are a frequent cause of antibiotic prescriptions in the ICU setting. They present in the form of severe sepsis or septic shock in intubated patients. Purulent respiratory secretions are required for diagnosis, but the differential diagnosis between pneumonia and tracheobronchitis is not easy. Both presentations are associated with prolonged duration of mechanical ventilation and ICU stay, providing rationale for antibiotic treatment initiation. Quantitative cultures might help to differentiate colonizers from true pathogens, being *Staphylococcus aureus* and *Pseudomonas aeruginosa* of great concern. Key management issues are the following: What is the pathogen, which is the initial empirical antibiotic choice, and decisions depending on the resolution pattern.

Words: 110

Introduction

In the point prevalence EPICII study that was conducted in 1,265 intensive care units (ICUs) from 75 countries worldwide,[1] 51% of adult ICU patients were infected and the respiratory tract (RT) was the focus of infection in 64% of the cases.[1] In the medical ICU, airway infections in intubated patients are the main reason for antibiotic prescription. Since there is no gold standard for the diagnosis of RT infections in intubated patients [2], prescription of antibiotics for patients with purulent respiratory secretions is a common clinical practice in the ICU setting. This article reviews ventilator-associated respiratory infections (VARI) in adult patients, placing particular emphasis on aspects of diagnosis, microbiological etiology and management.

The clinical challenge of respiratory infections in ventilated patients

The presentation of the patient in the vignette is suggestive of lower respiratory infection, presenting progressive hypoxemia and fever that is in contrast with the sudden onset of rigors and temperature rise of bloodstream infections.

In our view, the low sensitivity and specificity of the current diagnostic criteria is the most important problem in the assessment and diagnostic approach of mechanically ventilated patients with suspected lower airway infections.[3] The clinical syndrome definition based on VARI clinical presentation is a challenge for clinicians. Since the criteria include many subjective components (such as chest X-ray [CXR], assessment of respiratory secretions, and even auscultation) the inter-rater variability for identifying VAP is high.[4,5] A recent prospective survey that was conducted in a nationally representative group of US hospitals asked the participants to classify standardized vignettes of possible cases of VAP as pneumonia or no pneumonia. This study reported that the agreement among hospitals about classification of cases as

ventilator-associated pneumonia or not was nearly random, highlighting the limitations of the current definitions.[6]

The clinical pulmonary infection score (CPIS) was created to predict the pre-test probability of pneumonia.[7] It combines information on body temperature, volume and appearance of tracheal secretions, CXR, WBC count, oxygenation, and tracheal aspirate culture.[7] Many randomized clinical trials have used the CPIS score to identify patients with pneumonia as it allows an objective assessment of clinical variables for pneumonia diagnosis[8,9]. Unfortunately, despite of using objective data as WBC count or oxygenation, CPIS also includes variables that are either subjective or retrospective, such as CXR findings and microbiological data what might compromise its utility in some sub-groups (e.g. SDRA patients). We consider that individual diagnostic decisions should not be made based on scores. Diagnostic scores are helpful for providing probabilities for comparisons between groups, but seems not appropriate for the assessment of the probability of pneumonia in individual patients.

Differentiate VAP and VAT based only on clinical signs might be a difficult task at bedside. The cut-off points for colonization, tracheobronchitis and pneumonia in MV patients have not been conclusively defined, and there is also a clear need to assess vasopressor requirement and complications such as the effect of the respiratory infection on oxygenation. The US Centres for Disease Control and Prevention (CDC) diagnosis of ventilator-associated tracheobronchitis (VAT) is based on the absence of CXR infiltrates and the presence of signs consistent with respiratory inflammation along with at least 1 microbiologic criterion.[4] However the lack of objectivity and the inherent variability in the interpretation of CXR in MV patients makes it difficult to take decisions based on CXR. Dallas and colleagues reported a median onset of VAT 7.5 days after intubation and initiation of MV compared with 5 days of VAP,

suggesting that VAT and VAP might be two distinct entities, and that VAT is not necessarily a precursor of VAP, although a high percentage of patients initially diagnosed as VAT evolved to VAP. In addition, it suggests antibiotic use might be an important factor influencing whether VAT progresses to VAP. [10] Pathophysiological aspects of VAT and VAP correlation are proposed in figure 1 and are also discussed in VAT management section.

Beyond clinical differences between VAT and VAP, a pilot translational study comparing gene expression profiles in VAP and VAT identified that 5,595 genes expressed differently in the pre-infection period.[11] A significant depression of the complement system signalling pathway was identified in the VAP group, along with a depression of cAMP and calcium signalling pathways during the pre-infection phase.[11]

Epidemiological features

The epidemiology of respiratory infections varies, depending on whether the patient is mechanically ventilated with a tracheostomy or an endotracheal tube. The role of the biofilm is important in tracheostomized patients.[12,13] Aspiration constitutes the main pathophysiological event. Avoiding an artificial airway is the best method of prevention. In contrast with community-acquired pneumonia (CAP) episodes, respiratory infections in MV patient are heterogeneous. Poor comparison can be established when a patient with intra-abdominal surgery is compared with another who underwent cardiac surgery or trauma. Moreover, medical patients are a different subset, and when VAP develops as a complication of severe CAP, *P. aeruginosa* is the most frequent organism.

While the attributable mortality of VAP is controversial, it certainly does prolong mechanical ventilation and the length of stay in the ICU. [14] In a recent metanalysis,[15] the overall attributable mortality of VAP was estimated to be 13%. Admission diagnosis, age, causative pathogens and adequacy of therapy are also influencing outcomes. Higher attributable mortality rates were reported in surgical patients and patients with mid-range severity-of-illness at admission, whereas attributable mortality close to zero was reported in trauma, medical patients, and patients with low or high severity-of-illness scores.[15] However, there is a huge variability on incidence-rate and attributable mortality in different studies[16-22]. Data from low-income and developing countries suggest the incidence-rates and attributable mortality might be higher [16-22] .

A particular challenge is the development of pneumonia in the postoperative period of lung transplantation because its presentation may overlap with acute rejection that requires an opposite therapeutic approach (increase versus decrease immunosuppressors).[23] Interestingly, Riera and colleagues reported that episodes of tracheobronchitis doubled episodes of pneumonia in this subset of patients.[23] Pneumonia was related with increased in-hospital death (42.9% vs 11.5%; p=0.01), while tracheobronchitis was not related to this increased mortality (14.0% vs 14.7%; p=0.9).[23] In a prospective observational study of 2,436 patients from 27 ICUs in nine European countries,[24] mortality of VAP was 73% lower in trauma compared to non-trauma patients. In addition, Melsen and colleagues reported that VAP development decreased the daily probability of discharge from the ICU by 26%, indicating that the disorder extends the length of ICU stay.[25]

In addition, variability on VAP rates might be due to lack of a diagnostic gold standard. The standard definition used to measure VAP rates is based on several

nonspecific clinical signs with the addition of microbiologic criteria aiming to improve specificity, but may be severely limited by lack of sensitivity and specificity of current criteria[14]. Also, differences between surveillance strategies and the clinical definition of VAP are crucial for understanding such variability. It impacts on appropriate assessment of prevention studies, as lower rates might be associated to different criteria in subjective aspects of diagnosis. An attempt to design a simple, objective surveillance definition for ventilator-associated complications (VAC) was presented by Klompas and colleagues, which shifted the focus of surveillance from pneumonia alone to complications of MV,[26] but impact on clinical practice of adopting such new criteria is not available.

Our view is that prevention trials and recommendations should no longer focus on VAP rates. Only measures associated with improved outcomes (particularly lower duration of MV) and reduced costs should be implemented. A recent Spanish multicenter cohort study have reported that full VAP prevention care bundle compliance was associated with an incidence risk ratio of VAP of 0.78 (95% CI 0.15-0.99), as well as with a reduction of both median ICU length of stay (LOS) from 10 to 6 days and MV duration from 8 to 4 days.[27] Key interventions were oral care, maintaining pressure of the cuff, hand hygiene before artificial airways manipulation and strategies to avoid hyper-sedation. Prolongation of ICU stay is associated with increased (preventable) healthcare costs, as it was reported in a large matched cohort study,[28] and emphasizes the interest of giving priority to prevention measures, that have demonstrated potential costs' reduction, (rather than rates' reduction).

Pathogens

Less than ten organisms are implicated in the vast majority of VARI cases and it should be noted that a significant percentage are polymicrobial infections.[29] In the recent years a shift in the pattern of respiratory pathogens have been towards Gram-negative infections. The EUVAP study [29] identified *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the most commonly isolated pathogens in VAP.[29] Core organisms, such as methicillin susceptible *S. aureus* (MSSA)*, Haemophilus influenzae or Streptococcus pneumoniae* are common causes of early-onset VAP in trauma patients, but VAP improves quickly (within three days) when adequate therapy is promptly started. A recently published secondary analysis of the EUVAP study reported that elderly ICU patients with VAP had increased rates of *Enterobacteriaceae* compared to younger age groups.[30] Table 1 details the top three pathogens of VAP reported in six studies published during the last decade.[29,31-35] Figure 2 depicts the median onset of VAP by pathogen.

Antibiotic resistance

Nosocomial infections are commonly caused by ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and Enterobacter species).[36] Sandiumenge and colleagues reported *S. aureus, P. aeruginosa and A. baumannii* as the top three pathogens.[37] Enteroccus sp. and Candida sp, on the other hand, should be interpreted as oral contaminants. The risk of MDR pathogens causing VAP is mainly determined by comorbidity and prior exposure to more than two antibiotics. The increased mortality of VAP caused by MDR as compared with non-MDR pathogens is explained by more severe comorbidity and presence of organ failures[38]. Resistant ESKAPE VAP mortality was double (RR, 2.25; 95% CI, 1.67-9.48) compared with the mortality of

the remaining patients with VAP.[37] Therefore, we will focus the discussion on MRSA and *P.aeruginosa* that constitutes a major concern, in terms of both outcomes and costs. Emergence of extended spectrum beta lactamases (ESBL) or *Klebsiella pneumoniae* carbapenemases (KPC) is of concern between Enterobacteriaceae, but these resistant pathogens are more frequently involved in extra-pulmonary infections. Also, we will discuss briefly *A.baumannii*, which is endemic in some ICUs.

Severity-of-illness seems not to affect the etiology of VAP, therefore, risk factors for multi-drugs resistance (MDR) rather than the severity-of-illness should guide the initial empirical antibiotic therapy.[39] On the other hand, it has been reported that patients with higher severity scores and septic shock at onset of pneumonia had significantly lower survival and higher systemic inflammatory response.[39] In relation to VAP caused by MRSA, it is interesting to note that variables influencing decisions for anti-methicillin resistant *S. aureus* (MRSA) empiric prescription differ from risk factors.[40] Factors associated with MRSA VAP development include prior antibiotic exposure, prolonged hospitalization, underlying COPD and steroid use.[41] On the contrary, age younger than 25 years and neurologic impairment, such as head trauma, were associated with methicillin-susceptible strains. It is crucial to highlight that the baseline prevalence in a specific ICU should be to be taken into consideration before choosing the initial empirical antibiotic therapy on pneumonia suspicion. Bacteraemic VAP is independently associated with MRSA and mortality.[42] Moreover, mortality is higher for MRSA versus methicillin-susceptible *S.aureus* (MSSA) ICU infections; a secondary analysis of the EPIC II study reported that MRSA was independently associated with an almost 50% higher likelihood of hospital death compared with MSSA infection.[43]

In regards to MRSA VAP treatment, there is a lot of controversy in relating to glycopeptides' versus linezolid's use, that has been fueled by the vancomycin's minimal inhibitory concentration (MIC) creep, the poor alveolar penetration of vancomycin, the potential adverse events, linezolid's cost and the high rate of poor clinical resolution of MRSA VAP[44]. Regarding resolution, MRSA VAP traditionally has poor resolution and half of the patients need MV for more than 3 weeks after pneumonia onset.[44] Vidaur et al reported that the resolution of MRSA VAP was associated with longer need for respiratory support compared to VAP due to other pathogens, regardless of the appropriateness of initial antibiotic therapy.[44] Interestingly, a prospective, double-blind, controlled, multicenter trial involving hospitalized adult patients with nosocomial MRSA pneumonia reported that, although 60-day mortality was similar between linezolid and dose-optimized vancomycin, clinical response was significantly higher with linezolid.[45] Moreover, acute kidney injury has been associated with vancomycin's use in patients with glomerular filtration rate (GFR) above 50 ml/min (18.8% for vancomycin vs. 5.6% for linezolid).[45] This study however, is not definite as presents some potential biases, including trend to unequal comorbidities distribution between groups and bacteremia [46]. A practical approach that we suggested was to use linezolid in patients with immunosuppression, concomitant administration of nephrotoxic drugs, vasopressors, severe sepsis or in elderly patients, and to administer glycopeptides in the absence of these factors.

Further research is required in adjunctive therapy, neutralizing virulence factors (alginate, pantovalentin leukocidine or alfatoxin) to improve outcomes and minimize injury.

With respect to *P. aeruginosa,* over the last decade an increase in the frequency of MDR *P. aeruginosa* (MDR-PSA) strains has been recorded.[47] In the ICU, MDR-PSA represents a major issue regarding infections management, especially VAP.[48] Patients at risk of PSA infection should receive combination therapy with two agents from pneumonia onset, due to the probability of initial wrong therapy, which was associated with statistically significant higher mortality.[49] However, when susceptible, an agent has comparable outcomes than two, and simplifying to a single agent can be implemented after susceptibility is available.[49] For empirical therapy choice, prescribers should bear in mind the factors reported to be associated with isolation of MDR-PSA. These factors include admission from chronic care facilities,[50] advanced age, diabetes, prolonged hospitalization,[50-52] using invasive devices,[50,53,54] recent surgery,[52] and predominantly prolonged ICU stay, prolonged ventilation periods, and higher severity-of-illness scores.[54-56] It has been reported that *Candida* spp airway colonization may promote pneumonia development, especially when caused by PSA, perhaps linked to the biofilm environment in the artificial airway.[57-60] In episodes with clinical suspicion of VAP, *Candida* spp airway colonization was associated with increased mortality risk (OR:1.72).[58] Moreover, yeasts have been reported as an independent risk factor for identification of MDR microorganisms (OR: 1.79). Further research is needed to understand if *Candida* airways colonization should be a variable influencing selection of VAP empiric therapy.[58] Cross-infection also may contribute to emergence of MDR-PSA strains.[54, 61-]Indeed, a key role for acquisition of MDR strains are prior antibiotic exposure.[61-63] Indeed, aminoglycosides exposure has been identified as risk factor;[50]however, other reports also identified the importance of anti-pseudomonal cephalosporins,[50, 64] fluoroquinolones (levofloxacin more than ciprofloxacin), [52, 65,66] and carbapenems, [53,67] It has been reported that imipenem might

have the greatest potential for MDR strains selection,[65] whereas ertapenem does not induce carbapenem resistance to *Pseudomonas* strains.[62] Prior treatment with anti-pseudomonal penicillin/β-lactamase inhibitor combinations does not seem to increase isolation of MDR organisms.[66] However, antibiotic therapy is not the only factor associated with the acquisition of MDR organism; a study of meropenem high-level-resistant *Pseudomonas* strains reported an association between factors associated with higher severity and MDR strains, but failed to identify prior antibiotic exposure.[68]

Implications of MDR-PSA infection, particularly respiratory infection, remain controversial. In 2006, it was reported that infection by MDR-PSA was associated with increased mortality (OR: 4.4) and hospital stay (HR: 2.0), when compared with controls.[50] However, further reports suggested that MDR *per se* does not directly affect outcomes, being associated with factors related with MDR strains isolation. [69,70] Presence of organ dysfunction (OR= 10.4),[55] more comorbidities and inappropriate empiric antibiotic therapy increased mortality (RR: 1.59), ICU and mechanical ventilation periods (at least 4 days) and hospital length of stay (13 days).[47] Piperacillin-resistance does not influenced outcomes in episodes of VAP.[69,70]

Interestingly, recent research has emphasized the contribution of virulence factors in *P.aeruginosa* pneumonia. Quorum sensing and biofilm formation[71] have been studied. These data suggest that type III secretion system (TTSS) encoded by PSA might play a substantial role. The needle-like TTSS mechanism allows bacteria to inject toxins directly into cytoplasm of cells' host. Therefore, toxins are not exposed extra-cellularly, evading direct recognition by the host immune system.[72] These findings fueled the hypothesis than failure to eradicate *Pseudomonas sp* in pneumonia might be due to the TTSS. In pneumonia caused by *P.aeruginosa*, despite appropriate antimicrobial treatment, above 50% of the strains expressing at least one type of

TTSS protein (TTSS+) were recovered one week later. In contrast, eradication was documented in all episodes caused by TTSS- strains.[73] The group leaded by Rouby, in a retrospective cohort of 143 patients with *P.aeruginosa* VAP, reported that O6 and O11 were the most prevalent strains. Moreover, mortality tended to be worse with 01 or 011 serotypes and better with 02 or 06 serotypes.[74] Moreover, clones exhibiting ExoU, one of the toxins secreted by the TTSS were frequently serotyped as O11, in contrast with serotype O6 strains, which were often associated with a negative exo U serotype.[75,76] These findings highlight the importance of immunomodulatory adjunctive therapy in the future management of severe pneumonia.[77] Elective *P. aeruginosa* vaccination in patients at high risk of late onset pneumonia represents another future way of prevention that warrants priority research.

Acinetobacter baumannii is a non-fermentative Gram-negative bacilli (GNB) that has caused large outbreaks in contaminated ICUs. Compared with *P aeruginosa, A.baumannii* has different risk factors and lacks virulence factors. Independent risk factors for *A.baumannii* pneumonia in intubated patients include ARDS, head trauma, large-volume pulmonary aspiration,[78] presence of tracheostomy,[79] and prolonged ICU stay.[80] Prolonged antibiotic course is a frequent risk factor for *A.baumannii* infections. Presence of a resistant phenotype that often involves carbapenems is of great concern. For carbapenem resistant strains, high doses of nebulized (aerosolized) colistin has been associated with good resolution and shorter periods of hospitalization.[81] It has been claimed that the high doses of colistin can be delivered by nebulization without significant systemic exposure because of the fact that, even in the presence of severe lung infection, colistin does not easily cross the alveolar-capillary membrane.[81]

Management Strategies

Management Strategies of VAP

The priority in pneumonia management is to avoid any delays in the administration of adequate antibiotics; inadequate treatment increases mortality and, in survivors, it increases healthcare costs. The use of broad-spectrum antibiotics as initial empirical has been advocated, however academics have concerns on resistance emergence. Indeed, the most effective strategy against resistance development should be based on prompt and unequivocal killing of the microbes and thereby defeating resistance before it starts ('dead bugs don't mutate'). In addition, the de-escalation strategy that allows the use use of broad-spectrum antibiotics as initial empirical, maximizing the odds to an appropriate antibiotic therapy associated with a early de-escalation, using a more strict-spectrum coverage after pathogen identification, minimizing exposure and risk for resistance emergence, have demonstrated benefit on clinical outcomes in ventilated patients. [82,83] The 'right first time' concept and short duration of therapy whenever possible, is the 'two-steps' strategy for VAP management.

There is controversy regarding the best diagnostic method for VAP (invasive versus non-invasive sampling techniques). A meta-analysis by Shorr and colleagues concluded that the use of invasive strategies did not alter mortality, but it affected antibiotic utilization, leading to modifications in the antibiotic regimen in more than half of patients.[84] On the other hand, findings from Canadian Critical Care Trials Group study showed no difference in clinical and microbiolgical outcomes comparing an invasive and non-invasive diagnostic approach, suggesting endotracheal aspirate might be as effective as bronchoalveolar lavage for etiological diagnosis in VAP. [85] Nevertheless, using quantitative culture technique might help to evaluate probability for colonization or infection, although no unequivocal cut-off could be found. [86]

The optimal length of treatment has not been conclusively established, but in the EU-VAP study,[40] a large European multicentre cohort, standard of care was an 8-day antibiotic regimen, according to current recommendations [87]. The EU-VAP study listed the causes of antibiotic prescription for intubated patients in Europe, including reasons for anti-MRSA prescription. Further studies are now required to evaluate more recently devised treatment strategies and their impact on emerging resistance. Careful antibiotic monitoring is recommended in the ICU setting, but it is not known which monitoring practices are associated with benefits. In fact, the real impact of stewardship on the emergence of resistance and on patients' outcomes is still to be established, but it seems that changing our practices to individualize management, avoid homogeneous selective pressure and employ the entire potential of our antimicrobial choices are useful strategies to escape the adverse consequences associated with the emerging resistance [88].

Management Strategies of VAT

More controversial is the use of antibiotics in VAT. In a recent survey,[89] only the 24.3% of prescribers routinely prescribed antibiotics for VAT; conversely, 26% considered that VAT should not be treated with antibiotics, whereas only 24% indicated a preference for an antibiotic course lower than 7 days.[89] Nseir and colleagues on the other hand, reported lower mortality rates and more MV free-days when VAT was treated with IV antibiotics (45% of the cohort were COPD patients).[90] Palmer and colleagues conducted a double-blind, randomized, placebo-controlled study, reporting decrease of VAP development rates, faster weaning, reduced use of systemic antibiotics, and reduce of bacterial resistance when nebulized antibiotics were administered for VAT.[91] Dallas and colleagues reported that patients diagnosed

with VAT had similar outcomes to those with VAP, suggesting that administration of antimicrobial therapy might be appropriate for VAT.[10] Consistent reports of increased length of ICU stay in VAT due to prolonged MV need, provide a strong rationale for antibiotic administration. The duration of targeted VAT treatment has not been established, but VAT may respond to shorter courses.[92]Further research is warranted to identify the subgroup of patients with VAT that would benefit from antimicrobial treatment and the subgroup that could safely have antimicrobial therapy withheld or limited.

Key issues for management of VAP and VAT (Figure 3) are the following: a) Identification when to start an antibiotic; b) what microbiologic test can be of help to guide antibiotic prescriptions, being advisable to perform quantitative respiratory cultures of a high quality respiratory specimen; c) What organism should be covered, based on direct staining and the presence of potential risk factors; d) What initial agent should be prescribed and at what dosage, based on baseline susceptibilities, patient's conditions and prior antibiotic exposure; e) duration of therapy.

The currently standard on duration of antibiotic therapy for VAP is one week, although patients with core pathogens present quick resolution and might benefit from even ultra-short courses. On the other hand, in cases of *P. aeruginosa* VAP that receive inappropriate initial treatment or in cases of MRSA VAP, the resolution is usually delayed and more than 10 days of antibiotics are required.[44] Improvement in oxygenation and defervescence occurs within three days in the majority of patients. Assessment of the delta value of a biomarker may contribute to more objective decisions, but increases costs.[93] Resolution of CXR, WBCs count or clearance of

respiratory secretions does not help.[94] In VAT, at present, there is no evidence that can support an objective decision to prolong therapy.

Areas of uncertainty

A long-standing problem in ICU care is the differential diagnosis between the inflammatory response and the infection. In many cases, the challenge is to establish whether bacteria are merely colonising the patient or whether they are in fact the cause of disease. There are two main issues here. First, how can we conclusively determine that the bacterial growth in the respiratory tract sample is the cause of the inflammatory response in a ventilated patient? Second, how can we establish that the microorganism isolated from an upper airway sample is the cause of the disease in the lower airway?

The biomarkers required for pre-emptive treatment are often insufficient to resolve these problems.[95] Pro-calcitonin is currently the most widely used,[96,97] but the search is on for other biomarkers offering better sensitivity and specificity.[97,98] In the future, genomics may provide a better answer to these problems,[98,99] but at the moment further research is needed on gene expressions before this marker (or others) can be widely used in clinical practice.

Another current focus of research is the identification of the causative microorganism in a timely fashion. [96,100,101] Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry or MALDI-TOF MS (sometimes without the MS) is a particularly promising technique. It can identify either Gram-positive or Gram-negative bacteria (to species level) within a matter of minutes, and only a relatively low bacterial load is needed for identification. A large recent observational study

demonstrated the clinical benefit of this rapid turn-around time.[101] Indeed, a system of this kind, able to identify the pathogen and its sensitivity quickly and accurately in a point-of-care test, would signal a new era for the management of respiratory infections.

In contrast with community-acquired respiratory infections, where there are different scores to stratify by severity-of-illness. It is not a common practice to stratify the severity of an episode of respiratory infection in MV patients. However, it is obvious that VARIs are heterogeneous and they need to be compared. Lisboa and colleagues designed the VAP PIRO score to stratify risk of death. It combines information on **P**redisposition (comorbidities), **I**njury (bacteremia), **R**esponse (systolic blood pressure under 90 mmHg or use of vasopressors) and **O**rgan failure (ARDS) This is a single score that classifies patients in three categories (0-1, 2 and 3-4) depending the risk of ICU mortality: low (1 of 8), intermediate (1 of 2) and high (4 of 5).[102] A cohort validation, demonstrated a good correlation with health care resources use.[102] Further research should be conducted to refine the tool, and perhaps to add biomarkers in the intermediate severity in order to improve its stratification capacity.

Regarding the possibility to avoid VARI with antibiotic prophylaxis, in a prospective randomised study of major heart surgery patients, Bouza et al. assigned patients to either "standard of care" or to three days of prophylactic antibiotics (meropenem and linezolid).[103] No patient-centred outcome benefits (i.e. mortality, ventilatory days, ICU length of stay) were found, but the authors observed a significantly lower incidence of VARI (combined VAP and VAT) in the intervention group and a 4.5-day delay in the onset of pneumonia.[103] They also found an association between three days of pre-emptive treatment and an increase in resistance to linezolid.[103] In a previous study our group established that an antimicrobial regimen of more than two days may

be associated with increased resistance,[104] and may modify the gut flora. Our group's recent finding that VAP can be prevented with a single dose, as is the case in surgical prophylaxis, underlines the importance of appropriate stewardship in the ICU.[105]

Lastly, inhalation is likely to establish itself as a new means of antibiotic delivery. Rationale for using inhaled therapy include ability to achieve high lung tissue concentrations minimizing systemic absorption [106, 107]. In humans, Lu et al. [108] described a study using nebulized ceftazidime (15 mg·kg(-1)·3 h(-1)) and amikacin (25 mg·kg(-1)·d(-1)) and showed that nebulization of antibiotics provided high lung tissue concentrations and rapid bacterial killing in ventilator-associated pneumonia caused by Pseudomonas aeruginosa. A recent study indicated comparable outcomes in patients with multidrug-resistant non-fermentative GNB when high dose colistin (5MU/8h) was nebulized, either in isolation or combined with parenteral treatment. [109] This method for administering high concentrations of antibiotics in the distal airways enhances bacterial killing in the case of organisms with very high MICs and customized use according to the pathogen and the MIC is an opportunity for further research. Potential adverse events, such as blocking of the expiratory limb of the ventilator or bronchospasm and contraindications in severe hypoxemia are potential limitations that require further research.

Conclusions

Respiratory infections in mechanically ventilated patients present in the form of severe sepsis or septic shock in intubated patients. Purulent respiratory secretions are required for diagnosis, but differentiating between pneumonia and tracheobronchitis based only on clinical findings is a clinical challenge. Both presentations are

associated with prolonged MV period and ICU stay, providing rationale for therapy. Key VARI management issues are: what is the pathogen, initial antibiotic choice and decisions depending on resolution pattern and criteria. New opportunities for research include role for biomarkers, earlier etiological diagnosis with molecular diagnosis techniques and optimization and customization of therapy.

REFERENCES

1. Vincent JL, Rello J, Marshall J, et al.; EPIC II Group of Investigators. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009; 302:2323-29.

2. Klompas M. Does this patient has ventilator-associated pneumonia. JAMA 2007; 297:1583-93.

3. Klompas M. What can we learn from international ventilator-associated pneumonia rates? *Crit Care Med* 2012; 40:3303-04.

4. Niederman MS. Hospital-acquired pneumonia, health care-associated pneumonia,ventilator-associated pneumonia, and ventilator-associated tracheobronchitis: definitions and challenges in trial design. *Clin Infect Dis* 2010; 51 Suppl 1:S12-17.

5. Improving surveillance for ventilator-associated events in adults. Centers for Disease Control and Prevention website. http://www.cdc.gov/nhsn/PDFs/pscManual/10-VAE_FINAL.pdf Accessed April 13, 2014

6. Stevens JP, Kachniarz B, Wright SB, et al. When policy gets it right: variability in u.s. Hospitals' diagnosis of ventilator-associated pneumonia. *Crit Care Med* 2014; 42:497-503.

7. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1991; 143:1121-29.

8. Luna CM, Blanzaco D, Niederman MS, et al. Resolution of ventilator associated pneumonia: prospective evaluation of the clinical pulmonary

infection score as an early clinical predictor for outcome. Crit Care Med 2003; 31: 676-82.

9. Parks NA, Magnotti LJ, Weinberg JA, et al. Use of the clinical pulmonary infection scopre to guide therapy for ventilator-associated pneumonia risks antibiotic overexposure in patients with trauma. J Trauma Acute Care Surg 2012; 73:52-8.

10. Dallas J, Skrupky L, Abebe N, Boyle WA 3rd, Kollef MH. Ventilator-associated tracheobronchitis in a mixed surgical and medical ICU population. *Chest* 2011; 139:513-18.

11. Martin-Loeches I, Papiol E, Almansa R, López-Campos G, Bermejo-Martin JF, Rello J. Intubated patients developing tracheobronchitis or pneumonia have distinctive complement system gene expression signatures in the pre-infection period: a pilot study. *Med Intensiva* 2012; 36:257-63

12. Solomon DH, Wobb J, Buttaro BA, Truant A, Soliman AM. Characterization of bacterial biofilms on tracheostomy tubes. *Laryngoscope* 2009; 119:1633-8.

13. Meslemani D, Yaremchuk K, Rontal M. Presence of biofilm on adult tracheostomy tubes. *Ear Nose Throat J* 2010; 89:496-504.

14. Blot S, Lisboa T, Angles R, Rello J. Prevention of VAP: Is zero rate possible? *Clin Chest Med* 2011; 32: 591-599.

15. Melsen WG, Rovers MM, Groenwold RH, et al. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. *Lancet Infect Dis* 2013; 13:665-71.

16. Cuellar LE, Fernandez-Maldonado E, Rosenthal VD, Castaneda-Sabogal A, Rosales R, Mayorga-Espichan MJ, et al. Device-associated infection rates and mortality in intensive care units of Peruvian hospitals: findings of the

International Nosocomial Infection Control Consortium. Rev Panam Salud Publica 2008 Jul; 1:16-24

17. Guanche-Garcell H, Requejo-Pino O, Rosenthal VD, Morales-Perez C, Delgado-Gonzalez O, Fernandez-Gonzalez D. Device-associated infection rates in adult intensive care units of Cuban university hospitals: International Nosocomial Infection Control Consortium (INICC) findings. Int J Infect Dis May; 5:e357-362.

18. Hu B, Tao L, Rosenthal VD, Liu K, Yun Y, Suo Y, et al. Device-associated infection rates, device use, length of stay, and mortality in intensive care units of 4 Chinese hospitals: International Nosocomial Control Consortium findings. Am J Infect Control Apr; 4:301-306

19. Mehta A, Rosenthal VD, Mehta Y, Chakravarthy M, Todi SK, Sen N, et al. Device-associated nosocomial infection rates in intensive care units of seven Indian cities. Findings of the International Nosocomial Infection Control Consortium (INICC). J Hosp Infect 2007 Oct; 2:168-174.

20. Moreno CA, Rosenthal VD, Olarte N, Gomez WV, Sussmann O, Agudelo JG, et al. Device-associated infection rate and mortality in intensive care units of 9 Colombian hospitals: findings of the International Nosocomial Infection Control Consortium. Infect Control Hosp Epidemiol 2006 Apr; 4:349-356.

21. Salomao R, Rosenthal VD, Grimberg G, Nouer S, Blecher S, Buchner-Ferreira S, et al. Device-associated infection rates in intensive care units of Brazilian hospitals: findings of the International Nosocomial Infection Control Consortium. Rev Panam Salud Publica 2008 Sep; 3:195-202.

22. Rosenthal VD, Guzman S, Crnich C. Device-associated nosocomial infection rates in intensive care units of Argentina. Infect Control Hosp Epidemiol

2004;25:251-255.

23. Riera J, Caralt B, Augustin S, et al. Complications in the immediate postoperatory of lung transplantation: three years of practice at a high-experienced center. *Chest* 2014; 145(3 Suppl):631A

24. Magret M, Amaya-Villar R, Garnacho J, et al.; EU-VAP/CAP Study Group. Ventilator-associated pneumonia in trauma patients is associated with lower mortality: results from EU-VAP study. *J Trauma* 2010; 69:849-54.

25. Melsen WG, Rovers MM, Groenwold RH, et al. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. *Lancet Infect Dis* 2013; 13:665-71

26. Muscedere J, Sinuff T, Heyland DK, et al.; Canadian Critical Care Trials Group. The clinical impact and preventability of ventilator-associated conditions in critically ill patients who are mechanically ventilated. *Chest* 2013; 144:1453-60

27. Rello J, Afonso E, Lisboa T, et al.; FADO Project Investigators. A care bundle approach for prevention of ventilator-associated pneumonia. *Clin Microbiol Infect* 2013; 19:363-69.

28. Rello J, Ollendorf DA, Oster G, et al.; VAP Outcomes Scientific Advisory Group. Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. *Chest* 2002; 122:2115-21.

29. Koulenti D, Lisboa T, Brun-Buisson C, et al.; EU-VAP/CAP Study Group. Spectrum of practice in the diagnosis of nosocomial pneumonia in patients requiring mechanical ventilation in European intensive care units. *Crit Care Med* 2009; 37:2360-68.

30. Blot S, Koulenti D, Dimopoulos G, et al.; EU-VAP Study Investigators.

Prevalence, risk factors, and mortality for ventilator-associated pneumonia in middle-aged, old, and very old critically ill patients. *Crit Care Med* 2014; 42:601-09.

31. Esperatti M, Ferrer M, Theessen A, et al. Nosocomial pneumonia in the intensive care unit acquired by mechanically ventilated versus nonventilated patients. Am J *Respir Crit Care Med* 2010; 182:1533-39.

32. Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS. Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. *Chest* 2005; 128:3854-62.

33. Lee MS, Walker V, Chen LF, Sexton DJ, Anderson DJ. The epidemiology of ventilator-associated pneumonia in a network of community hospitals: a prospective multicenter study. *Infect Control Hosp Epidemiol* 2013; 34:657-62.

34. Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques for ventilator-associated pneumonia. *N Engl J Med* 2006; 355:2619-30

35. Bekaert M, Timsit JF, Vansteelandt S, et al.; Outcomerea Study Group. Attributable mortality of ventilator-associated pneumonia: a reappraisal using causal analysis. *Am J Respir Crit Care Med* 2011; 184:1133-39.

36. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J Infect Dis* 2009; 197:1079-81

37. Sandiumenge A, Lisboa T, Gomez F, Hernandez P, Canadell L, Rello J. Effect of antibiotic diversity on ventilator-associated pneumonia caused by ESKAPE Organisms. *Chest* 2011; 140:643-51.

38. Depuydt PO, Vandjick DM, Bekaert MA, et al. Determinants and impact of multidrug antibiotic resistance in pathogens causing ventilator-associated pneumonia. Crit Care 2008; 12: R142.

39. Di Pasquale M, Ferrer M, Esperatti M, et al. Assessment of severity of ICU-acquired pneumonia and association with etiology. *Crit Care Med* 2014; 42:303-12

40. Rello J, Ulldemolins M, Lisboa T, et al.; EUVAP/CAP Study group. Determinants of prescription and choice of therapy in hospital-acquired pneumonia and ventilator-associated pneumonia. *Eur Resp J* 2011; 37:1332-39.

41. Rello J, Torres A, Ricart M, et al. Ventilator-associated pneumonia by Staphylococcus aureus. Comparison of methicillin-resistant and methicillin-sensitive episodes. *Am J Respir Crit Care Med* 1994; 150:1545-49.

42. Agbaht K, Diaz E, Muñoz E, et al. Bacteremia in patients with ventilator-associated pneumonia is associated with increased mortality: A study comparing bacteremic vs. nonbacteremic ventilator-associated pneumonia. *Crit Care Med* 2007; 35:2064-70.

43. Hanberger H, Walther S, Leone M, et al.; EPIC II Group of Investigators. Increased mortality associated with methicillin-resistant Staphylococcus aureus (MRSA) infection in the intensive care unit: results from the EPIC II study. *Int J Antimicrob Agents* 2011; 38:331-35.

44. Vidaur L, Planas K, Sierra R, et al. Ventilator-associated pneumonia: impact of organisms on clinical resolution and medical resources utilization. *Chest* 2008; 133:625-32.

45. Wunderink RG, Niederman MS, Kollef MH, et al. Linezolid in methicillin-

resistant Staphylococcus aureus nosocomial pneumonia: a randomized, controlled study. *Clin Infect Dis* 2012; 54:621-29.

46. Lahey T. Questionable superiority of linezolid for methicillin-resistant Staphylococcus aureus nosocomial pneumonia: watch where you step. Clin Infect Dis. 2012 Jul;55(1):159-60

47. Tumbarello M, De Pascale G, Trecarichi EM, et al. Clinical outcomes of Pseudomonas aeruginosa pneumonia in intensive care unit patients. *Intensive Care Med* 2013; 39:682-92.

48. Sandiumenge A, Rello J. Ventilator-associated pneumonia caused by ESKAPE organisms: cause, clinical features, and management. *Curr Opin Pulm Med* 2012; 18:187-93

49. Garnacho-Montero J, Sa-Borges M, Sole-Violan J, et al. Optimal management therapy for Pseudomonas aeruginosa ventilator-associated pneumonia: an observational, multicenter study comparing monotherapy with combination antibiotic therapy. *Crit Care Med* 2007; 35:1888-95.

50. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. Antimicrob Agents Chemother 2006; 50:43-48

51. Parker CM, Kutsogiannis J, Muscedere J, et al.; Canadian Critical Care Trials Group.Ventilator-associated pneumonia caused by multidrug-resistant organisms or Pseudomonas aeruginosa: prevalence, incidence, risk factors, and outcomes. *J Crit Care* 2008; 23:18-26.

52. Nakamura A, Miyake K, Misawa S, et al. Meropenem as predictive risk factor for isolation of multidrug-resistant Pseudomonas aeruginosa. *J Hosp Infect* 2013; 83:153-55.

53. Liew YX, Tan TT, Lee W, et al. Risk factors for extreme-drug resistant Pseudomonas aeruginosa infections in patients with hematologic malignancies. *Am J Infect Control* 2013; 41:140-44.

54. Park YS, Lee H, Chin BS, et al. Acquisition of extensive drug-resistant *Pseudomonas aeruginosa* among hospitalized patients: risk factors and resistance mechanisms to carbapenems. *J Hosp Infect* 2011; 79:54-58.

55. Peña C, Gómez-Zorrilla S, Oriol I, et al. Impact of multidrug resistance on Pseudomonas aeruginosa ventilator-associated pneumonia outcome: predictors of early and crude mortality. *Eur J Clin Microbiol Infect Dis* 2013; 32:413-20.

56. Depuydt PO, Vandijck DM, Bekaert MA, et al. Determinants and impact of multidrug antibiotic resistance in pathogens causing ventilator-associated-pneumonia. *Crit Care* 2008; 12:R142.

57. Azoulay E, Timsit JF, Tafflet M, et al.; Outcomerea Study Group. Candida colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. *Chest* 2006; 129:110-17.

58. Hamet M, Pavon A, Dalle F, et al. Candida spp. airway colonization could promote antibiotic-resistant bacteria selection in patients with suspected ventilator-associated pneumonia. *Intensive Care Med* 2012; 38:1272-79.

59. Ricard JD, Roux D. Candida colonization in ventilated ICU patients: no longer a bystander! *Intensive Care Med* 2012; 38:1243-5

60. Nseir S, Jozefowicz E, Cavestri B, et al. Impact of antifungal treatment on Candida-Pseudomonas interaction: a preliminary retrospective case-control study. *Intensive Care Med* 2007; 33:137–142

61. Boyer A, Doussau A, Thiébault R, et al. Pseudomonas aeruginosa acquisition on an intensive care unit: relationship between antibiotic selective pressure

and patients' environment. *Crit Care* 2011; 15:R55.

62. Cook PP, Gooch M, Rizzo S. Reduction in fluoroquinolone use following introduction of ertapenem into a hospital formulary is associated with improvement in susceptibility of Pseudomonas aeruginosa to group 2 carbapenems: a 10-year study. *Antimicrob Agents Chemother* 2011; 55:5597-01.

63. Augustin P, Kermarrec N, Muller-Serieys C, et al. Risk factors for multidrug resistant bacteria and optimization of empirical antibiotic therapy in postoperative peritonitis. *Crit Care* 2010; 14:R20

64. Ginn AN, Wiklendt AM, Gidding HF, George N, O'Driscoll JS, Partridge SR, O'Toole BI, Perri RA, Faoagali J, Gallagher JE, Lipman J, Iredell JR. The ecology of antibiotic use in the ICU: homogeneous prescribing of cefepime but not tazocin selects for antibiotic resistant infection. *PLoS One* 2012; 7:e38719.

65. Kanj SS, Kanafani ZA. Current concepts in antimicrobial therapy against resistant gram-negative organisms: extended-spectrum beta-lactamase-producing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae, and multidrug-resistant Pseudomonas aeruginosa. *Mayo Clin Proc* 2011; 86:250-59.

66. Yang K, Zhuo H, Guglielmo BJ, Wiener-Kronish J. Multidrug-resistant Pseudomonas aeruginosa ventilator-associated pneumonia: the role of endotracheal aspirate surveillance cultures. *Ann Pharmacother* 2009; 43:28-35.

67. Plüss-Suard C, Pannatier A, Kronenberg A, Mühlemann K, Zanetti G. Impact of antibiotic use on carbapenem resistance in Pseudomonas aeruginosa: Is

there a role for antibiotic diversity? *Antimicrob Agents Chemother* 2013; 57:1709-13.

68. Eagye KJ, Kuti JL, Nicolau DP. Risk factors and outcomes associated with isolation of meropenem high-level-resistant Pseudomonas aeruginosa. *Infect Control Hosp Epidemiol* 2009; 30:746-752.

69. Kaminski C, Timsit JF, Dubois Y, et al.; OUTCOMEREA study group. Impact of ureido/carboxypenicillin resistance on the prognosis of ventilator-associated pneumonia due to Pseudomonas aeruginosa. *Crit Care* 2011; 15:R112

70. Combes A, Luyt CE, Fagon JY, Wolff M, Trouillet JL, Chastre J. Impact of piperacillin resistance on the outcome of Pseudomonas ventilator-associated pneumonia. *Intensive Care Med* 2006; 32:1970-78.

71. Hueck CL,Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol Mol Biol Rev* 1998; 62:379-33.

72. Veesenmeyer JL, Hauser AR, Lisboa T, Rello J. Pseudomonas aeruginosa virulence and therapy: evolving translational strategies. *Crit Care Med* 2009; 37:1777-86.

73. El Solh AA, Akinnusi ME, Wiener-Kronish JP, Lynch SV, Pineda LA, Szarpa K. Persistent infection with Pseudomonas aeruginosa in ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2008; 178:513-519

74. Lu Q, Eggimann P, Luyt CE, et al. Pseudomonas aeruginosa serotypes in nosocomial pneumonia: prevalence and clinical outcomes. *Crit Care* 2014; 18:R17.

75. Le Berre R, Nguyen S, Nowak E, et al.; Pyopneumagen Group. Relative contribution of three main virulence factors in Pseudomonas aeruginosa

pneumonia. *Crit Care Med* 2011; 39:2113-20.

76. Faure K, Shimabukuro D, Ajayi T, Allmond LR, Sawa T, Wiener-Kronish JP. O-antigen serotypes and type III secretory toxins in clinical isolates of *Pseudomonas aeruginosa. J Clin Microbiol* 2003; 41:2158-60.

77. Lu Q, Rouby JJ, Laterre PF, et al. Pharmacokinetics and safety of panobacumab: specific adjunctive immunotherapy in critical patients with nosocomial Pseudomonas aeruginosa O11 pneumonia. *J Antimicrob Chemother* 2011; 66:1110-16.

78. Baraibar J, Correa H, Mariscal D, Gallego M, Vallés J, Rello J. Risk factors for infection by Acinetobacter baumannii in intubated patients with nosocomial pneumonia. *Chest* 1997; 112:1050-54

79. Mah MW, Memish ZA, Cunningham G, Bannatyne RM. Outbreak of *Acinetobacter baumannii* in an intensive care unit associated with tracheostomy. *Am J Infect Control* 2001; 29:284-88.

80. Playford EG, Craig JC, Iredell JR. Carbapenem-resistant Acinetobacter baumannii in intensive care unit patients: risk factors for acquisition, infection and their consequences. *J Hosp Infect* 2007; 65:204-11.

81. Rouby JJ, Bouhemad B, Monsel A, Brisson H, Arbelot C, Lu Q; Nebulized Antibiotics Study Group. Aerosolized antibiotics for ventilator-associated pneumonia: lessons from experimental studies. *Anesthesiology* 2012; 117:1364-80.

82. Rello J, Vidaur L, Sandiumenge A et al. De-escalation therapy in ventilator associated pneumonia. *Crit Care Med* 2004; 32: 2183-90.

83. Raman K, Nailor MD, Nicolau DP, et al. Early antibiotic discontinuation in patients with clinically suspected ventilator-associated pneumonia and

negative quantitative bronchoscopy cultures. *Crit Care Med* 2013; 41: 1656-63.

84. Shorr AF, Sherner JH, Jackson WL, Kollef MH. Invasive approaches to the diagnosis of ventilator-associated pneumonia: a meta-analysis. *Crit Care Med* 2005; 33:46-53.

85. Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques for ventilator associated pneumonia. *N Engl J Med* 2006; 355: 2619-30.

86. Lisboa T, Rello J. Diagnosis of ventilator-associated pneumonia: is there a gold standard and a simple approach?. *Curr Opin Infect Dis* 2008; 21:174-178.

87. Chastre J, Wolff M, Fagon JY, et al. Comparison of 8 vs 15 days of antibiotic therapy for ventilator associated pneumonia in adults: a randomized trial. *JAMA* 2003; 290: 2588-98.

88. Lisboa T, Nagel F. Infection with multdrugi-resistant agents in the ICU: how to escape? *Rev Bras Ter Intensiva*. 2011; 23(2):120-124.

89. Rodríguez A, Póvoa P, Nseir S, Salluh J, Curcio D, Martín-Loeches I. Incidence and diagnosis of ventilator-associated tracheobronchitis (VAT) in the intensive care unit: an international online survey. *Crit Care 2014*; 18:R32.

90. Nseir S, Favory R, Jozefowicz E, et al.; VAT Study Group. Antimicrobial treatment for ventilator-associated tracheobronchitis: a randomized, controlled, multicenterstudy. *Crit Care* 2008;12:R62.

91. Palmer LB, Smaldone GC, Chen JJ, et al. Aerosolized antibiotics and ventilator-associated tracheobronchitis in the intensive care unit. *Crit Care Med* 2008; 36:2008-13

92. Craven DE, Chroneou A, Zias N, Hjalmarson KI. Ventilator-associated

tracheobronchitis: the impact of targeted antibiotic therapy on patient outcomes. *Chest* 2009; 135:521-28.

93. Seligman R, Meisner M, Lisboa TC, et al. Decreases in procalcitonin and C-reactive protein are strong predictors of survival in ventilator-associated pneumonia. *Crit Care* 2006;10:R125

94. Vidaur L, Gualis B, Rodriguez A, et al. Clinical resolution in patients with suspicion of ventilator-associated pneumonia: a cohort study comparing patients with and without acute respiratory distress syndrome. *Crit Care Med* 2005; 33:1248-53.

95. Calfee CS, Pugin J. The search for diagnostic markers in sepsis: many miles yet to go. *Am J Respir Crit Care Med* 2012; 186:2-4.

96. Póvoa P, Salluh JI. Biomarker-guided antibiotic therapy in adult critically ill patients: a critical review. *Ann Intesnsive Care* 2012; 2:32.

97. Mitsuma SF, Mansour MK, Dekker JP. Promising new assays and technologies for the diagnosis and management of infectious diseases. *Clin Infect Dis* 2013; 56:996-02.

98. Sutherland A, Thomas M, Brandon R, et al. Development and validation of a novel molecular biomarker diagnostic test for the early detection of sepsis. *Critical Care* 2011; 15:R149

99. Johnson SB, Lissauer M, Bochicchio GV, Moore R, Cross AS, Scalea TM. Gene expression profiles differentiate between sterile SIRS and early sepsis. *Ann Surg* 2007; 245:611-21.

100.   Prod'hom G, Bizzini A, Durussel C, Bille J, Greub G. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for direct bacterial

identification from positive blood culture pellets. *J Clin Microbiol* 2010; 48:1481-83.

101.   Clerc O, Prod'hom G, Vogne C, Bizzini A, Calandra T, Greub G. Impact of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry on the Clinical Management of Patients With Gram-negative Bacteremia: A Prospective Observational Study. *Clin Infect Dis* 2013; 56:1101-07.

102.   Lisboa T, Diaz E, Sa-Borges M, et al. The ventilator-associated pneumonia PIRO score: a tool for predicting ICU mortality and health-care resources use in ventilator-associated pneumonia. *Chest* 2008; 134:1208-16.

103.   Bouza E, Perez-Granda MJ, Hortal J, Barrio JM, Cercenado E, Munoz P. Pre-emptive broad-spectrum treatment for ventilator-associated pneumonia in high-risk patients. *Intensive Care Med* 2013; 39:1547-55.

104.   Rello J, Ausina V, Ricart M, Castella J, Prats G. Impact of prior antibiotic therapy on etiology and outcomes of Ventilator-associated pneumonia. *Chest* 1993; 104:1230-35.

105.   Rello J. Antibiotic stewardship in the ICU. *Chest* 2013; 143:1195-96.

106.   Rouby JJ, Bouhemad B, Monsel A, et al. Aerosolized antibiotics for ventilator associated pneumonia: lessons from experimental studies. *Anesthesiology* 2012; 117: 1364-80.

107.   Luyt CE, Combes A, Nieszkowska A, et al. Aerosolized antibiotics to treat ventilator associated pneumonia. *Curr Opin Infect Dis* 2009; 22:154-8.

108.   Lu Q, Yang J, Liu Z, et al. Nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by *Pseudomonas aeruginosa. Am J Respir Crit Care Med* 2011; 184:106-15.

109.   Lu Q, Luo R, Yang J, et al. Efficacy of high-dose nebulized colistine in ventilator-associated pneumonia caused by multidrug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* *Anesthesiology* 2012; 117:135-47

Table 1. Top three pathogens of VAP reported in six studies published during the past decade.[20,22-26]

Koulenti et al. EUVAP/CAP study group, 2009 Prospective multicenter study; 27 ICUs of 9 European countries; 465 cases of VAP[*]	Overall	Early-onset (< 5 days)	
	S.aureus 32.6% ------------(MRSA 18.0%) ------------(MRSA 14.6%) *P.aeruginosa* 22.8% *A.baumannii* 20.2%	MSSA 27.6% *P.aeruginosa* 17.9% MRSA 12.4%	
		Late-onset (≥5 days)	
		A.baumannii 26.5% *P.aeruginosa* 26.1% MRSA 16.1 %	
Esperatti et al., 2010 Prospective single center Spanish study; 164 VAP cases [**]	*P.aeruginosa* 24.0% MSSA 14.0% MRSA 9.0%		
Kollef et al., 2005 Retrospective multicenter study, 59 US hospitals, 499 culture-positive VAP cases	MSSA 28.5% *P,aeruginosa* 21.2% MRSA 19.0%		
Lee MS et al., 2013 Prospective multicenter study; 31 US community hospitals; 247 VAP cases[**]	MRSA 24.5% *Pseudomonas spp* 14.0% *Klebsiella spp* 11.9%		
Canadian Critical Care Trials, 2006 Group, 2006 Prospective, multicenter study; 28 ICUs in Canada & USA; 739 VAP cases**	*S.aureus* 17.2% *H.influenzae* 13.4% Enterobacter spp 9.3%		
Bekaert M et al.; OUTCOMEREA Study Group, 2011[***] Longitudinal prospective French multicenter Outcocomerea database; 685 patients with microbiologically confirmed VAP***	*P.aeruginosa* 26.2% MSSA 9.7% *A. baumannii* 8.2%		

Percentages refer to microbiologically confirmed VAP cases (n=356); **Percentages refer to all VAP cases; ***Percentages refer to the total number of isolated microorganisms (n=868)

Figure 1. Depiction of the main proposed pathogenetic correlations between VAT and VAP.
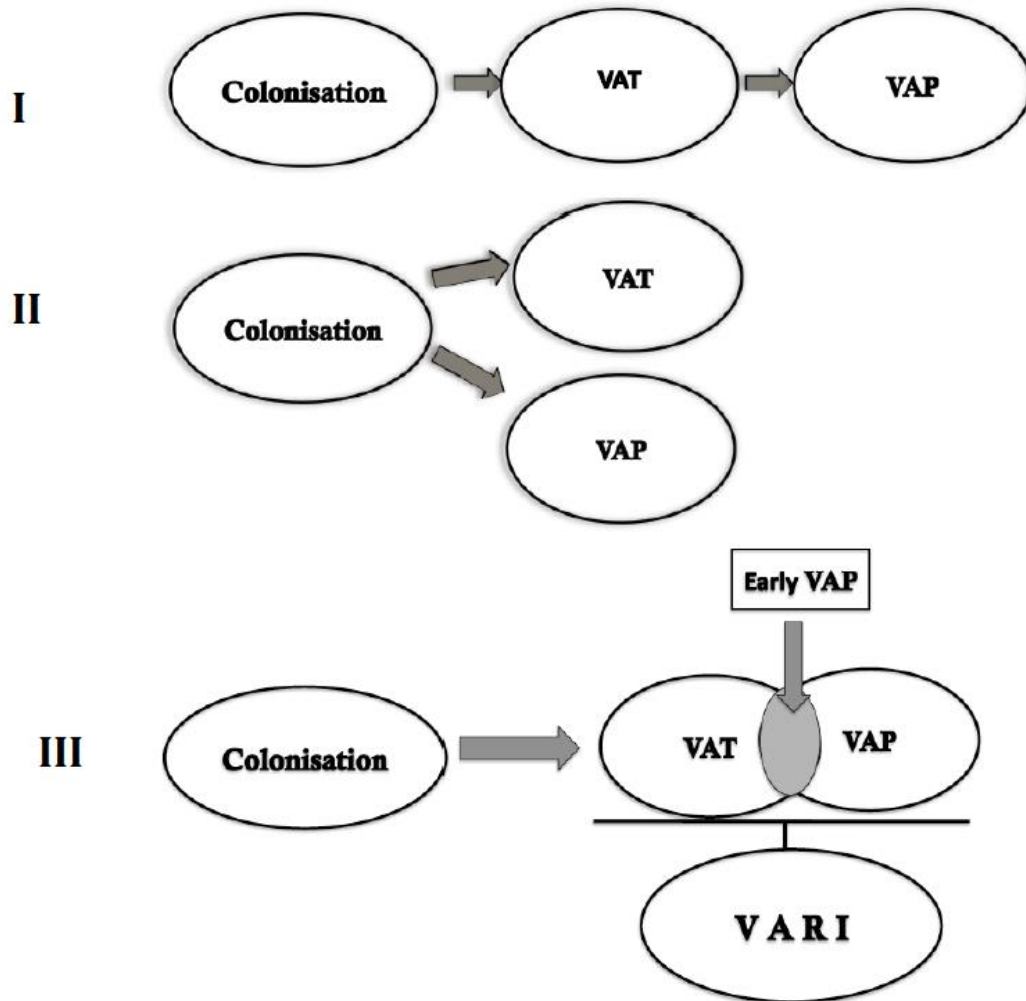
Figure 2. Median (25-75 percentiles) onset of VAP by pathogen (onset as days after intubation). Data of 465 episodes of VAP from the EU-VAP/CAP Study database (only the first episodes of pneumonia were included in the analysis).
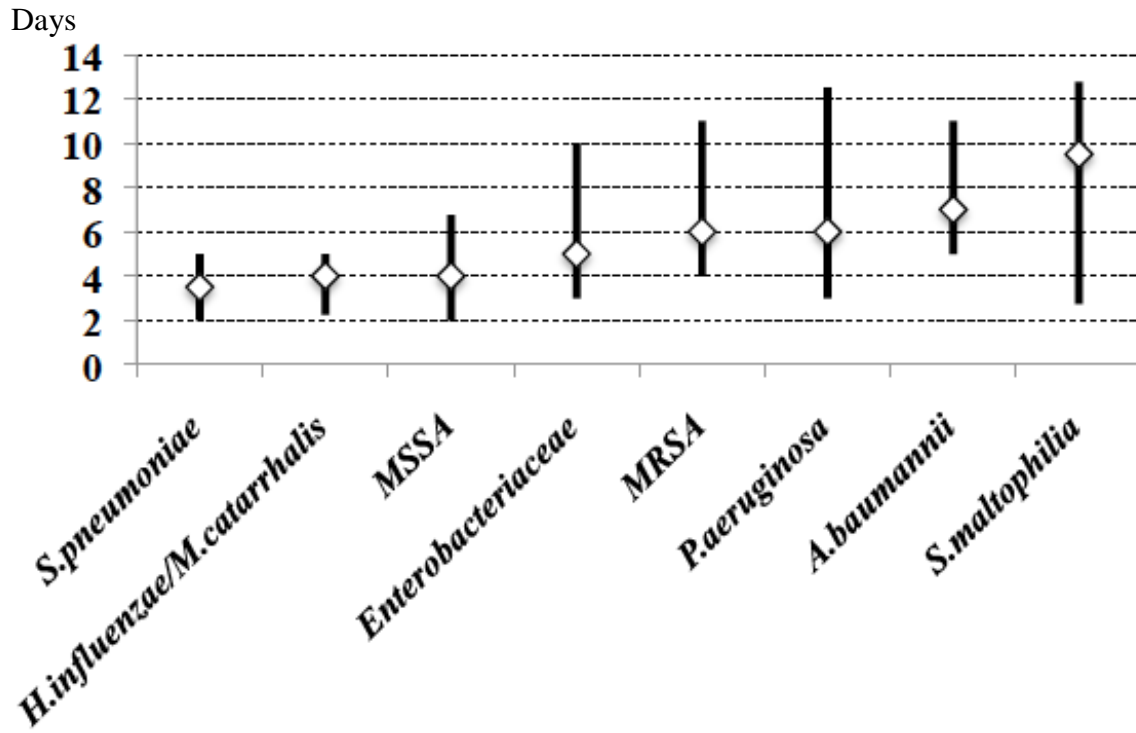
Figure 3. Approach to the work-up of Ventilator-Associated Respiratory Infections (VARI).

Therapeutic Management of VARI

Risk factors for specific organism?

Known organism colonization?

YES

EMPIRIC THERAPY

Baseline MDR? MV > 5 days. COPD?

IC, Vasopressors, Severe hypoxemia?

NO — Narrow Spectrum

YES — Broad Spectrum

CLINICAL RESPONSE

FAVORABLE

De-escalate

Combo therapy to Monotherapy

Culture negative -> stop antibiotic

GNB or other: adapt antibiotic

DELAYED

Switch to broader spectrum

-Add second agent

-Increase Dosage

-Rule out EMPYEMA, ABSCESS..

Resultados

8. ARTIGOS ORIGINAIS

Using CRP to evaluate clinical response in chronic critically ill patients with nosocomial pneumonia: still useful?

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INTRODUCTION

Ventilator-associated pneumonia (VAP), a clinical situation associated with high morbidity and mortality, is the most prevalent infectious complication in intensive care unit (ICU) patients (1 - 3). Microbiological data, obtained using an invasive or non-invasive strategy are essential to the evaluation of the appropriateness of antibiotic therapy, an important determinant of outcome in these patients (4-6). Moreover, the monitoring of biomarkers may play a role in prognosis assessment (7-9), although its use and its relation to the inflammatory response have been discussed.

Many studies (9-11) have focused on the search for prognostic markers in septic patients, particularly in those with VAP, and have proposed strategies for individualizing and optimizing treatment. Several recent studies assessed biomarkers as useful tools to evaluate VAP patients evolution, either using procalcitonin or C-reactive protein (CRP (11, 12). The use of CRP as a marker of evolution and/or appropriateness of antibiotic treatment may be a promising strategy to anticipate the evaluation of antibiotic effectiveness. However, some specific populations has never been studied.

Many critically ill patients survive their initial acute illness but go on to experience persistent organ failures necessitating prolonged intensive care, a syndrome known as chronic critical illness (CCI) (13). CCI is characterized by high hospitalization costs, frequent post-acute care use, and poor long-term survival (14). CCI patients are particularly susceptible to infections, for many reasons. It includes an immunologically deficient state commonly referred to as "immune exhaustion," in which diminished physiological reserves impair the patient's ability to fight infections or risk for acquiring virulent nosocomial organisms because they are cared for in an environment where multidrug-resistant organisms thrive (15,16). No data on clinical

or particular characteristics of VAP or biomarkers evolution in this specific subset of patients is available.

We designed this study to evaluate: 1) the patients characteristics and outcomes in chronic critically ill patients developing VAP; 2) the evolution of biomarkers according to the presence of CCI using serum C-reactive protein as a biochemical marker of inflammatory response.

We hypothesized that CRP peak values and its kinetics would be altered in patients with chronic critically illness developing VAP.

MATERIALS AND METHODS

Study Setting and Population

A secondary analysis of a prospective observational study including patients with suspected ventilator-associated pneumonia (VAP) in the ICU for surveillance purpose. The cohort included mechanically ventilated patients from Hospital de Clinicas de Porto Alegre (Brazil), a large urban hospital affiliated to teaching institution, during 2008-2013. The data collection was approved by institutional ethics committee.

Baseline Assessment, Definitions and Data Collection

Suspicion of VAP required the radiographic appearance of a new, persistent pulmonary infiltrate in conjunction with purulent respiratory secretions, and at least one of the following criteria: temperature>38°C or <35,5°C, white blood cell count >10.000/mm^3 or < 4000/ mm^3(1).

Microbiological data on all these episodes were obtained from quantitative tracheal aspirate performed on the day of pneumonia onset (baseline). Tracheal aspirates were required to have more than 25 neutrophils present on Gram stain, with

ten epithelial cells or fewer per high-power field to be accepted for culture of potential pathogens. Microorganisms were identified by standardized laboratory methods. Tracheal aspirate cultures and other study variables were collected within 8h of clinical suspicion in all patients with criteria of suspected VAP. Empirical antimicrobial therapy was considered appropriate when all isolates were susceptible in vitro to at least one antibiotic in use and institution has an empirical therapy protocol based on local microbiologic data and specific patient risk factors as previous exposure to antibiotics, previous pathology and time to onset of VAP.

All laboratory and physical examination data allowing the determination of APACHE II score were recorded prospectively in a computerized database 24h after ICU admission as well as all comorbidities. The following data were collected at ICU admission: age, gender, baseline diagnostic, admission type, presence of comorbidities (cardiovascular, diabetes, Chronic obstructive pulmonary disease, renal dysfunction, cirrhosis, cancer, dementia, presence of AIDS), ICU and hospital length of stay. Mortality rate was evaluated at ICU and hospital discharge. Chronic critical illness was evaluated in those patients with more than 14 days (14) on mechanical ventilation.

CRP levels were measured in serum using an automated nephelometric technique. CRP basal level was defined as that measured at day of VAP diagnosis. CRP variation was evaluated through a CRPratio defined as the ratio between CRP levels on follow-up (72-96h) and CRP levels on baseline. CRPmax was the higher CRP value identified within first 72h of diagnosis, based on this biomarker kinetics. Also, the ratio between CRPmax/CRPbasal was measured as an index of the maximum amplitude of CRP variation within 72h.

Statistical Analysis

Descriptive statistical analysis was performed. Continuous data were compared using the unpaired Student's t-test or Mann-Whitney test when appropriate. Proportions were compared using the Chi-square and Fisher's test when necessary. The correlation was checked with Spearman's correlation test. The variation of values from baseline was compared using paired t-test. Survival analysis was performed using Cox proportional hazard analysis. Analysis of covariance (ANCOVA) has been performed for paired values on baseline and follow-up. All p values were two-tailed and a $p < 0.05$ was considered significant. Statistics were computed with the STATA for Mac 14.0 statistical package.

RESULTS

We included 405 patients with VAP diagnosis. Most were male (61.1%). Mean APACHE II score was $19.9 \pm 10,5$ and median age was 60 (IQR 45;71) years. Median LOS in ICU was 22 (IQR 15;36) days and length of mechanical ventilation median was 9 (IQR 6-13) days. Overall mortality rate was 55.7%. Mean days of mechanical ventilation before VAP diagnosis were 11.6 ± 11.2 days. The baseline characteristics of survivors and non-survivors are described in **Table 1**. Etiology of VAP episodes is described in **Table 2,** showing an increased prevalence of *Acinetobacter species* and *Pseudomonas aeruginosa* in CCI patients. Multidrug-resistant pathogens were isolated in 171 patients (42.2%). Overall, appropriateness of empirical therapy was 81.4%, with a higher rate of appropriateness in CCI patients when compared to non-CCI patients (**Table 3**).

We found 99 patients (24.4%) with more than 14 days of mechanical ventilation, defined as CCI. When compared episodes in patients with and without chronic critical illness, presence of chronic renal disease, dementia, presence of ≥ 2

comorbidities and previous use of antibiotic were identified as risk factors associated with CCI (**Table 3**). Also, CCI patients present more frequently MDR pathogens than no-CCI patients (63.6% vs 35.3%, OR 3.21 95%CI 2.00-5.14).

Outcomes of CCI patients VAP episodes were not significantly different compared to no CCI patients, with similar crude ICU-mortality (58.6% vs 54.6%, OR 1.18 95%CI 0.74-1.86). However, after Cox regression analysis with adjustment for severity of illness at admission and MDR pathogen etiology of VAP episode, we found a significant increase on the hazard risk in CCI patients (HR 2.35 95% CI 1.71-3.22).

CRP evolution

CRP levels and kinetics were not significant different when comparing survivors and non-survivors without adjustment for antimicrobial appropriateness (**Table 1**). However, CRP ratio estimated marginal means were different when comparing survivors and non-survivors, after adjustment for covariance – ANCOVA – with basal levels (0.82 95% CI 0.68-0.95 vs 1.02 95% CI 0.90-1.13, p<0.05). When assessing CRP as a surrogate of inflammatory response in CCI patients, we found no difference in the response pattern, compared to patients without CCI.

Basal serum CRP levels were not different when comparing CCI and no CCI patients (167.3±77.0 vs 168.7±87.6, p=0.94) at time of diagnosis. Also, CRPmax reached within 72h of diagnosis (175.0±79.2 vs 182.9±87.2, p=0.58) or relative increase in CRP levels (CRPratio and CRPmax/CRPbasal) (0.86±0.49 vs 0.93±0.70, p=0.46; 1.09±0.37 vs 1.20±0.56, p=0.21) were not significant different between patients presenting CCI and those without CCI (**Figure 1**). Even when analyzing only patients with appropriate empirical antimicrobial therapy, no difference was found in comparison between CCI and non-CCI patients (**Table 4**).

In addition, the analysis of covariance showed no significant differences on follow-up levels of serum CRP after adjustment for baseline values (**Table 4**).

DISCUSSION

This is an original study assessing characteristics of VAP episodes in CCI patients. CCI population is increasing in ICUs. Although advances in critical care have enabled more patients to survive an acute critical illness, they also have created a large and growing population of chronically critically ill patients with prolonged dependence on mechanical ventilation and other intensive care therapies. This high dependent population, needing prolonged respiratory support is at higher risk for developing nosocomial infections, particularly ventilator-associated pneumonia. Our findings suggest some characteristics such as age, severity of illness at ICU admission, some comorbidities are associated with worse outcomes in this subgroup of patients. However, interestingly, inflammatory response to infectious damage assessed using CRP as a surrogate, were not different when comparing VAP episodes in CCI and no-CCI patients. Nor basal serum CRP or peak levels obtained within 72h, or the ratio of increase in CRP levels were different comparing both groups.

The burden of chronic critical illness is respiratory failure, its increasing prevalence and epidemiological aspects described in the last years is in close relation with the increasing requirement of prolonged dependence on mechanical ventilation in ICUs around the globe. Long-term mortality is high, approaching rates of 40% to 60% at one-year in inclusive cohorts (17). Patients have a very high symptom burden during the weeks of prolonged ventilation and chances of living at home with functional independence at the end of the year are as low as 10% (18). Although the term ''prolonged mechanical ventilation'' has been used in the literature to describe periods of ventilator dependence ranging from 2 days to 4 weeks (19,20), a clear

definition of CCI is still missing in the literature, and several studies purposed different definitions (21,22). A period of mechanical ventilation, ranging from 2 to 30 days, has been used to define the majority of cohorts for longitudinal studies (19-21). In our study, we identified patients with more than 14 days of mechanical ventilation to define CCI, according to Hough et al. et al (14).

Besides prolonged ventilator dependence, evidence suggests that chronic critical illness is a syndrome comprising additional characteristics including high susceptibility to complications, brain dysfunction manifesting as coma or delirium; skin breakdown associated with nutritional deficiencies, edema, incontinence, and prolonged immobility (15,21). All together, these characteristics also are associated with a higher vulnerability to develop nosocomial infections, frequently by multidrug resistant (MDR) microorganisms.

As expected, our cohort show that patients with prolonged mechanical ventilation present a microbiological profile in which multidrug resistant pathogens are more predominant when comparing CCI patients with VAP with episodes in patients without CCI, more specifically non-fermenting Gram-negative bacilli – *Acinetobacter* and *Pseudomonas* species. A higher prevalence of MDR pathogens is expected in this population, as colonization of critically ill patients by these pathogens is a progressive phenomena developing during the stay of patient in ICU (23). Also, as already described in the literature, outcomes in patients developing CCI are significantly worse, compromising ICU survival in VAP episodes in patients with CCI. This finding was not surprisingly in our study, but difference was present only after adjustment for severity of illness and MDR pathogens. This might be due to some aspects: First, patients developing VAP within the first 2 weeks of mechanical ventilation might be at higher risk for worse outcomes as VAP is considered a

preventable phenomena and its occurrence might be associated with higher severity at admission or failures on process of care – we adjust for one of these variables as process of care variables were not available for this analysis; second, VAP episodes early on evolution are associated with more virulent pathogens, and attributable mortality in early episodes might be more relevant; third, patients with CCI have more episodes due to MDR pathogens, and some of these low virulence pathogens such as Stenotrophomonas might be only an epiphenomena, without increment on CCI patients risk for worse outcome. So, effect of CCI only appears after adjustment for some of these conditions. Although attributable mortality in VAP is a controversial issue, it does prolong mechanical ventilation and length of stay in the ICU (24), causing a positive feedback with risk and outcomes associated with CCI.

A unique aspect of our study is to assess whether developing CCI would affect response from an infectious insult, such as a VAP episode. Several studies has used biomarkers such as CRP and procalcitonin as surrogates for host response in severe infections (10-12). CRP is one of the most important acute-phase reactant in humans and the most used and studied biomarker of inflammation due to the wide availability, diagnostic accuracy, and relatively low costs of laboratory assays. Its main biological functions include activation of the classical complement pathway and binding of bacteria with subsequent activation of leukocyte-mediated cytotoxicity (25). Strategies based on biomarkers such as CRP in evolution assessment after treatment might be useful to evaluate appropriateness of empirical therapy and even antimicrobial duration. We found a significant difference when comparing CRP levels between survivors and non-survivors, as already shown in other studies (11). Values of serum CRP for evolution and prognosis assessment of patients with severe infections, and VAP specifically, have been evaluated in several studies (10-12). A

decrease in serum CRP level on the fourth day of evolution was predictive of survival and appropriateness of antimicrobial therapy (12) in VAP patients. Povoa et al. (10) identified patterns of serum CRP response and its relationship with prognosis in VAP and suggested that serum CRP response may be useful in recognition the host response and anticipation of individual clinical course. No data, however, is available in CCI patients.

Cabrera-Cancio M (15) describes alterations not completely understood in immune response occurring during CCI. Following the acute or initial hyper-inflammatory response to sepsis, an immune system down-regulation can lead to prolonged immune dysfunction. This period of "immune paralysis" has consequences: it limits the ability to fight infections and predisposes the patient to nosocomial infections and multi-organ dysfunction. Patients who survive this initial systemic inflammatory response syndrome enter a state of immune suppression and dysfunction (16). Additionally, these patients frequently have comorbidities that precede the acute event. Their defenses might be already impaired at the beginning of the ICU admission by preexisting illnesses (15). Considering these potential confounders, we assessed CRP behavior in this subgroup of patients. Our data show no difference in basal CRP levels at time of diagnosis, nor at CRP peak value obtained within 72h of diagnosis, nor the CRP ratio in 96h in comparison with basal CRP serum levels, when comparing VAP episodes in patients with CCI and patients without CCI, suggesting that, at least regarding CRP role in the host response, CCI patients has the same response than non CCI patients. These findings are more robust as confirmed by analysis of covariance, adjusting for potential baseline imbalance. This absence of difference persisted, even when considering only patients with appropriate empirical antibiotic therapy.

Our study has several limitations. It is an observational study and no causal inference can be determined. We did not collect any new severity assessment score such as SAPS3, only APACHE II. It is possible that undiscovered variables might explain the apparent absence of effect of CCI on CRP behavior in VAP patients. In addition, we could only assess response in VAP patients using CRP levels. Perhaps, other biomarkers such as procalcitonin, interleukins, cytokines or other PAMPs and DUMPs could identify more specific qualitative or quantitative alterations in host response in this population. But as CRP is part of innate immune response and it is a mechanism well preserved in evolutionary chain, the suggestion that it is preserved might be useful to define characteristics or subgroups of patients developing CCI at higher risk to develop VAP or with worse associated outcomes. Also, our study could not assess or speculate on basic mechanisms responsible for the persistence of CRP response in CCI patients. Still, several CCI definitions are available in the literature, but we select 14 days as the most sensitive criteria available.

CONCLUSION

In conclusion, in this cohort of patients with VAP, we described that those patients developing CCI present VAP with worse prognosis, with higher hazard risk for ICU negative outcome after adjustment for severity of illness at admission, MDR pathogens and more comorbidities. However, such findings does not appear related to a compromised response to infectious episodes as assessed by CRP serum levels at moment of diagnosis, nor its evolution within the first 72-96h. Our data suggest that using CRP as a surrogate for clinical evolution in patients with CCI might be still appropriate as we were not able to find changes in response pattern comparing patients with or without CCI. Further studies should prospectively assess CRP and other biomarkers role in management strategies in CCI patients.

References:

1. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171: 388–416.

2. Torres A, Ewig S, Lode H, et al. Defining, treating and preventing hospital acquired pneumonia: European perspective. Intensive Care Med 2009; 35: 9–29.

3. Rello J, Diaz E. Pneumonia in the intensive care unit. Crit Care Med. 2003: 31; 2544-2551.

4. Diaz E, Ulldemolins M, Lisboa T, Rello J. Management of ventilator-associated pneumonia. Infect Dis Clin North Am. 2009; 23: 521-533.

5. Lisboa T, Craven D, Rello J. Should Ventilator-associated pneumonia be a quality indicator for patient safety. Clin Pulm Med 2009; 16: 28-32

6. Rello J, Ollendorf DA, Oster G, Vera-Llonch M, Bellm L, Redman R, Kollef MH. Epidemiology and outcomes of ventiladar-asociated pneumonia in a large US database. Chest 2002; 122: 2115-2121.

7. Póvoa P, Salluh JIF. Biomarker-guided antibiotic therapy in adult critically ill patients: a critical review. Ann Intensive Care 2012; Jul 23;2(1):32.

8. Moreno MS, Nietmann H, Matias CM, Lobo SM. C-reactive protein: a tool in the follow-up of nosocomial pneumonia. Journal of Infection 2010 Sep;61(3):205–11.

9. Oliveira CF, Botoni FA, Oliveira CRA, Silva CB, Pereira HA, Serufo JC, et al. Procalcitonin versus C-reactive protein for guiding antibiotic therapy in sepsis: a randomized trial. Crit Care Med 2013 Oct;41(10):2336–2343.

10. Povoa P, Coelho L, Almeida E, et al. C-reactive protein as a marker of ventilator-associated pneumonia resolution: a pilot study. Eur Respir J 2005; 25: 804–812.

11. Seligman R, Meisner M, Lisboa TC, et al. Decreases in procalcitonin and C-reactive protein are strong predictors of survival in ventilator-associated pneumonia. Crit Care 2006; 10: R125.

12. Lisboa T, Seligman R, Diaz E, Rodriguez A, Teixeira PJ, Rello J. C-reactive protein correlates with bacterial load and appropriate antibiotic therapy in suspected ventilator-associated pneumonia. Crit Care Med. 2008;36(1):166-71.

13. Kahn JM, Le T, Angus DC, et al. The epidemiology of chronic critical illness in the United States. Crit Care Med 2015; 43:282–287.

14. Hough CL, Caldwell ES, Cox CE, et al. Development and Validation of a

Mortality Prediction Model for Patients Receiving 14 Days of Mechanical Ventilation. Crit Care Med 2015; 43:2339-2345.

15. Cabrera-Cancio MR. Infections and the Compromised Immune Status in the Chronically Critically Ill Patient: Prevention Strategies. Respir Care 2012; 57: 979-992.

16. Kalb TH, Lorin S. Infection in the chronically critically ill: unique risk profile in a newly defined population. Crit Care Clin 2002;18:529–552.

17. Nelson JE, Cox CE, Hope AA, et al: Chronic critical illness. Am J Respir Crit Care Med 2010; 182:446–454

18. Estenssoro E, Reina R, Canales HS, Saenz MG, Gonzalez FE, Aprea MM, Laffaire E, Gola V, Dubin A. The distinct clinical profile of chronically critically ill patients: a cohort study. Crit Care 2006;10:R89.

19. Combes A, Costa MA, Trouillet JL, Baudot J, Mokhtari M, Gilbert C, et al. Morbidity, mortality, and quality-of-life outcomes of patients requiring .= 14 days of mechanical ventilation. Crit Care Med. 2003; 31:1373-81.

20. Cox CE, Carson SS, Hoff JA, Olson MK, Govert JA, Chelluri L. Differences in one-year health outcomes and resource utilization by definition of prolonged mechanical ventilation: a prospective cohort study. Crit Care. 2007;11:R9, doi: 10.1186/cc5667.

21. Carson SS: Definitions and epidemiology of the chronically critically ill. Respir Care 2012; 57:848–56; discussion 856–858

22. Boniatti M, Friedman G, Castilho RK, et al. Characteristics of chronically critically ill patients: comparing two definitions. Clinics 2011;66(4):701-704.

23. Trouillet JL, Chastre J, Vuagnat A, et al. Ventilator-associated pneumonia caused by potentially drug-resistant bacteria. Am J Respir Crit Care Med 1998; 157: 531-539.

24. Bekaert M, Timsit JF, Vansteelandt S, et al. Attributable mortality of ventilator associated pneumonia: a reappraisal using causal analysis. Am J Respir Crit Care Med 2011; 184: 1133-9.

25. Ticinesi A, Lauretani F, Nouvenne A, et al. C-reactive protein measurement in geriatric patients hospitalized for acute infections. Eur J Intern Med 2016; http://dx.doi.org/10.1016/j.ejim.2016.08.026

Table 1. Clinical characteristics of VAP patients comparing survivors and non-survivors

	Survivors	Non-survivors	p
Number of patients	180	226	
Age (mean (SD))	54.4 (17.3)	59.8 (17.4)	<0.001
Gender (male/female) (%)	63,3/36,7%	59,3/40,7	0,42
APACHE II (mean (SD))	20.7 (7.6)	23.7 (7.6)	<0.001
LOS MV before PAV (median [IQR])	8 (4-12)	9 (6-14)	0.03
Admission type (%)			0.76
Medical	108 (60.0%)	139 (61.5%)	
Surgery	72 (40.0%)	87 (38.5%)	
Heart failure class IV (n (%))	12 (6.7%)	26 (11.5%)	0.13
AIDS (n (%))	11 (6.1%)	18 (8.0%)	0.60
Cirrhosis (n (%))	12 (6.7%)	18 (8.0%)	0.76
CRD (n (%))	9 (5.0%)	18 (8.0%)	0.32
Diabetes (n (%))	22 (12.2%)	38 (16.8%)	0.25
Hypertension (n (%))	50 (27.8%)	63 (27.9%)	0.99
Dementia (n (%))	4 (2.2%)	13 (5.8%)	0.12
Cancer (%)	26 (14.4%)	60 (26.5%)	<0.001
COPD (%)	11 (6.1%)	22 (9.7%)	0.25
More than 2 Comorbidities (n (%))*	85 (47.2%)	140 (61.9%)	<0.001
CCI (n (%))	41 (22.8%)	58 (25.8%)	0.56
ICU LOS, days, median [IQR]	22 (11-38)	22(11-34)	0.98
MV duration, days, median [IQR]	16 (11-29)	20(13-31)	0.04
Appropriateness of ATB therapy (%)	147 (81.7%)	184(81.4%)	0.99
Previous ATB (%)	72 (40.0%)	103 (45.6%)	0.27
MDR pathogen (%)	69 (38.3%)	103 (45.6%)	0.16
Basal serum CRP (median (IQR))	163 (108-196)	168 (126-213)	0.77
CRP maximum within 72h (median (IQR))	168 (119-201)	179 (135-243)	0.30
CRP at 96h (median (IQR))	116 (81-165)	128 (83-178)	0.29

ICU – Intensive Care unit; CRP – C-reactive protein; SD – Standard deviation; IQR – Interquartile range; LOS – length of stay; MV – mechanical ventilation; CRD – Chronic renal disease; COPD – chronic obstructive pulmonary disease; ATB – antibiotic therapy; CCI – chronic critical illness; MDR – multidrug resistant.

Table 2 – Etiology of VAP episodes in CCI and non-CCI patients

ETIOLOGY	CCI	Non-CCI	p
Enterobacteriaceae	33 (33.3%)	91 (29.7%)	0.58
E. coli	3 (3.0%)	10 (3.3%)	
Enterobacter spp.	10 (10.1%)	29 (9.5%)	
Klebsiella spp.	14 (14.1%)	43 (14.1%)	
Other Enterobacteriaceae	6 (6.1%)	9 (2.9%)	
S. aureus	16 (16.2%)	59 (19.3%)	0.59
MSSA	10 (10.1%)	48 (15.7%)	
MRSA	6 (6.1%)	11 (3.6%)	
Pseudomonas aeruginosa	29 (29.3%)	40 (13.1%)	<0.001
Acinetobacter sp.	28 (28.3%)	60 (19.6%)	0.06
Streptococcus pneumoniae	0	5 (1.6%)	0.49
Haemophilus influenzae	1 (1.0%)	12 (3.9%)	0.26
Serratia sp.	4 (4.0%)	12 (3.9%)	0.99
Stenotrophomonas maltophila	8 (8.1%)	17 (5.6%)	0.40
Polymicrobial episodes	25 (25.3%)	82 (26.8%)	0.87

Table 3. Comparison of clinical characteristics and outcomes according to presence of chronic critical illness (CCI) in ICU patients

|	CCI	No-CCI	p
Number of patients	99	306	
Age (mean (SD))	57.1 (18.4)	58.2 (17.5)	0.72
Gender (male/female) (%)	65,7/34,3%	59,5/40,5%	0.29
APACHE II (mean (SD))	21.4 (10.9)	21.1 (9.7)	0.86
LOS MV before PAV (median [IQR])	19 (17-27)	7 (4-10)	<0.001
Admission type (%)			0.41
Medical	64 (64.6%)	183 (59.8%)	
Surgery	35 (35.3%)	123 (40.2%)	
Heart failure class IV (n (%))	9 (9.1%)	29 (9.5%)	0.99
AIDS (n (%))	9 (9.1%)	20 (6.5%)	0.51
Cirrhosis (n (%))	4 (4.0%)	26 (8.5%)	0.20
CRD (n (%))	11 (11.1%)	16 (5.2%)	0.04
Diabetes (n (%))	19 (19.2%)	41 (13.4%)	0.21
Hypertension (n (%))	27 (27.3%)	86 (28.1%)	0.98
Dementia (n (%))	8 (8.1%)	9 (2.9%)	0.04
Cancer (%)	26 (26.3%)	60 (19.6%)	0.21
COPD (%)	8 (8.1%)	25 (8.2%)	0.99
More than 2 Comorbidities (n (%))*	76 (76.7%)	149 (48.7%)	<0.001
ICU LOS, days, median [IQR]	40 (26-52)	19 (14-28)	<0.001
Appropriateness of ATB therapy (%)	90 (90.9%)	240 (78.4%)	<0.05
Previous ATB (%)	66 (66.7%)	108 (35.3%)	<0.001
MDR pathogen (%)	63 (63.6%)	108 (35.3%)	<0.001
Basal serum CRP (median (IQR))	154 (124-193)	167 (108-206)	0.88
CRP maximum within 72h (median (IQR))	161 (129-195)	175 (132-216)	0.45
CRP at 96h (median (IQR))	124 (87-168)	125 (79-171)	0.83
ICU Mortality (n(%))	58 (58.6%)	167 (54.6%)	0.56

ICU – Intensive Care unit; CRP – C-reactive protein; SD – Standard deviation; IQR – Interquartile range; LOS – length of stay; MV – mechanical ventilation; CRD – Chronic renal disease; COPD – chronic obstructive pulmonary disease; ATB – antibiotic therapy; CCI – chronic critical illness; MDR – multidrug resistant.

Table 4. Comparison of CRP response according to presence of chronic critical illness (CCI) in ICU patients

	CCI	Non-CCI	p
CRUDE			
Basal serum CRP (mean (SD))	167.6 (77.0)	168.7 (87.6)	0.94
CRP maximum within 72h (mean (SD))	175.0 (79.2)	183.0 (87.2)	0.58
CRP at 96h (mean (SD))	135.5 (79.2)	130.7 (75.4)	0.71
CRP ratio (median [IQR])	0.96 (0.72-1.36)	1.08 (0.80-1.49)	0.30
ONLY APPROPRIATE ANTIBIOTIC THERAPY			
Basal serum CRP (mean (SD))	167.4 (74.0)	171.0 (88.0)	0.81
CRP maximum within 72h (mean (SD))	172.4 (78.5)	186.0 (87.4)	0.36
CRP at 96h (mean (SD))	134.5 (81.2)	133.9 (76.0)	0.96
CRP ratio (median [IQR])	0.84 (0.58-1.00)	0.76 (0.56-1.05)	0.93
ANALYSIS OF COVARIANCE – ESTIMATED MARGINS ADJUSTED BY BASAL CRP LEVELS			
CRP maximum within 72h (mean (95% CI))	175.7 (164.9-186.5)	182.7 (176.3-189.1)	0.27
CRP at 96h (mean (SD))	135.9(117.6-154.1)	130.6(119.8-141.4)	0.63

ICU – Intensive Care unit; CRP – C-reactive protein; SD – Standard deviation; IQR – Interquartile range

Figure 1. CRP evolution in CCI and non-CCI patients with VAP
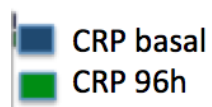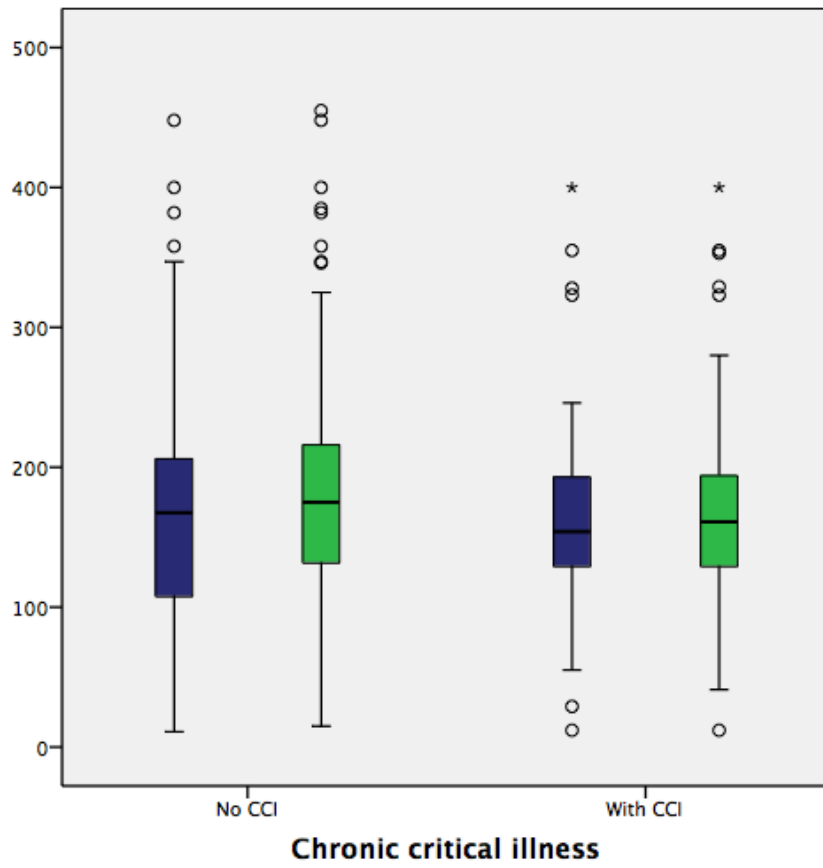
Effects of age on CRP evolution in critically ill patients with nosocomial pneumonia

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INTRODUCTION

VAP is the most common nosocomial infection in ICU and represents 25% of all ICU infections, with high cost and impact on outcomes (1-3). Although the attributable mortality VAP is a controversial issue, it does prolong mechanical ventilation and length of stay in the ICU (3-5). Moreover, the monitoring of biomarkers may play a role in prognosis assessment (6), although its use and its relation to the inflammatory response have been discussed. Many studies (7 -10) have focused on the search for prognostic markers in septic patients, particularly in those with VAP, and have proposed strategies for individualizing and optimizing treatment. Several recent studies assessed biomarkers as useful tools to evaluate VAP patients evolution, either using procalcitonin or C-reactive protein (CRP (7-10). In addition, biomarkers usefulness is some specific subsets of patients have been discussed.

The impact of age on the outcome of critically ill patients remains controversial (11). Older patients now receive a substantial share of health care resources, including those related to intensive care (12). In addition, the physiology of inflammatory response is modified by the aging process and is substantially affected by multimorbidity and disability (13). So, the clinical significance of serum CRP determination has not been completely clarified in older subjects with acute infection, especially in the light of the age-related rearrangements in immunity and cytokine production (14). Few data is available regarding CRP kinetics in older patients with infection (15-19). As such, even if many data are present about the CRP and VAP patients, the current knowledge does not allow recommending serial CRP measurements to guide therapeutic choices in older VAP patients.

We designed this study to evaluate: 1) the impact of age on outcomes in critically ill patients developing VAP; 2) the evolution of biomarkers according to age

in these patients using serum C-reactive protein levels and kinetics as a biochemical marker of inflammatory response.

We hypothesized that CRP peak values and its kinetics would be altered in older patients with critically illness developing VAP.

MATERIALS AND METHODS

Study Setting and Population

A secondary analysis of prospective observational study including patients with suspected ventilator-associated pneumonia (VAP) in the ICU for surveillance purpose. From 2008 to 2013 all patients with suspected VAP were included. The cohort included mechanically ventilated patients from Hospital de Clinicas de Porto Alegre (Brazil), a large urban hospital affiliated to teaching institution. The data collection was approved by institutional ethics committee.

Baseline Assessment, Definitions and Data Collection

Suspicion of VAP required the radiographic appearance of a new, persistent pulmonary infiltrate in conjunction with purulent respiratory secretions, and at least one of the following criteria: temperature$>38_oC$ or $<35,5_oC$, white blood cell count $>10.000/mm_3$ or $< 4000/ mm_3$ (1).

Microbiological data on all these episodes were obtained from quantitative tracheal aspirate, performed on the day of pneumonia onset (baseline). Microorganisms were identified by standardized laboratory methods. Tracheal aspirate cultures and other study variables were collected within 8h of clinical suspicion in all patients with criteria of suspected VAP.

Empirical antimicrobial therapy was considered appropriate when all isolates were susceptible in vitro to at least one antibiotic in use and institution has an empirical therapy protocol based on local microbiologic data and specific patient risk factors as previous exposure to antibiotics, previous pathology and time to onset of VAP.

All laboratory and physical examination data allowing the determination of APACHE II score were recorded prospectively in a computerized database 24h after ICU admission as well as all comorbidities. Pre-existing **Chronic obstructive pulmonar disease** was defined as a disease state characterized by the presence of airflow limitation due to chronic bronchitis or emphysema (15). **AIDS, dementia and cancer** presence were clinically defined. **Chronic Heart Failure** was considered in patients admitted with New York Heart association (NYHA) class III and IV. **Chronic Hepatopathy** was considered in patients with documented biopsy proven cirrhosis, documented portal hypertension, episodes of past upper gastrointestinal bleeding attributed to portal hypertension or previous episodes of hepatic encephalopathy. **Chronic Renal Failure** was considered in patients receiving chronic hemodialysis.

CRP levels were measured in serum using an automated nephelometric technique. CRP basal level was defined as that measured at day of VAP diagnosis. CRP variation was evaluated through a CRPratio defined as the ratio between CRP levels on follow-up (72-96h) and CRP levels on baseline. CRPmax was the higher CRP value identified within first 72h of diagnosis, based on this biomarker kinetics. Also, the ratio between CRPmax/CRPbasal was measured as an index of the maximum amplitude of CRP variation within 72h.

Statistical Analysis

Descriptive statistical analysis was performed. Continuous data were compared using the unpaired Student's t-test or Mann-Whitney test when appropriate. Proportions were compared using the Chi-square and Fisher's test when necessary. Logistic regression was also performed to identify factors associated with ICU mortality after univariate evaluation and Hosmer-Lemeshow goodness-of-fit statistics was used to evaluate its calibration. The correlation was checked with Spearman's correlation test. The variation of values from baseline was compared using paired t-test. Analysis of covariance (ANCOVA) has been performed for paired values on baseline and follow-up. Impact of age in survival was tested using Cox proportional hazards analysis. Null model Martingale residuals were used to assess the functional form of age impact (12), and showed an upward bend around the age of 65 yrs. A model including a smoothing function of age was tested, but the nonlinearity was not significant, indicating that a linear fit was acceptable. To assess outcome predictors in young and elderly patients, the dataset was divided in two groups (<65 and >65 yrs), based on the functional form of age (Fig. 1). All p values were two-tailed and a $p<0.05$ was considered significant. Statistics were computed with the STATA for Mac 14.0 statistical package and SPSS 20.0.

RESULTS

In the study period, 405 patients fulfilled the eligibility criteria. The median age was 60.5 (IQR, 45.0; 71.0) years. The main patients' characteristics are depicted in **Table 1**. Distribution of admission diagnosis category included: 247 (61.0%) medical, 155 (38.3%) surgical; and 6 (1.7%) trauma patients. Overall mortality rate was 55.7%. Overall, appropriateness of empirical antibiotic therapy was 81.4%. Etiology of VAP episodes is described in **Table 2**, suggesting a higher prevalence of

Enterobacteriaceae in older patients.

Age was independently associated with survival time, adjusted by gender, severity of illness and comorbidities. In **Figure 1**, age was plotted against the martingale residuals showing that risk of death substantially influenced by age only after 65 yrs. From this point upward, there was an increase in the residual values. Based on the analysis of Figure 1, patients were stratified in two groups: younger (<65 yrs, 242, 59.6%) and elderly (>65 yrs, 164, 40.4%). Baseline characteristics comparison between younger and older patients is presented in the **table 3**. In univariate analysis, a higher ICU mortality was associated with older patients (OR 1.94 95%CI 1.29-2.92). Also, in multivariable analysis, age >65 yrs was associated with higher ICU mortality (OR 1.72 95%CI 1.13-2.62) after adjustment for severity of illness at admission (Hosmer-Lomeshow goodness of fit p=0.62).

CRP evolution and age

Age, assesed as a continuous variable, has no significant correlation with basal serum CRP or CRPmax in VAP patients (**Figure 2**). Also, when assessing CRP as a surrogate of inflammatory response in patients according to age, we found no difference in the response pattern, comparing patients older than 65 with those with less than 65 years old (**Figure 3**).

Basal serum CRP levels were not different when comparing older and younger patients (173.1±79.5 vs 164.9±88.6, p=0.51) at time of diagnosis. Also, CRPmax reached within 72h of diagnosis were not significant different between patients older than 65 and those younger than 65 years (177.2±76.6 vs 184.0±91.1, p=0.59). Even after considering only patients with appropriate empirical therapy, no difference was found (**Table 4**).

In addition, the analysis of covariance showed no significant differences on

follow-up according to age group in the levels of serum CRP, after adjustment for baseline values (**Table 4**).

DISCUSSION

Our analysis evaluated the impact of age on outcomes in critically ill patients developing VAP and the evolution of biomarkers according to age in these patients using serum CRP levels and kinetics as a biochemical marker of inflammatory response. We found no difference in CRP levels at baseline, maximum CRP level within 72h, ratio between CRP levels at 96h and baseline, or variation of CRP levels within 96h when comparing older and younger patients. We hypothesized that age would affect outcomes in VAP patients and identified impact of age on survival only after 65 years as suggested by Martingale residual analysis resulting from a null-model of Cox survival hazard analysis. Although older patients had a higher rate of comorbidities, more severe burden of disease and worse outcomes, with higher mortality and prolonged length of stay, CRP levels and kinetics were not different. Our findings suggest that the same biomarker-variation based strategies used for VAP patients in ICU might keep its validity for an older VAP-patients population.

Several specific subgroups of patients with VAP have been evaluated in the literature. Trauma (20,21), patients with cancer (22), COPD patients (23) are some of the specific conditions evaluated in VAP. Our study is novel as we assessed and compare outcomes according to age in critically ill patients with VAP diagnosis and how it affects host-response, based on CRP evolution. We evaluated age as a continuous variable and could identify a specific cut-off in which age begins to affect outcome in VAP patients around 65 years. We used a statistical approach already published in critically ill cancer patients (12). We found some difference in

microbiology in older patients, but our sample was not large enough to determine a causal effect between age and risk for specific pathogens as many confounding factors such as comorbidities and severity of illness were more important in older patients subgroup. Regarding age and VAP, Blot et al. (24) assessed prevalence and associated outcomes in VAP patients according to age. In this study, an arbitrary cut-off of age was used and 3 groups were defined (middle-aged (45-64ys), old (65-74ys) and very old patients (≥75ys)). Main findings in this study were no difference on VAP prevalence within three groups, an increasing of associated mortality along the three age-ranges, and a higher prevalence of *Enterobacteriaceae* in older patients, in accordance with our data. No difference was described on clinical symptoms except by a lower presence of new onset of fever in older patients. No data on biomarkers was assessed. Also, regional variations are expected for age effect on mortality and this study included only European patients.

We decided to assess age impact on CRP behavior in VAP patients based on pathophysiological changes described in older patients with acute infection. The aging process has an important effect on immunity and inflammation, affecting host-response, leading to chronic low-grade activation of inflammatory pathways and decreased response to novel antigens (immunosenescence) (13), affecting host-pathogen interaction. Ticinesi et al. (14) describes that these differences include poorer T helper cell function, poorer B cell humoral response to neoantigens, reduced neutrophil, and macrophage cytotoxic function, and expansion of natural killer cells with apparent reduced functionality (25). Despite this, during acute infection, older subjects have a generally intact production of proinflammatory cytokines, including IL-1, TNF-α, and IFN-γ. Acute IL-6 production is even increased compared to adult subjects, and the duration of this response is generally longer (14,25). In fact,

immunosenescence mainly affects innate immunity in terms of reduced cell function (i.e. reduced adhesion, chemotaxis, and phagocytosis), but not in terms of systemic mediator release (26). It is suggested that these alterations on response might have a clinical impact on infection recognition, delay on treatment and inability to assess clinical response in older patients population (14). Despite many studies are available assessing CRP and acute infection in critically ill patients (14-19), and particularly, in respiratory infections (8-10), no study assessed specifically older patients with VAP and impact of age on CRP evolution.

In VAP patients, higher peak CRP values are generally associated with a higher intensity of inflammation, reflecting a more severe disease and thus a higher risk for adverse outcomes. The utility of serial biomarkers measurements during VAP treatment has been studied, suggesting that biomarkers may help guiding duration and quality of antibiotic therapy in sepsis. Values of serum CRP for evolution and prognosis assessment of patients with VAP have been evaluated in several studies (7-10). A decrease in serum CRP level on the fourth day of evolution was predictive of survival and appropriateness of antimicrobial therapy (9) in VAP patients. Povoa et al. (10) identified patterns of serum CRP response and its association with prognosis in VAP patients and suggested that serum CRP kinetics might be useful in the recognition the host response and anticipation of the individual clinical course. Few data are available regarding serum CRP levels, its kinetics and older patients.

In older patients, CRP kinetics is not fully understood. Wester et al (27) evaluated patients hospitalized with acute bloodstream infections due to *Streptococcus pneumoniae* and *Escherichia coli* and observed a decline in CRP levels from the fourth day of stay onwards in both adult and geriatric patients, while CRP levels were generally comparable to baseline during the second and third day of stay.

However, the possible association of CRP kinetics with clinical outcomes was not verified in that study. Koppensteiner et al. evaluated surgical patients after hip or knee arthroplasthy and found that a decrease in CRP levels between day 2-4 after procedure predict a positive outcome in older patients (28).

Our findings suggest no correlation between age and any measure of serum CRP at baseline, peak of concentration within 72h or CRP levels at 96h. In addition, we found no difference on CRP kinetics and response pattern when comparing older and younger patients. It suggests CRP serial measurements remain a valid strategy to assess evolution of VAP in older patients, as our sample seems not to be affected by immunosenescence.

Our study has several limitations. It is an observational study and no causal inference can be determined. Also, as a single-center study, specific aspects on admission restriction of older patients to the ICU could impact on results through selection bias. However, as we assessed a complication developed during ICU stay, this bias is minimized. Another potential selection bias derived from single-center design is that we did include a very small number of trauma patients. It is known that trauma patients, although younger than medical patients in epidemiological studies, are at higher risk for developing VAP (20). However, VAP associated outcomes in trauma patients are better when compared to medical patients (20). So, inclusion of a more substantial sample of this subset of patients could potentially change our results. Also, progressive increasing age of admitted patients in ICU, and as consequence, in the risk population and regional variability might have potentially affected the cut-off we found in our population. Still, we did not compare other cut-off age rather than 65 years old. It is possible also that undiscovered confounding factors might explain the apparent absence of effect of age on CRP behavior in VAP patients. In addition, we

could only assess and compare host-response in older and younger VAP patients using CRP levels. Perhaps, other biomarkers such as procalcitonin, specific interleukins, cytokines or other PAMPs and DUMPs could identify more specific qualitative or quantitative alterations in host response in this population. Also, our study could not assess or speculate on basic mechanisms responsible for the persistence of CRP response in older patients.

CONCLUSION

In conclusion, our findings in this cohort of patients with VAP described that those older patients developing VAP had worse prognosis, with higher mortality, more severity of illness at admission and more comorbidities. We suggest this effect begins at 65 years old. Also, we found no difference on CRP serum levels at moment of diagnosis, nor its evolution within the first 72-96h when comparing older and younger patients using 65 years old as cut-off. No correlation was found between age and any CRP levels at baseline or kinetics. Our data suggest that using CRP as a surrogate for clinical response in older VAP patients might still be adequate as we were not able to find changes in response pattern comparing patients younger or older than 65 years. Further studies should prospectively assess CRP and other biomarkers role in management strategies in older critically ill patients.

References:

1. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171: 388–416.

2. Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS. Epidemiology and outcomes of health-care-associated pneumonia: Results from a large US database of culture-positive pneumonia. Chest 2005; 3854- 3862.

3. Muscedere JG, Martin CM, Heyland DK. The impact of ventilator-associated pneumonia on the Canadian health care system. J Crit Care Med 2008; 23:5-10.

4. Bekaert M, Timsit JF, Vansteelandt S, et al. Attributable mortality of ventilator associated pneumonia: a reappraisal using causal analysis. Am J Respir Crit Care Med 2011; 184: 1133- 1139.

5. Koulenti D, Lisboa T, Brun-Buisson C, et al; EU-VAP/CAP Study Group: Spectrum of practice in the diagnosis of nosocomial pneumonia in patients requiring mechanical ventilation in European intensive care units. Crit Care Med 2009; 37:2360–2368.

6. Póvoa P, Salluh JIF. Biomarker-guided antibiotic therapy in adult critically ill patients: a critical review. Ann Intensive Care 2012; Jul 23;2(1):32.

7. Oliveira CF, Botoni FA, Oliveira CRA, Silva CB, Pereira HA, Serufo JC, et al. Procalcitonin versus C-reactive protein for guiding antibiotic therapy in sepsis: a randomized trial. Crit Care Med 2013 Oct;41(10):2336–2343.

8. Seligman R, Meisner M, Lisboa TC, et al. Decreases in procalcitonin and C-reactive protein are strong predictors of survival in ventilator-associated pneumonia. Crit Care 2006; 10: R125

9. Povoa P, Coelho L, Almeida E, et al. C-reactive protein as a marker of ventilator-associated pneumonia resolution: a pilot study. Eur Respir J 2005; 25: 804–812.

10. Lisboa T, Seligman R, Diaz E, Rodriguez A, Teixeira PJ, Rello J. C-reactive protein correlates with bacterial load and appropriate antibiotic therapy in suspected ventilator-associated pneumonia. Crit Care Med. 2008;36(1):166-171.

11. Martin GS, Mannino DM, Moss M: The effect of age on the development and outcome of adult sepsis. Crit Care Med 2006; 34:15–21.

12. Soares M, Carvalho MS, Salluh JIF, et al. Effect of age on survival of critically ill patient with cancer. Crit Care Med 2006; 34:715-721.

13. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol SciMed Sci 2014;69(S1):S4–9.

14. Ticinesi A, Lauretani F, Nouvenne A, et al. C-reactive protein measurement in geriatric patients hospitalized for acute infections. Eur J Intern Med 2016; http://dx.doi.org/10.1016/j.ejim.2016.08.026.

15. Cox ML, Rudd AG, Gallimore R, Hodkinson HM, Pepys MB. Real-time measurement of serum C-reactive protein in the management of infection in the elderly. Age Ageing 1986;15(5):257–266.

16. Stucker F, Herrmann F, Graf JD, Michel JP, Krause KH, Gavazzi G. Procalcitonin and infection in elderly patients. J Am Geriatr Soc 2005;53:1392–1395.

17. Liu A, Bui T, Van Nguyen H, Ong N, Shen Q, Kamalasena D. Serum C-reactive protein as a biomarker for early detection of bacterial infection in the older patient. Age Ageing 2010;39: 559–565.

18. Porfyridis I, Georgiadis G, Vogazianos P, Mitis G, Georgiou A. C-reactive protein, procalcitonin, clinical pulmonary infection score, and pneumonia severity scores in nursing home acquired pneumonia. Respir Care 2014;59(4):574–581.

19. Pinato DJ, Bains J, Irkulla S, et al. Advanced age influences the dynamic changes in circulating C-reactive protein following injury. J Clin Pathol 2013;66: 695–699.

20. Agbaht K, Lisboa T, Pobo A, et al. Management of ventilator-associated pneumonia in a multidisciplinary intensive care unit: does trauma make a difference? Intensive Care Med 2007; 33: 1387- 1395.

21. Hedrick T, Smith R, McElearney S, et al. Difference in early- and late-onset ventilator-associated pneumonia between surgical and trauma patients in a combined surgical or trauma intensive care unit. J Trauma 2008; 64: 714-720.

22. Póvoa P, Souza-Dantas VC, Soares M, Salluh JF. C-reactive protein in critically ill cancer patients with sepsis: influence of neutropenia. Crit Care 2011; May 19;15(3):R129.

23. Koulenti D, Blot S, Dulhunty JM, et al. COPD patients with ventilator-associated pneumonia: implications for management. Eur J Clin Microbiol Infect Dis. 2015 Dec;34(12):2403-2411.

24. Blot S, Koulenti D, Dimopoulos G, et al. Prevalence, Risk Factors, and Mortality

for Ventilator-Associated Pneumonia in Middle-Aged, Old, and Very Old Critically Ill Patients. Crit Care Med 2014; 42:601–609.

25. Opal SM, Girard TD, Ely EW. The immunopathogenesis of sepsis in elderly patients. Clin Infect Dis 2005;41:S504–12.

26. Gomez CR, Nomellini V, Faunce DE, Kovacs EJ. Innate immunity and aging. Exp Gerontol 2008;43: 718–728.

27. Wester AL, Blaasaas KG,Wyller TB. Is the concentration of C-reactive protein in bacteremia associated with age? Immun Ageing 2008;5: 8.

28. Koppensteiner W, Auersperg V, Halwachs-Baumann G. The use of inflammatory markers as a method for discharging patients post hip or knee arthroplasty. Clin Chem Lab Med 2011;49(10):1647–1651.

Table 1. Comparison of baseline characteristics between survivors and non-survivors

	Survivors	Non-survivors	p
Number of patients	180	226	
Age (mean (SD))	54.4 (17.3)	59.8 (17.4)	<0.001
Gender (male/female) (%)	63,3/36,7%	59,3/40,7	0,42
APACHE II (mean (SD))	20.7 (7.6)	23.7 (7.6)	<0.001
LOS MV before PAV (median [IQR])	8 (4-12)	9 (6-14)	0.03
Admission type (%)			0.76
Medical	108 (60.0%)	139 (61.5%)	
Surgery	72 (40.0%)	87 (38.5%)	
Heart failure class IV (n (%))	12 (6.7%)	26 (11.5%)	0.13
AIDS (n (%))	11 (6.1%)	18 (8.0%)	0.60
Cirrhosis (n (%))	12 (6.7%)	18 (8.0%)	0.76
CRD (n (%))	9 (5.0%)	18 (8.0%)	0.32
Diabetes (n (%))	22 (12.2%)	38 (16.8%)	0.25
Hypertension (n (%))	50 (27.8%)	63 (27.9%)	0.99
Dementia (n (%))	4 (2.2%)	13 (5.8%)	0.12
Cancer (%)	26 (14.4%)	60 (26.5%)	<0.001
COPD (%)	11 (6.1%)	22 (9.7%)	0.25
More than 2 Comorbidities (n (%))*	85 (47.2%)	140 (61.9%)	<0.001
CCI (n (%))	41 (22.8%)	58 (25.8%)	0.56
ICU LOS, days, median [IQR]	22 (11-38)	22(11-34)	0.98
MV duration, days, median [IQR]	16 (11-29)	20(13-31)	0.04
Appropriateness of ATB therapy (%)	147 (81.7%)	184(81.4%)	0.99
Previous ATB (%)	72 (40.0%)	103 (45.6%)	0.27
MDR pathogen (%)	69 (38.3%)	103 (45.6%)	0.16
Basal serum CRP (median (IQR))	163 (108-196)	168 (126-213)	0.77
CRP maximum within 72h (median (IQR))	168 (119-201)	179 (135-243)	0.30
CRP at 96h (median (IQR))	116 (81-165)	128 (83-178)	0.29

ICU – Intensive Care unit; CRP – C-reactive protein; SD – Standard deviation; IQR – Interquartile range; LOS – length of stay; MV – mechanical ventilation; CRD – Chronic renal disease; COPD – chronic obstructive pulmonary disease; ATB – antibiotic therapy; CCI – chronic critical illness; MDR – multidrug resistant.

Table 2 – Etiology of VAP episodes in older and younger patients

ETIOLOGY	Younger	Older	p
Enterobacteriaceae	63 (26.0%)	59 (36.0%)	0.04
E. coli	4 (1.7%)	9 (5.5%)	
Enterobacter spp.	19 (7.8%)	20 (12.2%)	
Klebsiella spp.	32 (13.2%)	23 (14.0%)	
Other Enterobacteriaceae	8 (3.3%)	7 (4.3%)	
S. aureus	41 (16.9%)	34 (20.7%)	0.40
MSSA	34 (14.0%)	24 (14.6%)	
MRSA	7 (2.9%)	10 (6.1%)	
Pseudomonas aeruginosa	38 (15.7%)	31 (18.9%)	0.48
Acinetobacter sp.	53 (21.9%)	35 (21.3%)	0.99
Streptococcus pneumoniae	3 (1.2%)	2 (1.2%)	0.99
Haemophilus influenzae	11 (4.5%)	2 (1.2%)	0.10
Serratia sp.	12 (4.9%)	4 (2.4%)	0.30
Stenotrophomonas maltophila	15 (6.2%)	10 (6.1%)	0.99
Polymicrobial episodes	63 (26.0%)	40 (24.4%)	0.80

Table 3. Comparison of characteristics between older and younger ICU patients

	Younger	Older	p
Number of patients	242	164	
Age (mean (SD))	45.6 (13.5)	73.6 (6.3)	<0.001
Gender (male/female) (%)	60.3/39.7%	62.2/37.8%	0.76
APACHE II (mean (SD))	19.1 (9.8)	23.8 (9.6)	<0.001
LOS MV before PAV (median [IQR])	8 (5-13)	9 (6-14)	0.14
Admission type (%)			0.03
Medical	158 (65.3%)	89 (54.3%)	
Surgery	84 (34.7%)	75 (45.7%)	
Heart failure class IV (n (%))	16 (6.6%)	22 (13.4%)	0.03
AIDS (n (%))	28 (11.6%)	1 (0.6%)	<0.001
Cirrhosis (n (%))	25 (10.3%)	5 (3.0%)	<0.001
CRD (n (%))	12 (5.0%)	15 (9.1%)	0.14
Diabetes (n (%))	32 (13.2%)	28 (17.1%)	0.35
Hypertension (n (%))	49 (20.2%)	64 (39.0%)	<0.001
Dementia (n (%))	5 (2.1%)	12 (7.3%)	0.02
Cancer (%)	48 (19.8%)	38 (23.2%)	0.49
COPD (%)	11 (4.5%)	22 (13.4%)	<0.001
More than 2 Comorbidities (n (%))*	115 (47.5%)	110(67.1%)	<0.001
CCI (n (%))	57 (23.6%)	42 (25.8%)	0.64
ICU LOS, days, median [IQR]	21 (14-32)	24 (16-37)	0.04
MV duration, days, median [IQR]	18 (12-29)	20 (12-31)	0.21
Appropriateness of ATB therapy (%)	189 (78.1%)	142 (86.6%)	0.04
Previous ATB (%)	96 (39.7%)	79 (48.2%)	0.10
MDR pathogen (%)	96 (39.7%)	76 (46.3%)	0.18
Basal serum CRP (median (IQR))	167 (113-198)	164 (118-213)	0.49
CRP maximum within 72h (median (IQR))	175 (131-219)	168 (125-211)	0.64
CRP at 96h (median (IQR))	131 (83-173)	125 (85-165)	0.30
ICU Mortality (n(%))	119 (49.2%)	107 (65.2%)	<0.001

ICU – Intensive Care unit; CRP – C-reactive protein; SD – Standard deviation; IQR – Interquartile range; LOS – length of stay; MV – mechanical ventilation; CRD – Chronic renal disease; COPD – chronic obstructive pulmonary disease; ATB – antibiotic therapy; CCI – chronic critical illness.

Table 4. Comparison of CRP response according to age in ICU patients

	Younger	Older	p
CRUDE			
Basal serum CRP (mean (SD))	164.9 (88.6)	173.1 (84.6)	0.51
CRP maximum within 72h (mean (SD))	184.0 (91.1)	177.2 (76.7)	0.59
CRP at 96h (mean (SD))	139.5 (84.6)	123.3 (63.2)	0.15
CRP ratio (median [IQR])	1.22 (0.84-1.67)	0.96 (0.70-1.25)	0.09
ONLY APPROPRIATE ANTIBIOTIC THERAPY			
Basal serum CRP (mean (SD))	166.6 (88.3)	174.8 (79.1)	0.52
CRP maximum within 72h (mean (SD))	185.5 (91.3)	178.8 (76.4)	0.60
CRP at 96h (mean (SD))	141.7 (85.5)	125.1 (64.0)	0.14
CRP ratio (median [IQR])	0.85 (0.61-1.15)	0.71 (0.47-0.96)	0.07
ANALYSIS OF COVARIANCE – ESTIMATED MARGINS ADJUSTED BY BASAL CRP LEVELS			
CRP maximum within 72h (mean (95% CI))	187.2 (180.0-194.5)	174.1 (165.0-182.7)	0.05
CRP at 96h (mean (95%CI))	142.3 (128.0-153.5)	122.0 (108.3-135.0)	0.07

ICU – Intensive Care unit; CRP – C-reactive protein; SD – Standard deviation; IQR – Interquartile range

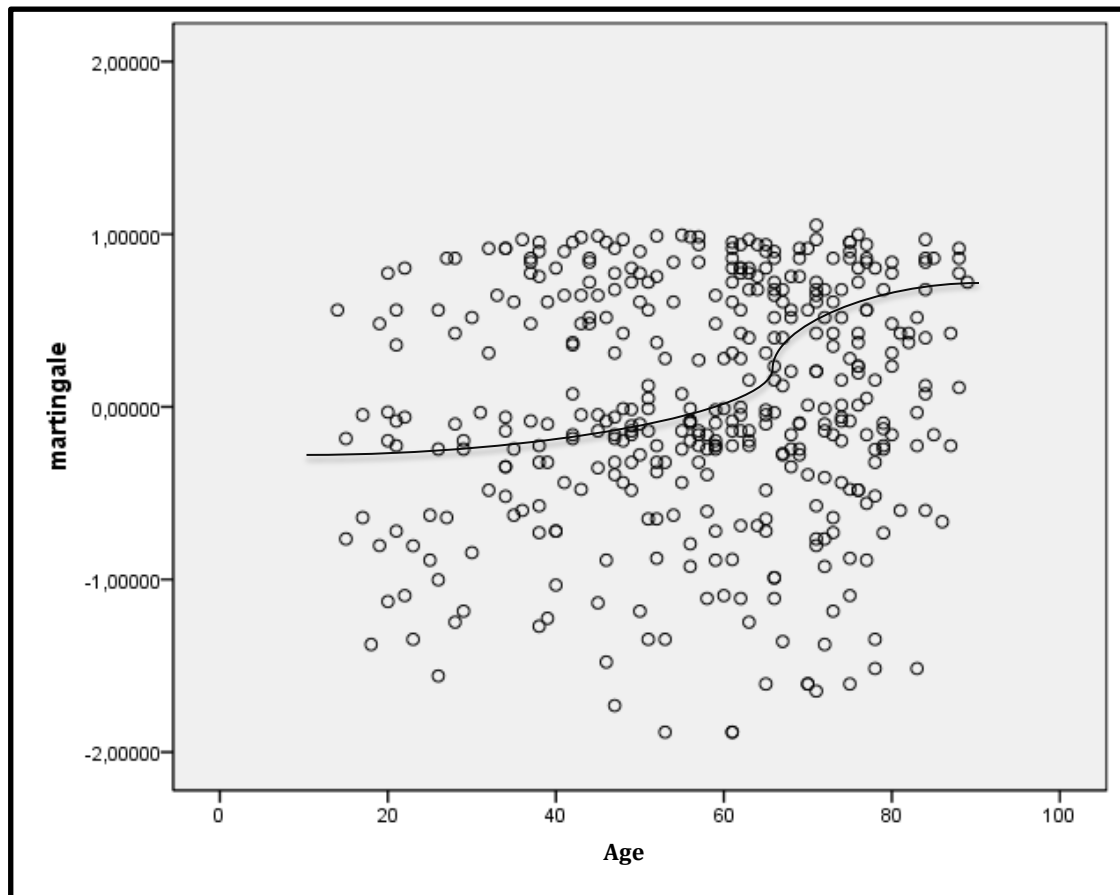**Figure 1**. Age plotted agains Martingale residuals in VAP patients

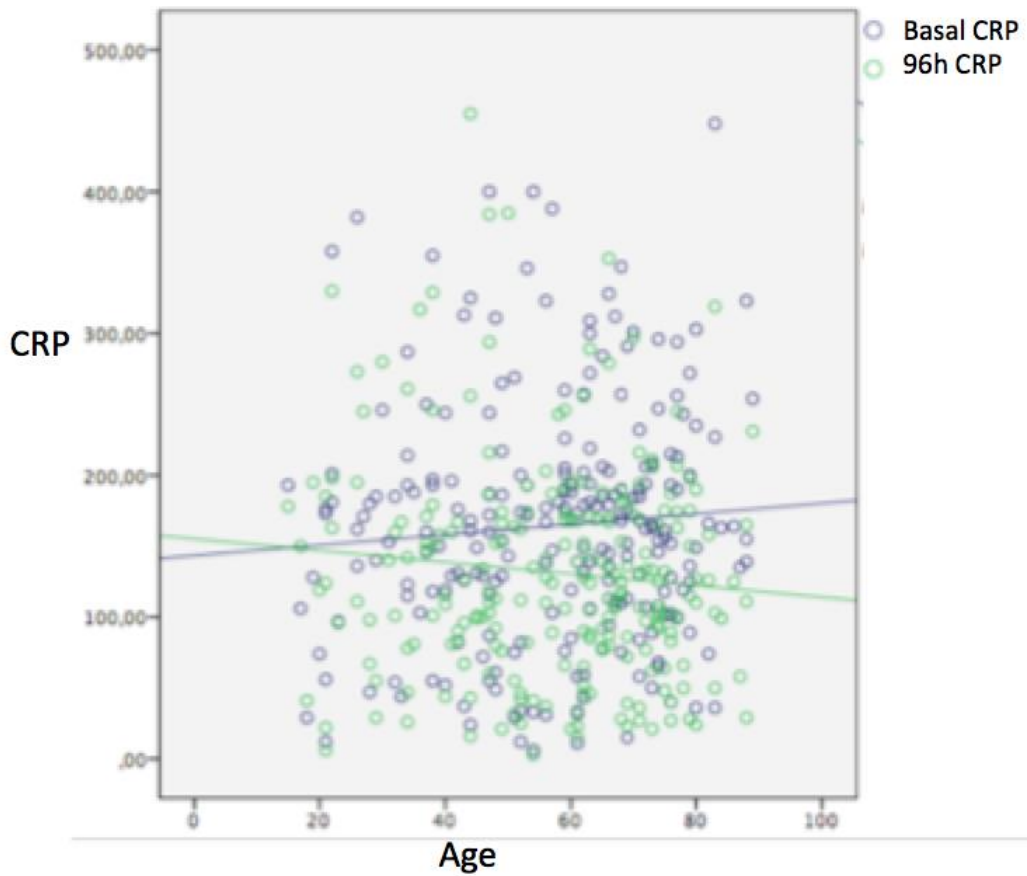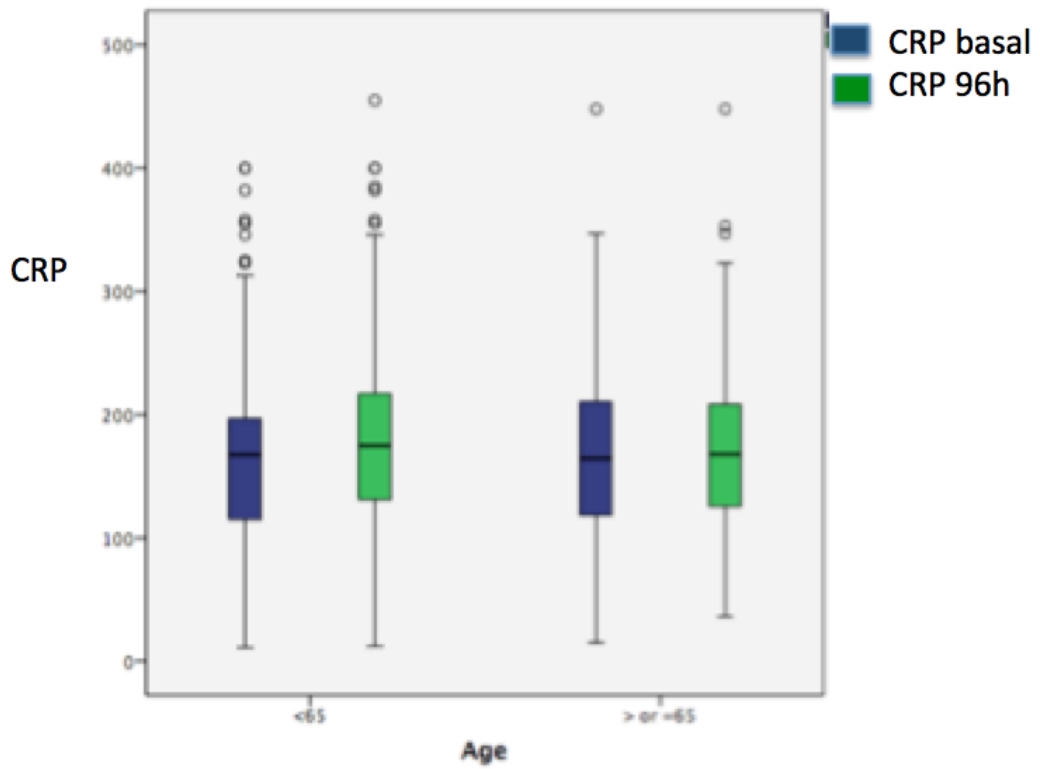**Figure 2**. Correlation between age and CRP levels in VAP patients

Figure 3. CRP evolution in older and younger patients with VAP

CONCLUSÕES

Em conclusão, nesta tese avaliamos uma coorte de pacientes com diagnóstico de pneumonia nosocomial em ventilação mecânica e a evolução da PCR em duas populações específicas, não avaliadas previamente na literatura.

Descrevemos que pacientes que desenvolvem PAV num cenário de doença crítica crônica tem pior prognóstico, apresentando maior *hazard risk* para mortalidade na UTI após ajuste pra gravidade de doença na admissão, presença de patógenos multi-resistentes e comorbidades. Ainda, não encontramos uma alteração na cinética ou comprometimento na resposta inflamatória, medida pela evolução dos níveis de PCR, sugerindo que na população de pacientes com doença crítica crônica, o uso de PCR como um biomarcador da evolução dos pacientes permanence uma estratégia válida.

De maneira análoga, avaliamos também os episódios de PAV em diferentes faixas etárias. Pacientes idosos que desenvolvem PAV tem pior prognóstico, com maior mortalidade, maior gravidade na admissão e mais comorbidades. O efeito no prognóstico parece iniciar a partir de 65 anos. Ainda, não encontramos diferença nos niveis de PCR ou na sua cinética, quando comparamos pacientes com diferentes faixas etárias (> ou <65 anos). Também não detectamos correlação entre a idade e os níveis de PCR, sugerindo que a PCR mantem suas características como biomarcador de evolução em pacientes com PAV, independente da faixa etária.

CONSIDERAÇÕES FINAIS

A pneumonia nosocomial permanece uma condição clínica prevalente com alta morbimortalidade associadas. Neste trabalho, revisamos diversos aspectos de sua epidemiologia, fisiopatologia, etiologia e manejo, além de levantar diversas áreas de incerteza na literatura.

Pudemos ainda avaliar, em dois estudos originais, o uso da proteína C-reativa (PCR) como marcador da evolução dos pacientes com pneumonia nosocomial associada a ventilação mecânica em duas populações previamente não estudadas na literatura. Nos pacientes com doença crítica crônica, condição cuja prevalência vem aumentando dramaticamente nas unidades de terapia intensiva, pudemos verificar que a despeito de um questionamento empírico sobre a viabilidade de manutenção da resposta inflamatória nestes pacientes, a produção e a cinética das primeiras 96h deste biomarcador não foram diferentes daqueles pacientes sem doença crítica crônica. Isso sugere que possíveis mudanças qualitativas e quantitativas da resposta inflamatória neste grupo de pacientes, associada ao possível "esgotamento" da capacidade de reação do sistema imunológico, não parecem afetar a PCR, permitindo seu uso na avaliação da evolução de eventos infecciosos nesta população. Além disso, a descrição das características dos pacientes com PAV nesta população não foi devidamente avaliado na literatura até então.

Uma segunda análise, avaliou o efeito da idade na evolução dos pacientes com pneumonia associada a ventilação mecânica, bem como seu efeito na evolução da PCR como marcador da resposta inflamatória nesses pacientes. Embora a idade, a

partir dos 65 anos, pareça ter um efeito na mortalidade dos pacientes com PAV, não houve alteração na resposta da PCR ou na sua cinética nas primeiras 96h quando comparamos pacientes com diferentes faixas etárias a partir de um ponto de corte de 65 anos. Esta informação auxilia no manejo dos pacientes idosos, pois sugere que o uso da PCR como marcador da evolução dos pacientes com pneumonia parece válida e não parece ser afetada pelo fenômeno de "imunosenescencia" descrito em outros aspectos qualitativos e quantitativos da resposta inflamatória em idosos.

Nossos resultados, portanto, além de revisar e pontuar diversas áreas de incerteza na literatura e oportunidades de investigação, puderam também, de maneira original, esclarecer alguns aspectos do uso de biomarcadores, especificamente de PCR, em duas populações especiais que não haviam sido estudadas previamente. Isto permite que os estudos futuros que avaliem intervenções baseadas em biomarcadores em pacientes com pneumonia nosocomial possam levar em consideração estas populações de pacientes, dado o comportamento similar na evolução dos biomarcadores nestes subgrupos em comparação com a população geral de pacientes criticamente doentes.

Infelizmente, nesta amostra não foi possível avaliar outros biomarcadores como procalcitonina, limitando as conclusões apenas ao universo da PCR.

As perspectivas que se abrem a partir desta análise incluem testar intervenções baseadas na PCR nestes dois grupos de pacientes. Além disso, o uso de biomarcadores como a PCR para identificar potenciais candidatos a intervenções especificas é uma estratégia promissora descrita na literatura. Nossos dados podem contribuir para a generalização destas estratégias aos pacientes com doença critica crônica e idosos.