Strategies to optimise the treatment of community-acquired pneumonia

Wittermans, E.

STRATEGIES TO OPTIMISE THE TREATMENT OF COMMUNITY-ACQUIRED PNEUMONIA

Esther Wittermans

ISBN: 978-94-6483-256-3
Cover design: Irene Legters
Layout: Anna Bleeker | persoonlijkproefschrift.nl
Printing: Ridderprint | Ridderprint.nl

Financial support for the research described in this thesis was kindly provided by St. Antonius Research Fund and St. Antonius Hospital.

Printing of this thesis was financially supported by SBOH

Strategies to optimise the treatment of community-acquired pneumonia

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van de rector magnificus prof. dr. ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op donderdag 28 september 2023
klokke 13.45 uur

door

Esther Wittermans
geboren te St. Leonards on Sea (Verenigd Koninkrijk)
in 1992

Promotores
Prof. dr. W.J.W. Bos
Prof. dr. J.C. Grutters *UMC Utrecht*

Co-promotor
Dr. E.M.W. van de Garde *Universiteit Utrecht*

Leden promotiecommissie
Prof dr. D.H. Biesma
Prof dr. C.A.J. Knibbe
Prof. dr. W.J. Wiersinga *Amsterdam UMC*
Prof dr. G.T. Rijkers *University College Roosevelt, Middelburg*
Dr. W.G. Boersma *Noordwest Ziekenhuisgroep, Alkmaar*

TABLE OF CONTENTS

CHAPTER 1

General introduction, aim and outline of the thesis

COMMUNITY-ACQUIRED PNEUMONIA

Pneumonia is an infection of the alveoli of the lungs, which is clinically characterised by a pulmonary infiltrate on chest radiograph accompanied by symptoms such as cough, fever, sputum production and dyspnoea.[1] Pneumonia can be divided into three types based on place of acquirement: hospital-acquired pneumonia, ventilator-acquired pneumonia and community-acquired pneumonia (CAP).[2] This thesis will only focus on CAP, pneumonia acquired outside the hospital. In the Netherlands, CAP is most frequently caused by *Streptococcus pneumonia* followed by *Haemophilus influenza* and atypical bacteria. Viruses such as Influenza virus, are responsible for 3-5% of hospitalised CAP cases and approximately one third of viral pneumonia is also accompanied by bacterial pneumonia.[3–5] In the period in which the studies presented in this thesis were performed, severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) emerged as a new pathogen for CAP and led to the COVID-19 pandemic. Therefore, this thesis will distinguish between two types of CAP: non-COVID-19 pneumonia (from here on referred to as CAP) and hospitalised COVID-19 (from here on referred to as COVID-19). Both will be discussed, though CAP is the main focus of this thesis.

Globally pneumonia is among the leading causes of death due to infectious diseases.[6] The incidence of CAP is highest in young children and the elderly. In the Netherlands, there were 156.000 new cases of CAP in 2020. In that same year 24.205 patients were hospitalised with CAP and 2.726 patients died due to CAP. The annual health care costs for CAP are substantial, in the Netherlands the healthcare cost for CAP was estimated at 584 million euros in 2019 of which 61,3% was allocated to hospital care.[7] Identifying strategies to optimise the management of CAP might aid in lightening the burden of CAP.

The cornerstones of CAP treatment are early diagnosis and swift initiation of appropriate empiric antimicrobial treatment, preferably within 4 hours of hospital presentation.[8] Empiric antimicrobial treatment is based on the most likely causative organism and disease severity.[9] Empiric antimicrobial treatment should be switched to targeted treatment once the causative pathogen is identified. Appropriate antimicrobial therapy is essential in the treatment of CAP as it improves clinical outcomes and reduces the selective pressure for antimicrobial resistance.[10–12] Yet, in some patients appropriate antibiotic therapy is not enough to prevent unfavourable clinical outcomes. An excessive or dysregulated host immune response can lead to severe disease accompanied by lung injury, sepsis and eventually multi-organ damage and death.[13,14] This has led to an interest in immunomodulation in CAP. In COVID-19 a similar mechanism is thought to play a role: dysregulation of the host immune response triggered by SARS-COV-2 is thought to cause inflammatory organ injury resulting in unfavourable clinical outcomes.[15]

ADJUNCTIVE CORTICOSTEROID TREATMENT IN CAP

The host inflammatory response in CAP is a complex interaction between the numerous cells and soluble mediators of the immune system. The response is regulated by cytokines and chemokines, the messengers of the immune system. Cytokines are produced by multiple cell types (e.g., neutrophils, macrophages, epithelial cells). They recruit, regulate and activate immune cells such as neutrophils and initiate local repair processes. The interaction between pro-inflammatory cytokines and anti-inflammatory cytokines determines the nature, duration and intensity of the host immune response.[16] Corticosteroids are potent non-specific inhibitors of the immune system. Corticosteroids have multiple anti-inflammatory mechanisms. The most important being the deactivation of genes that encode for pro-inflammatory cytokines (e.g., IL-6 and IL-8) and the activation genes that encode for anti-inflammatory cytokines (e.g., IL-10).[17,18] In CAP, corticosteroids have shown to downregulate the cytokine response.[19] Furthermore, corticosteroids inhibit the migration of neutrophils to the site of infection.[20] It is hypothesised that downregulation of the host immune response by corticosteroids can reduce pulmonary damage and inhibit the development of sepsis ultimately reducing unfavourable clinical outcomes.[21]

The first well-designed randomised clinical trial investigating adjunctive corticosteroid treatment in CAP was published in 2005 by Confalonieri et al.[22] The study was terminated after the first interim analysis (n = 46) due to far better improvement of the PaO2/FiO2 ratio and a lower mortality rate (0% vs 30%) in the intervention group. Several large trials have followed most with positive albeit less spectacular results.[23–27] An individual patient data meta-analysis (IPDMA) including 6 trials showed that corticosteroids reduced length of hospital stay (LOS) by 1 day and improved time to clinical stability (TTCS). Yet, corticosteroids did not reduce mortality in CAP.[28] This begs the question if a 1-day LOS reduction outweighs the possible adverse event of corticosteroid use. This dilemma is further complicated by the fact that, in the IPDMA, CAP-related rehospitalisation was more frequent in patients treated with corticosteroids compared to placebo (5.0% vs 2.7%).[28] Currently treatment guidelines for CAP do not advise the routine use of adjunctive corticosteroid treatment.[9]

Nonetheless, it has been hypothesised that corticosteroids could be more effective in patients with severe disease as patients with severe disease show a more exuberant immune response.[29,30] Hence, a subgroup of CAP patients may exist for whom the benefits of corticosteroid treatment outweigh the risks. The IPDMA suggested a possible greater effect of corticosteroids on LOS in patients with severe disease based on pneumonia severity index (PSI) score.[28,31] No RCT has prospectively tested this hypothesis. Therefore **Chapter 2** aims to prospectively investigate whether the effect of adjunctive corticosteroid treatment depends on disease severity. In this randomised placebo-controlled trial (the Santeon-CAP study) randomisation is stratified by disease

severity based on PSI risk class. The Santeon-CAP study is the sequel to the Ovidius trial by Meijvis et al.[24] in which 6 mg dexamethasone reduced LOS by 1 day. In the Ovidius trial dexamethasone was administered intravenously. It was hypothesised that this may have hampered iv-to-oral switch of antibiotics thereby delaying discharge. Therefore, in the Santeon-CAP study, oral dexamethasone is investigated.

INFLAMMATORY SUBGROUPS FOR GUIDING CORTICOSTEROID TREATMENT IN CAP

As mentioned above, it is thought that corticosteroids are most effective in patients with more severe disease based on the rationale that severe disease is caused by higher levels of inflammation. In **Chapter 2**, PSI score is used to define subgroups. However, the PSI score is a mortality risk score and is greatly influenced by age.[31] Consequently, the PSI score does not necessarily correspond to level of inflammation. Parameters more indicative of inflammation may be more appropriate to identify patients in whom corticosteroid treatment is more effective. Yet identification of such a parameter has proven a challenge; Analysis of parameters such as C-reactive protein, ICU admission, or systemic inflammatory response criteria, have not resulted in the identification of a well-defined CAP subgroup benefitting more from corticosteroid treatment.[28] Therefore, besides PSI score, this thesis explores two additional methods for identifying CAP subgroups which might benefit more from corticosteroid treatment.

The first method is based on white blood cell (WBC) count differential parameters. WBCs are important effectors in the local and systemic inflammatory response in CAP.[32] Neutrophilia is widely used as a marker of inflammation in CAP. More recently, lymphocytopenia has been associated with disease severity and higher concentrations of inflammatory cytokines in CAP.[33] Furthermore, the neutrophil-lymphocyte count ratio (NLR) has been acknowledged as a marker of systemic inflammation.[34–36] In CAP, NLR has been associated with disease severity and has shown to predict mortality.[37,38] **Chapter 3** examines whether these parameters could also be used to guide corticosteroid treatment. In **Chapter 3** a post-hoc analysis of the Santeon-CAP study is performed to test whether the effect of adjunctive oral dexamethasone on clinical outcomes is modified by a high neutrophil count, low lymphocyte count and/or high NLR.

The second method used in this thesis to identify CAP subgroups is latent class analysis (LCA) of inflammatory and clinical parameters. LCA is a statistical modelling method used to identify “hidden" subgroups in a population by identifying individuals that share similar characteristics. Unlike other outcome modelling approaches LCA recognises the fact that some clinical factors can share variance as a constellation of observed variables for a common unobserved (latent) variable.[39] LCA uses this relationship between observed variables to group individuals together into mutually exclusive and collectively exhaustive subgroups. The subgroups identified by LCA are

called 'latent classes'. In general, LCA subgroups are solely based on baseline data or patient characteristics and thus are not dependent on an outcome variable. LCA is useful in defining the unobservable heterogeneity in a population.[39,40]

In acute respiratory distress syndrome LCA has successfully identified subgroups with different inflammatory profiles. These clinically distinct subgroups were shown to respond differently to ventilator and fluid management.[41,42] In COVID-19 related ARDS LCA also identified two clinically distinct subgroups. Both subgroups responded differently to corticosteroid treatment. Corticosteroids improved mortality in the hyperinflammatory subgroup but worsened mortality in the hypoinflammatory subgroup.[43] **Chapter 4** and **Chapter 5** examine whether LCA can also identify clinically distinct subgroups in CAP and if so, whether these subgroups respond differently to adjunctive corticosteroids. Therefore, in **Chapter 4** and **Chapter 5**, LCAs of baseline clinical and biomarker data collected at time of hospital admission are performed in three independent CAP cohorts.

DEXAMETHASONE FOR COVID-19

As discussed above adjunctive corticosteroids for CAP have been a subject of research for many years and no definitive conclusions have been made regarding its place in the treatment of CAP. In COVID-19 this is quite different. Dexamethasone has been studied as a stand-alone treatment for COVID-19. Trials studying corticosteroids for COVID-19 were commenced promptly after the Sars-CoV-2 virus emerged. The rationale for corticosteroids being that severe COVID-19 (defined as an oxygen saturation <94%), is caused by a dysregulated host immune response in reaction to the SARS-CoV-2 virus.[15] Just several months after COVID-19 was declared a pandemic the preliminary results from RECOVERY Trial and the results of the WHO react meta-analysis were published. The results showed that 6 mg dexamethasone reduced risk for mortality and mechanical ventilation.[44,45] The choice to investigate a 6 mg dose was partially based on the dose used in the Ovidius trial.[24] After publication of these studies, dexamethasone 6 mg for 10 days became standard treatment for hospitalised COVID-19 patients requiring oxygen therapy.[46,47]

Trials investigating corticosteroids for COVID-19 did not perform subgroup analyses based on BMI despite the fact that obesity had been associated with ICU admission and mortality.[48,49] Because dexamethasone is a lipophilic drug with a relatively large volume of distribution, one may hypothesise that serum dexamethasone levels are lower in patients with obesity compared to those with normal weight which might translate in the fixed 6 mg dexamethasone dose being less effective in patients with overweight or obesity.[50–52] To test this hypothesis, **Chapter 6** examines whether overweight and obesity are associated with worse clinical outcomes in a cohort of non-ICU COVID-19 patients treated with fixed-dose dexamethasone.

MICROBIOLOGICAL TESTING AND ANTIBIOTIC ALTERATIONS IN CAP

Though immunomodulation might improve outcomes for CAP patients, as mentioned earlier, appropriate antibiotic treatment is still the basis of CAP treatment. A quality indicator of appropriate antimicrobial treatment is the adjustment of the empirical antibiotic regimen guided by microbiological test results.[53] Yet to do so, the availability of actionable microbiological test results is necessary. In >60% of CAP cases a causative micro-organism is not identified.[9] Microbiological diagnostics consist of traditional cultures of blood and respiratory samples, newer techniques such as PCR assays on throat and nose swabs for identifying atypical bacteria and respiratory viruses, and urinary antigen tests for legionella and pneumococcus. In a research setting, the combination of traditional and newer tests had the potential to increase the percentage of identified pathogens up to 67%.[54–56] However, there is little evidence if the same is true in day-to-day clinical practice and more importantly, whether extensive testing leads to more alterations of the empirical antimicrobial regimen in individual patients. Therefore, **Chapter 7** examines whether extensive microbiological testing is associated with an increase in diagnostic yield and antibiotic treatment alterations in day-to-day clinical practice.

AIMS OF THE THESIS

The aim of this thesis was to identify strategies to improve the management of community-acquired pneumonia outside the intensive care unit with a focus on corticosteroid treatment. This thesis specifically focuses on the following three topics:

1. The effect of oral adjunctive dexamethasone on clinical outcomes in CAP and whether CAP subgroups exist in which the benefits of adjunctive corticosteroids outweigh the disadvantages of corticosteroid use.

2. The association between obesity and overweight and clinical outcomes in COVID-19 patients treated with dexamethasone.

3. The relationship between the extent of microbiological testing and early alterations of antibiotic therapy in CAP.

REFERENCES

1. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/ American Thoracic Society Consensus Guidelines on the management of community-acquired pneumonia in adults. *Clin Inf Dis*. 2007;44(suppl 2):S27-S72. doi:10.1086/511159
2. Anand N, Kollef MH. The alphabet soup of pneumonia: CAP, HAP, HCAP, NHAP, and VAP. *Semin Respir Crit Care Med*. 2009;30(1):3-9. doi:10.1055/S-0028-1119803
3. Postma DF, van Werkhoven CH, van Elden LJR, et al. Antibiotic Treatment Strategies for Community-Acquired Pneumonia in Adults. *N Engl J Med*. 2015;372(14):1312-1323. doi:10.1056/nejmoa1406330
4. Musher DM, Abers MS, Bartlett JG. Evolving Understanding of the Causes of Pneumonia in Adults, With Special Attention to the Role of Pneumococcus. *Clin Infect Dis*. 2017;65(10):1736-1744. doi:10.1093/cid/cix549
5. Bonten MJM, Huijts SM, Bolkenbaas M, et al. Polysaccharide Conjugate Vaccine against Pneumococcal Pneumonia in Adults. *N Engl J Med*. 2015;372(12):1114-1125. doi:10.1056/nejmoa1408544
6. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis*. 2018;18(11):1191-1210. doi:10.1016/S1473-3099(18)30310-4
7. Infecties van de onderste luchtwegen. VZinfo. Published September 1, 2022. Accessed October 27, 2022. https://www.vzinfo.nl/infecties-van-de-onderste-luchtwegen
8. Lim WS, Woodhead M. British Thoracic Society adult community acquired pneumonia audit 2009/10. *Thorax*. 2011;66(6):548-549. doi:10.1136/thoraxjnl-2011-200081
9. Wiersinga W, Bonten MJM, Boersma W, et al. Management of community-acquired pneumonia in adults: 2016 guideline update from the Dutch Working Party on Antibiotic Policy (SWAB) and Dutch Association of Chest Physicians (NVALT). *Neth J Med*. 2018;76(1):4-13.
10. Schuts EC, Hulscher MEJL, Mouton JW, et al. Current evidence on hospital antimicrobial stewardship objectives: a systematic review and meta-analysis. *Lancet Infect Dis*. 2016;16(7):847-856. doi:10.1016/S1473-3099(16)00065-7
11. Kothe H, Bauer T, Marre R, Suttorp N, Welte T, Dalhoff K. Outcome of community-acquired pneumonia: influence of age, residence status and antimicrobial treatment. *Eur Respir J*. 2008;32(1):139- 146.
12. Garcia-Vidal C, Fernández-Sabé N, Carratalà J, et al. Early mortality in patients with community-acquired pneumonia: causes and risk factors. *Eur Respir J*. 2008;32(3):733 - 739.
13. Fernandez-Botran R, Uriarte SM, Arnold FW, et al. Contrasting Inflammatory Responses in Severe and Non-severe Community-acquired Pneumonia. *Inflammation*. 2014;37(4):1158-1166. doi:10.1007/s10753-014-9840-2
14. Deng JC, Standiford TJ. The Systemic Response to Lung Infection. *Clin Chest Med*. 2005;26(1):1-9. doi:10.1016/j.ccm.2004.10.009
15. Hsu RJ, Yu WC, Peng GR, et al. The Role of Cytokines and Chemokines in Severe Acute Respiratory Syndrome Coronavirus 2 Infections. *Front Immunol*. 2022;13. doi:10.3389/fimmu.2022.832394
16. Moldoveanu B, Otmishi P, Jani P, et al. Inflammatory mechanisms in the lung. *J Inflamm Res*. 2009;2:1-11.
17. Barnes PJ. Anti-inflammatory Actions of Glucocorticoids: Molecular Mechanisms. *Clin Sci*. 1998;94(6):557-572. doi:10.1042/cs0940557

18. Rendon A, Rendon-Ramirez EJ, Rosas-Taraco AG. Relevant Cytokines in the Management of Community-Acquired Pneumonia. *Curr Infect Dis Rep.* 2016;18(3):10. doi:10.1007/s11908-016-0516-y

19. Remmelts HHF, Meijvis SCA, Biesma DH, et al. Dexamethasone downregulates the systemic cytokine response in patients with community-acquired pneumonia. *Clin Vaccine Immunol*. 2012;19(9):1532-1538. doi:10.1128/cvi.00423-12

20. Rhen T, Cidlowski JA. Antiinflammatory Action of Glucocorticoids — New Mechanisms for Old Drugs. *N Engl J Med.* 2005;353(16):1711-1723. doi:10.1056/nejmra050541

21. Martin-Loeches I, Torres A. Corticosteroids for CAP, influenza and COVID-19: when, how and benefits or harm? *European Respiratory Review*. 2021;30(159):1-9. doi:10.1183/16000617.0346-2020

22. Confalonieri M, Urbino R, Potena A, et al. Hydrocortisone Infusion for Severe Community-acquired Pneumonia. *Am J Respir Crit Care Med*. 2005;171(3):242-248. doi:10.1164/rccm.200406-808oc

23. Snijders D, Daniels JMA, de Graaff CS, van der Werf TS, Boersma WG. Efficacy of Corticosteroids in Community-acquired Pneumonia. *Am J Respir Crit Care Med*. 2010;181(9):975-982. doi:10.1164/rccm.200905-0808oc

24. Meijvis SC, Hardeman H, Remmelts HH, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. *The Lancet*. 2011;377(9782):2023-2030. doi:10.1016/S0140-6736(11)60607-7

25. Torres A, Sibila O, Ferrer M, et al. Effect of Corticosteroids on Treatment Failure Among Hospitalized Patients With Severe Community-Acquired Pneumonia and High Inflammatory Response: A Randomized Clinical Trial. *JAMA*. 2015;313(7):677-686. doi:10.1001/jama.2015.88

26. Fernández-Serrano S, Dorca J, Garcia-Vidal C, et al. Effect of corticosteroids on the clinical course of community-acquired pneumonia: a randomized controlled trial. *Crit Care*. 2011;15(2):R96-R96. doi:10.1186/cc10103

27. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: A multicentre, double-blind, randomised, placebo-controlled trial. *The Lancet*. 2015;385(9977):1511-1518. doi:10.1016/S0140-6736(14)62447-8

28. Briel M, Spoorenberg SMC, Snijders D, et al. Corticosteroids in Patients Hospitalized With Community-Acquired Pneumonia: Systematic Review and Individual Patient Data Metaanalysis. *Clin Infect Dis*. 2017;66(3):346-354. doi:10.1093/cid/cix801

29. Fernández-Serrano S, Dorca J, Coromines M, Carratalà J, Gudiol F, Manresa F. Molecular inflammatory responses measured in blood of patients with severe community-acquired pneumonia. *Clin Diagn Lab Immunol*. 2003;10(5):813-820. doi:10.1128/cdli.10.5.813-820.2003

30. Paats MS, Bergen IM, Hanselaar WEJJ, et al. Local and systemic cytokine profiles in nonsevere and severe community-acquired pneumonia. *Eur Respir J*. 2013;41(6):1378-1385. doi:10.1183/09031936.00060112

31. Fine MJ, Auble TE, Yealy DM, et al. A Prediction Rule to Identify Low-Risk Patients with Community-Acquired Pneumonia. *N Engl J Med*. 1997;336(4):243-250. doi:10.1056/nejm199701233360402

32. Pechous RD. With friends like these: The complex role of neutrophils in the progression of severe pneumonia. *Front Cell Infect Microbiol*. 2017;7:160. doi:10.3389/fcimb.2017.00160

33. Méndez R, Menéndez R, Amara-Elori I, et al. Lymphopenic community-acquired pneumonia is associated with a dysregulated immune response and increased severity and mortality. *J Infect*. 2019;78(6):423-431. doi:10.1016/j.jinf.2019.04.006

34. Huang Z, Fu Z, Huang W, Huang K. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. *Am J Emerg Med*. 2020;38(3):641-647. doi:10.1016/j.ajem.2019.10.023

35. Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: Experience in patients with cancer. *Crit Rev Oncol Hematol*. 2013;88(1):218-230. doi:10.1016/j.critrevonc.2013.03.010

36. Angkananard T, Anothaisintawee T, McEvoy M, Attia J, Thakkinstian A. Neutrophil Lymphocyte Ratio and Cardiovascular Disease Risk: A Systematic Review and Meta-Analysis. *Biomed Res Int*. 2018;2018: 2703518. doi:10.1155/2018/2703518

37. de Jager CPC, Wever PC, Gemen EFA, et al. The Neutrophil-Lymphocyte Count Ratio in Patients with Community-Acquired Pneumonia. *PLoS One*. 2012;7(10):4-11. doi:10.1371/journal.pone.0046561

38. Cataudella E, Giraffa CM, di Marca S, et al. Neutrophil-To-Lymphocyte Ratio: An Emerging Marker Predicting Prognosis in Elderly Adults with Community-Acquired Pneumonia. *J Am Geriatr Soc*. 2017;65(8):1796-1801. doi:10.1111/jgs.14894

39. Law E, Harrington R. A Primer on Latent Class Analysis. *Value & Outcome Spotlight*. 2016;2(6):18-19.

40. Sinha P, Calfee CS, Delucchi KL. Practitioner's Guide to Latent Class Analysis: Methodological Considerations and Common Pitfalls. *Crit Care Med*. 2021;49(1):e63-e79. doi:10.1097/ccm.0000000000004710

41. Famous KR, Delucchi K, Ware LB, et al. Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy. *Am J Respir Crit Care Med*. 2017;195(3):331-338. doi:10.1164/rccm.201603-0645OC

42. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med*. 2014;2(8):611-620. doi:10.1016/S2213-2600(14)70097-9

43. Sinha P, Furfaro D, Cummings MJ, et al. Latent Class Analysis Reveals COVID-19-related ARDS Subgroups with Differential Responses to Corticosteroids. 2021;204(11):1274-1285. doi:10.1164/rccm.202105-1302OC

44. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med*. 2021;384(8):693-704. doi:10.1056/nejmoa2021436

45. Sterne JAC, Murthy S, Diaz J v., et al. Association between Administration of Systemic Corticosteroids and Mortality among Critically Ill Patients with COVID-19: A Meta-analysis. *JAMA*. 2020;324(13):1330-1341. doi:10.1001/jama.2020.17023

46. Medicamenteuze behandeling voor patiënten met COVID-19 (infectie met SARS–CoV-2) | SWAB. Accessed December 16, 2021. https://swab.nl/nl/covid-19

47. Corticosteroids for COVID-19. Accessed December 16, 2021. https://www.who.int/publications/i/item/WHO-2019-nCoV-Corticosteroids-2020.1

48. Dessie ZG, Zewotir T. Mortality-related risk factors of COVID-19: a systematic review and meta-analysis of 42 studies and 423,117 patients. *BMC Infect Dis*. 2021;21(1):855. doi:10.1186/S12879-021-06536-3

49. Poly TN, Islam MM, Yang HC, et al. Obesity and Mortality Among Patients Diagnosed With COVID-19: A Systematic Review and Meta-Analysis. *Front Med*. 2021;8:620044. doi:10.3389/FMED.2021.620044

50. Loew D, Schuster O, Graul EH. Dose-dependent pharmacokinetics of dexamethasone. *Eur J Clin Pharmacol*. 1986;30(2):225-230. doi:10.1007/bf00614309

51. Tsuei SE, Moore RG, Ashley JJ, McBride WG. Disposition of synethetic glucocorticoids. I. Pharmacokinetics of dexamethasone in healthy adults. *J Pharmacokinet Biopharm*. 1979;7(3):249-264. doi:10.1007/BF01060016

52. Spoorenberg SMC, Deneer VHM, Grutters JC, et al. Pharmacokinetics of oral vs. intravenous dexamethasone in patients hospitalized with community-acquired pneumonia. *Br J Clin Pharmacol*. 2014;78(1):78-83. doi:10.1111/BCP.12295

53. van den Bosch CMA, Geerlings SE, Natsch S, Prins JM, Hulscher MEJL. Quality Indicators to Measure Appropriate Antibiotic Use in Hospitalized Adults. *Clin Infect Dis*. 2015;60(2):281-291. doi:10.1093/cid/ciu747

54. van der Eerden MM, Vlaspolder F, de Graaff CS, Groot T, Jansen HM, Boersma WG. Value of intensive diagnostic microbiological investigation in low- and high-risk patients with community-acquired pneumonia. *Eur J Clin Microbiol Infect Dis*. 2005;24(4):241-249. doi:10.1007/s10096-005-1316-8

55. Johansson N, Kalin M, Tiveljung-Lindell A, Giske CG, Hedlund J. Etiology of Community-Acquired Pneumonia: Increased Microbiological Yield with New Diagnostic Methods. *Clin Infect Dis*. 2010;50(2):202-209. doi: 10.1086/648678

56. Andreo F, Domínguez J, Ruiz J, et al. Impact of rapid urine antigen tests to determine the etiology of community-acquired pneumonia in adults. *Respir Med*. 2006;100(5):884-891. doi:10.1016/j.rmed.2005.08.020

CHAPTER 2

Adjunctive treatment with oral dexamethasone in non-ICU patients hospitalised with community-acquired pneumonia: A randomised clinical trial

E Wittermans, SMT Vestjens, SMC Spoorenberg, WL Blok, JC Grutters, R Janssen, GT Rijkers, FWJM Smeenk, GP Voorn, EMW van de Garde, WJW Bos, The Santeon-CAP study group

EUR RESPIR J. 2021;58(2):2002535.

ABSTRACT

Background

Adjunctive intravenous corticosteroid treatment has been shown to reduce length of stay (LOS) in adults hospitalised with community-acquired pneumonia (CAP). We aimed to assess the effect of oral dexamethasone on LOS and whether this effect is disease severity dependent.

Methods

In this multicentre, stratified randomised, double-blind, placebo-controlled trial, immunocompetent adults with CAP were randomly assigned (1:1 ratio) to receive oral dexamethasone (6 mg once daily) or placebo for 4 days in four teaching hospitals in the Netherlands. Randomisation (blocks of four) was stratified by CAP severity (pneumonia severity index class I–III and IV–V). The primary outcome was LOS.

Results

Between December 2012 and November 2018, 401 patients were randomised to receive dexamethasone (n=203) or placebo (n=198). Median LOS was shorter in the dexamethasone group (4.5 days, 95% CI 4.0–5.0 days) than in the placebo group (5.0 days, 95% CI 4.6–5.4 days; p=0.033). Within both CAP severity subgroups, differences in LOS between treatment groups were not statistically significant. The secondary ICU admission rate was lower in the dexamethasone arm (5 (3%) versus 14 (7%); p=0.030); 30-day mortality did not differ between groups. In the dexamethasone group the rate of hospital readmission tended to be higher (20 (10%) versus 9 (5%); p=0.051) and hyperglycaemia (14 (7%) versus 1 (1%); p=0.001) was more prevalent.

Conclusion

Oral dexamethasone reduced LOS and ICU admission rate in adults hospitalised with CAP. It remains unclear for which patients the risk–benefit ratio is optimal.

INTRODUCTION

Despite advances in antibiotic treatment and the availability of preventative measures such as vaccines, the burden of community-acquired pneumonia (CAP) remains high.[1] Therefore, nonantibiotic adjunctive therapies that modify the host response to microorganisms remain of interest.[2]

Excessive release of cytokines in response to invading pathogens is thought to contribute to high mortality and morbidity in patients with CAP.[3] Corticosteroids can inhibit inflammation by downregulating this cytokine response.[4] Through this mechanism, adjunctive treatment with corticosteroids might improve clinical outcomes.

Several randomised controlled trials (RCTs) show that adjunctive corticosteroid treatment reduces hospital length of stay (LOS).[5] However, most RCTs have studied intravenous corticosteroid treatment. Dexamethasone administered intravenously during the first 4 days of hospitalisation has been shown to reduce LOS by 1 day.[6] Oral administration of dexamethasone has several advantages over iv administration. It does not hamper an early iv-to-oral switch of antibiotics, causes patients less discomfort and carries no risk of phlebitis. Furthermore, a bioequivalence study showed that oral dexamethasone is feasible from a pharmacokinetics perspective.[7] Thus, we opted to investigate the effect of oral dexamethasone in patients with CAP.

Moreover, it is still debated which patients benefit most from corticosteroid treatment.[8] A recent individual patient data meta-analysis (IPDMA) suggested a greater effect of corticosteroids in patients with severe CAP, defined by a high pneumonia severity index (PSI) score.[5] So far, no RCT has prospectively investigated the effects of corticosteroids in pre-specified subgroups based on CAP severity.

The primary objective of this study was to investigate the effect of a short course of oral dexamethasone compared with placebo on LOS and to assess whether this effect depends on disease severity.

MATERIALS AND METHODS

Study design and patients

This multicentre, stratified randomised, double-blind, placebo-controlled trial was conducted in four non-academic teaching hospitals in the Netherlands. Patients presenting with CAP were screened and enrolled within 24 h of emergency department presentation. Inclusion criteria were age ≥18 years and the presence of new opacities on chest radiography, and two of the following signs and symptoms: cough, production of sputum, temperature >38.0°C or <36.0°C, abnormalities at auscultation consistent with pneumonia, C-reactive protein (CRP) >15 mg/L, white blood cell

count >10×10^9 or <4×10^9 cells/L, or >10% of bands in leukocyte differentiation. The following patients were excluded from study participation: patients with a congenital or acquired immunodeficiency, patients treated with chemotherapy <6 weeks prior to emergency department presentation, patients receiving corticosteroids or other immunosuppressive medication 6 weeks prior to emergency department presentation, patients requiring direct admission to the intensive care unit (ICU) at hospital presentation, patients with a known tropical worm infection, pregnant or breastfeeding females and patients with an intolerance for dexamethasone. Patients opting for palliative care, who did not receive active treatment for pneumonia, were also not eligible for study participation. All other patients with limitations in treatment (e.g. those who did not wish to be resuscitated, or did not want to be admitted to the ICU if necessary, or those who did not wish to be intubated) but who did seek active treatment for the pneumonia were eligible for study participation. Written informed consent was provided by all patients. This study was approved by the Medical Ethics Committee at St Antonius Hospital (Nieuwegein, The Netherlands) and is registered at ClinicalTrials.gov with identifier number NCT01743755.

Eligible patients were randomly allocated (1:1 ratio) to receive either 6 mg oral dexamethasone or placebo once a day for 4 days. A previous pharmacokinetics study showed that 6 mg dexamethasone orally equals the exposure of 5 mg dexamethasone phosphate (=4 mg dexamethasone) iv, as studied in the Ovidius trial.[6,7] Randomisation was performed in blocks of four using PASW Statistics software version 18.0.03 (IBM, Armonk, NY, USA). Patients were stratified by enrolling centre and by CAP severity (non-severe CAP and severe CAP). Non-severe CAP was defined as PSI class I–III and severe CAP was defined as PSI class IV–V.[9] Randomisation was set up to ensure that in each CAP severity subgroup, 50% of patients received dexamethasone and 50% of patients received placebo. After randomisation, patients were assigned a medication kit number using a central computer-assisted allocation system. Corresponding coded medication kits containing four tablets of 6 mg dexamethasone or placebo were available at the emergency department of each of the participating hospitals. Patients, treating physicians and investigators were masked to treatment allocation.

Methods

Patients in the dexamethasone group received 6 mg oral dexamethasone (Tiofarma, Oud-Beijerland, The Netherlands) once a day for 4 days and patients in the placebo group received one placebo tablet (Tiofarma) once a day for 4 days. Study treatment was initiated within 24 hours of emergency department presentation. Baseline blood samples for blood chemistry testing and haematology were obtained before initiation of study treatment in the emergency department as part of standard care. Measurements included CRP, electrolytes, glucose, renal function and a complete blood count. All patients received antibiotics prior to starting study medication. Decisions regarding antibiotic type, route of administration and treatment duration were made by the treating

physician, and were based on Dutch national guidelines.[10,11] Microbiological testing included sputum cultures, blood cultures, PCR assays for respiratory viruses and atypical pathogens, and urinary antigen tests for the detection of *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae*. The decision to transfer a patient to the ICU or to discharge a patient was made by the treating physician. The general rule for discharge in all hospitals was that patients were clinically stable (improvement of shortness of breath, consistent decrease in CRP concentrations, absence of hyperthermia or hypothermia, adequate oral intake and adequate gastrointestinal absorption) and in well enough condition to leave the hospital. Baseline characteristics included medical history and variables necessary to calculate the PSI score.[9]

Analysis

The primary outcome was LOS measured in 0.5 days. LOS was calculated from time of emergency department presentation to the day of discharge, day of death or day of ICU admission (study medication was stopped after ICU admission because patients are regularly treated with corticosteroids in the ICU). If the patient was admitted to the emergency department before 12:00, the day of presentation was counted as 1 day. If the patient was admitted to the emergency department after 12:00, the day of presentation was counted as 0.5 days. The discharge date was defined as the date that a patient was medically ready for discharge (hereby excluding waiting time for admission to a nursing home). Time of discharge was set at 12:00 for all patients as patients are generally discharged late morning or early afternoon depending on ward logistics. Secondary outcomes were admission to the ICU after initial admission to the general ward and all-cause mortality within 30 days of hospital admission.

Sample size estimation was based on our hypothesis that dexamethasone could reduce the median LOS in all patients with CAP by 1 day and reduce the median LOS in patients with severe CAP by 2 days. With sample data pseudo-randomly generated from available data from our previous trial[6] and assuming that 50% of patients have severe CAP, it was simulated that 300 patients were needed in each arm to provide >80% power maintaining a type I error rate of 0.05 (two-sided).

The primary analysis was a Kaplan-Meier analysis of time to discharge. The Kaplan-Meier method was used to estimate the median LOS with 95% confidence interval for each treatment group and to assess the difference in LOS between treatment groups by analysing time to discharge. Patients who died, who were transferred to a different hospital or who were admitted to the ICU after study enrolment were censored to show that time of reporting was cut off before the event of interest for the primary analysis (i.e. hospital discharge) occurred. Because the intervention was a short course of oral dexamethasone, a Gehan-Breslow-Wilcoxon test was used for the Kaplan–Meier method as this test emphasises early differences.[12] Furthermore, we performed an

extra sensitivity analysis in which patients who were admitted to the ICU after study enrolment were included in the time to discharge analysis.

To adhere to CONSORT (Consolidated Standards of Reporting Trials) guidelines on reporting results of randomised clinical trials we also calculated the unadjusted hazard ratio (HR) for discharge with 95% confidence interval using a Cox proportional hazards regression.[13] Differences in secondary outcomes between treatment groups were analysed with a Chi-squared test and risk ratios were calculated; a two-tailed p-value <0.05 was deemed significant. Statistical analyses were performed using SPSS version 24.0 (IBM). The primary analysis was performed according to the intention-to-treat principle after which the analysis was repeated in the per-protocol population. Patients who missed one or more doses of study medication while admitted to the general ward, whose diagnosis was altered, with exclusion criteria unknown at the time of study entry, or who were discharged on the day of study entry were excluded from the per-protocol analysis. The following predefined subgroup analyses were performed: 1) CAP severity (non-severe CAP versus severe CAP), 2) initial CRP level at emergency department presentation (above median versus below median) and 3) *S. pneumoniae* urinary antigen test result.

We added a sensitivity analysis to explore the effect of dexamethasone on hospital utilisation. The difference in hospital utilisation between treatment groups was assessed using a 30-day hospital-free approach (equivalent to the mechanical ventilator-free days approach). Hospital-free days (HFDs) were calculated by adding the number of days a patient was hospitalised during readmission (if a readmission occurred within 30 days of initial hospital admission) to the duration of initial hospital stay (including ICU admission) and subtracting this number from 30 days. If a patient died in hospital within 30 days of admission, the number of HFDs was 0. If a patient was not discharged within 30 days of admission, the number of HFDs was also 0. Because the effect of dexamethasone is primarily through shortened LOS rather than mortality, a Mann-Whitney U test was used to compare HFDs between groups.[14]

Categorical variables are shown as number (percentage). Continuous variables are presented as median (interquartile range (IQR)) or mean with standard deviation for variables with a nonparametric or parametric distribution, respectively.

Interim analyses to monitor the frequency of serious side-effects related to either dexamethasone or placebo were pre-planned at 200, 400 and 500 patients. The analyses and the review of the results were performed by an external independent Data Safety and Monitoring Board.

RESULTS

From 23 December 2012 to 28 November 2018, 1092 patients were screened for eligibility. For one hospital, screening logs were not available. 412 patients were randomly allocated to receive either dexamethasone or placebo; 11 patients were excluded post-randomisation (Figure 1). The study was prematurely terminated after the second interim analysis due to a slower inclusion rate than anticipated combined with a shorter LOS than used in our sample size calculation. Therefore, we did not expect a different outcome for LOS at 600 patients. Furthermore, for 30-day mortality we anticipated a 50% lower mortality rate in patients with severe CAP in the dexamethasone group compared with the placebo group (7.5% versus 15% based on results of an earlier trial).[6] Because there was no difference in 30-day mortality between treatment groups at 400 patients and the 30-day mortality was already lower than anticipated, we also did not expect a different outcome for 30-day mortality at 600 patients. The independent Data Safety and Monitoring Board found no grounds for early termination based on safety concerns.

There was no difference in baseline characteristics between the intervention and placebo groups (Table 1). The mean (±SD) PSI score calculated for all patients was 81±29. The severe CAP subgroup consisted of 156 (39%) patients. There was no difference in the distribution of causative organisms and initial antibiotic treatment between treatment groups (Supplementary Tables E1 and E2).

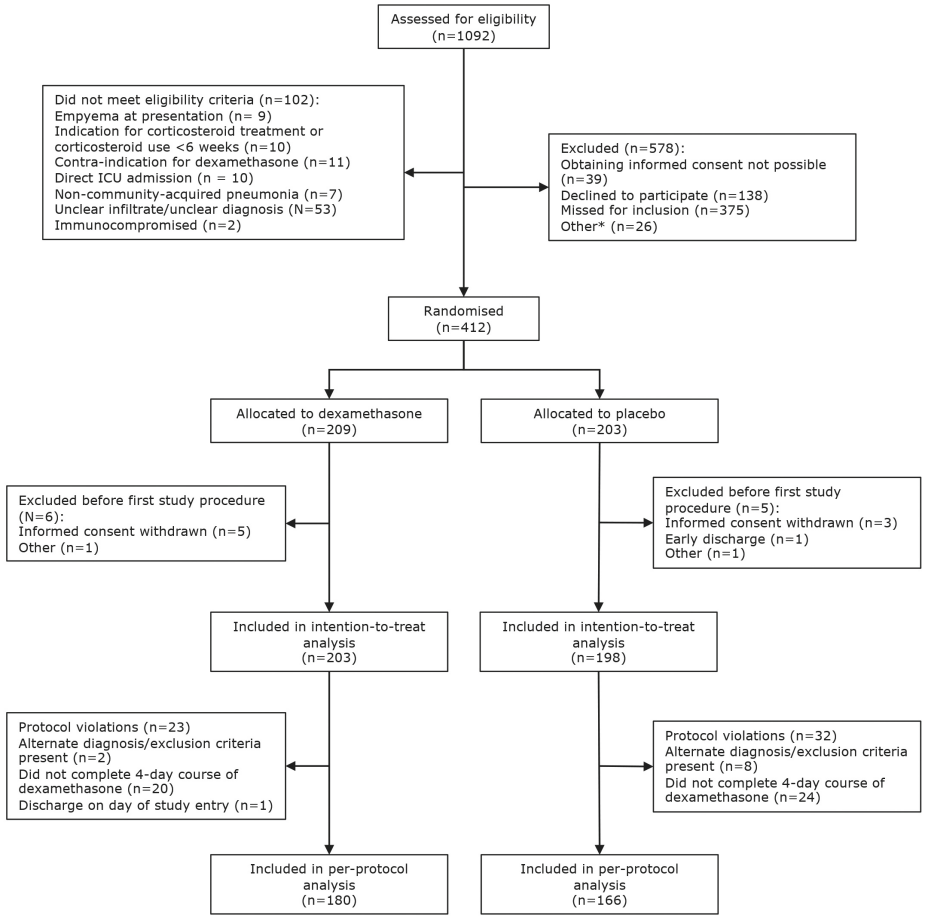


Figure 1 Study profile. No patient was lost to follow-up before reaching the primary endpoint. ICU: intensive care unit. *: e.g. transferred to another hospital or patient opting for palliative care.

Table 1 Baseline characteristics of enrolled patients

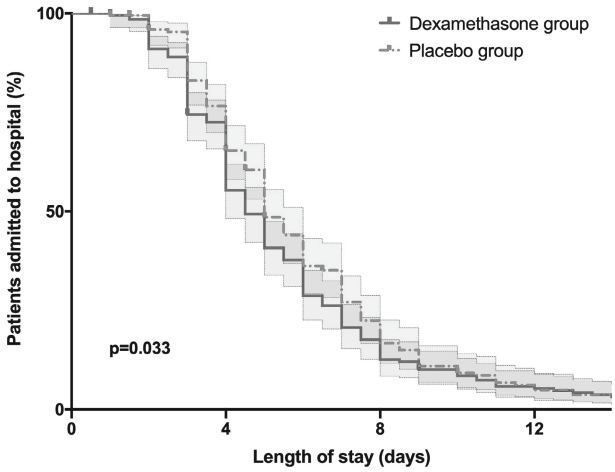
	All patients		PSI I-III		PSI IV-V	
	Placebo (n=198)	**Dexamethasone (n =203)**	**Placebo (n=119)**	**Dexamethasone (n = 126)**	**Placebo (n=79)**	**Dexamethasone (n =77)**
Men	120 (61)	116 (57)	58 (49)	63 (50)	62 (79)	53 (69)
Age (years)	67 [54-76]	68 [57-76]	61 [44-69]	61 [50-70]	77 [68-83]	76 [69-83]
Ethnicity						
Caucasian	186 (94)	197 (97)	111 (93)	122 (97)	75 (95)	75 (97)
Other	11 (6)	6 (3)	7 (6)	4 (3)	4 (5)	2 (3)
Elderly home resident	1 (1)	6 (3)	0 (0)	2 (2)	1 (1)	4 (5)
Current smoker	45 (23)	53 (26)	26 (22)	39 (31)	19 (24)	14 (18)
Antibiotic treatment prior to admission	57 (29)	56 (28)	40 (34)	35 (28)	17 (22)	21 (27)
Comorbidities						
Neoplastic disease	6 (3)	8 (4)	1 (1)	0 (0)	5 (6)	8 (10)
Liver disease	2 (1)	2 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Congestive heart failure	17 (9)	20 (10)	4 (3)	4 (3)	13 (17)	16 (21)
Renal disease	27 (14)	32 (16)	6 (5)	7 (6)	21 (27)	25 (33)
Diabetes Mellitus	47 (24)	41 (20)	14 (12)	22 (18)	33 (42)	19 (25)
COPD	35 (18)	40 (20)	20 (17)	22 (18)	15 (19)	18 (23)
Physical examination findings						
Temperature (°C)	38.3 (1.2)	38.4 (1.1)	38.3 (1.1)	38.4 (0.9)	38.3 (1.3)	38.4 (1.3)
SBP (mmHg)	128 (22)	130 (22)	127 (20)	131 (18)	121 [112-147]	130 [104-148]
Heart rate (bpm)	98 [87-110]	99 [87-111]	98 [90-110]	100 [90-111]	98 (20)	98 (23)
Resp rate (breaths/min)	20 [18-25]	20 [16-25]	21 (5)	20 (5)	23 (7)	23 (7)
Blood oxygen saturation	93.6 (4.1)	93.7 (4.2)	94.6 (3.7)	94.1 (4.4)	92.2 (4.2)	93.0 (3.6)
Altered mental status	14 (7)	13 (6)	0 (0)	1 (1)	14 (18)	12 (16)

Table 1 Continued

	All patients		PSI I-III		PSI IV-V	
	Placebo (n=198)	**Dexamethasone (n =203)**	**Placebo (n=119)**	**Dexamethasone (n = 126)**	**Placebo (n=79)**	**Dexamethasone (n =77)**
Inflammatory parameters						
CRP (mg/L)	198 [82-309]	211 [86-330]	190 [84-291]	249 [131-336]	203 [61-323]	153 [41-314]
WBC (10^9 cells/L)	13.0 [9.7-17.5]	13.7 [10.1-18.2]	13.0 [9.6-17.6]	14.0 [10.3-19.0]	13.0 [9.7-17.1]	13.1 [9.4-17.5]
PSI score	82 (29)	81 (29)	69 [52-76]	65 [52-76]	106 [97-115]	106 [97-120]
PSI risk class						
Class 1	25 (13)	27 (13)	25 (21)	27 (21)	-	-
Class 2	40 (20)	55 (27)	40 (34)	55 (44)	-	-
Class 3	54 (27)	44 (22)	54 (45)	44 (35)	-	-
Class 4	70 (35)	64 (31)	-	-	70 (90)	64 (82)
Class 5	9 (5)	13 (6)	-	-	9 (11)	13 (17)

Data are presented as n (%), median (interquartile range) or mean (±SD). PSI: pneumonia severity index; COPD: chronic obstructive pulmonary disease; SBP: systolic blood pressure; Resp rate: respiraoty rate; CRP: C-reactive protein; WBC: white blood cell.

In the intention-to-treat population, Kaplan-Meier analysis showed that median LOS was 0.5 days shorter in the dexamethasone group (4.5 (95% CI 4.0–5.0) days) than in the placebo group (5.0 (95% CI 4.6– 5.4) days) (Table 2). Kaplan-Meier analysis of time to discharge showed a significant difference between treatment groups (p=0.033) (Figure 2). Although non statistically significant, in the non-severe CAP subgroup LOS was 1.0 day shorter in the dexamethasone group compared with the placebo group (Table 2 and Figure 3). There was no difference in LOS between treatment groups in the severe CAP subgroup (Table 2 and Figure 3). Results were similar in the per-protocol population (Supplementary Table E3). In the Kaplan-Meier analysis in which ICU patients were not censored, median LOS was 5.0 (95% CI 4.5– 5.5) days in the dexamethasone group and 5.5 (95% CI 5.0–6.0) days in the placebo group (p=0.012) (Supplementary Figure E1). Using Cox regression, HR for discharge was 1.14 (95% CI 0.93–1.39) for all patients, 1.19 (95% CI 0.92–1.54) in the mild pneumonia group and 1.06 (95% CI 0.76–1.48) in the severe pneumonia group.



Number at risk				
Dexamethasone group	203	144	35	11
Placebo group	198	143	39	10

Figure 2 Kaplan–Meier analysis of the effect of dexamethasone on hospital length of stay in all enrolled patients. Patients who were admitted to the intensive care unit and/or died in hospital (n=21) and patients who were transferred to another hospital (n=2) were censored on the day of admission to the intensive care unit, day of death or day of transfer. The shading represents the confidence bands.

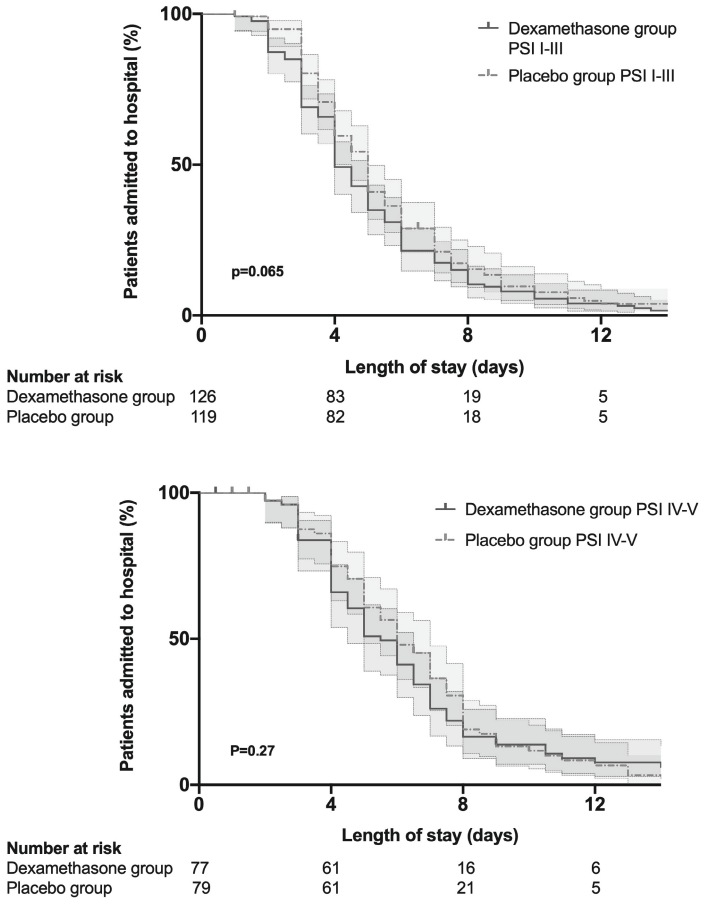


Figure 3 Kaplan–Meier analysis of the effect of dexamethasone on hospital length of stay stratified according to community-acquired pneumonia severity. Patients who died, were admitted to the intensive care unit or were transferred to a different hospital were censored on the day of death, day of admission to the intensive care unit or day of transfer. The shading represents the confidence bands.

For secondary outcomes, the secondary ICU admission rate was lower in the dexamethasone group (n=5 (3%)) than in the placebo group (n=14 (7%); p=0.030). Respiratory failure was the most common reason for ICU admission (Supplementary Table E5). The 30-day mortality rate did not differ between both treatment groups (Table 2). Causes of death are shown in Supplementary Table E6. The aforementioned results for the intention-to-treat population were similar in the per-protocol population (Supplementary Table E3). Results of predefined subgroup analyses are presented in Supplementary Table E4.

Table 2 Overview of primary and secondary end-points for the intention-to-treat population

	Dexamethasone[#] N = 203	Placebo[¶] N = 198	Risk ratio (95% CI)	p-value
Hospital LOS days				
All patients	4.5 (4.0–5.0)	5.0 (4.6–5.4)	-	0.033[+]
PSI class I-III	4.0 (3.6–4.4)	5.0 (4.5–5.5)	-	0.065[+]
PSI class IV-V	5.5 (4.6–6.4)	6.0 (5.1–6.9)	-	0.27[+]
Secondary ICU admission				
All patients	5 (3)	14 (7)	0.35 (0.13–0.95)	0.030[§]
PSI class I-III	0 (0)	6 (5)	-	0.011[§]
PSI class IV-V	5 (7)	8 (10)	0.64 (0.22–1.87)	0.41[§]
30-day mortality				
All patients	4 (2)	7 (4)	0.56 (0.17–1.87)	0.34[§]
PSI class I-III	1 (1)	2 (2)	0.47 (0.04–5.14)	0.53[§]
PSI class IV-V	3 (4)	5 (6)	0.62 (0.15–2.49)	0.49[§]

Data are presented as n, median (95% CI) or n (%), unless otherwise stated. LOS: length of stay; PSI: pneumonia severity index; ICU: intensive care unit. [#]: PSI class I–III n=126, PSI class IV–V n=77; [¶]: PSI class I–III n=119, PSI class IV–V n=79; [+]: Gehan–Breslow–Wilcoxon test; [§]: Chi-squared test.

Adverse events are shown in Table 3. The readmission rate within 30 days of study entry was higher in the dexamethasone group compared with the placebo group (20 (10%) versus 9 (5%); p=0.051). Reasons for readmission are shown in Supplementary Table E7. The median (IQR) number of HFDs was 25.0 (22.0– 26.0) in the dexamethasone group and 24.5 (22.5–26.5; p=0.061) in the placebo group. Hyperglycaemia was reported by physicians in 14 (7%) patients in the dexamethasone group and one (1%) patient in the placebo group (p=0.001). In the placebo group, one patient had a newly diagnosed myxoma and one patient was diagnosed with HIV. Both were transferred to an academic hospital. In the dexamethasone group, one patient had a perforated jejunal diverticulitis requiring surgical intervention. Abdominal complaints were present before study entry. Furthermore, in the dexamethasone group three patients had an ischaemic cerebrovascular accident and one patient developed deep venous thrombosis of the right leg.

Table 3 Overview of adverse events

	Dexamethasone N = 203	Placebo N = 198	Risk ratio (95% CI)	p-value[+]
Adverse event				
Readmission[#]	20 (10)	9 (5)	2.09 (0.98–4.47)	0.051
Empyema	3 (2)	5 (3)	0.59 (0.14–2.42)	0.45
Hyperglycaemia	14 (7)	1 (1)	13.7 (1.81–103)	0.001
Neuropsychiatric complaints (e.g. delirium, agitation)	10 (5)	7 (4)	1.39 (0.54–3.59)	0.49
Cardiac events (e.g. arrhythmia, congestive heart failure, myocardial infarction)	9 (4)[¶]	4 (2)	2.19 (0.69–7.01)	0.17

Data are presented as n or n (%), unless otherwise stated. [#]: n=201 patients analysed in the dexamethasone group and n=189 patients analysed in the placebo group (excluding missing (n=2) and patients who died in hospital (n=9)); [¶]: one patient suffered myocardial infarction and was admitted to the cardiac ward, and one patient was admitted to the cardiac ward after discharge due to ongoing angina pectoris and fatigue; [+]: Chi-squared test.

DISCUSSION

In the primary analysis of this trial, we observed a reduction in median LOS of 0.5 days in patients with CAP treated with oral dexamethasone compared with controls.

This finding supports our hypothesis that dexamethasone reduces LOS in patients with CAP. However, a 0.5-day reduction is lower than the hypothesised 1-day reduction. It is also lower than reported by Briel et al.[5] who also found a 1-day reduction of LOS in their IPDMA of six trials. The median LOS in our study was shorter compared with all trials included in the IPDMA by Briel et al.[5], which may explain the difference in absolute reduction in LOS. Still, the relative reduction in LOS was 10% in our trial compared with 12.5% found by Briel et al.[5] Thus, the relative effect of dexamethasone on LOS in our study was similar. The difference in overall LOS could be explained by the fact that most studies in the IPDMA used iv study medication; this may have hampered early iv-to-oral antibiotic switch and consequently an earlier discharge. Furthermore, there were fewer patients with severe CAP in our trial compared with the two trials in the IPDMA with similar inclusion criteria (39% versus 47% and 49%).[6,15]

The Cox regression analysis did not show a statistically significant difference in time to discharge between treatment groups. This analysis was included to adhere to CONSORT guidelines on reporting clinical trial results. However, the Cox regression requires the assumption of proportional hazards. Because we investigated a short course of dexamethasone and most patients were discharged during the first 5 days of hospital admission, the assumption of proportional hazards is not met.

This is the first study to show a reduction in the rate of secondary ICU admissions in patients with CAP receiving corticosteroids. However, as respiratory failure was the main reason for ICU admission (n=14 (74%)), this finding is in line with the meta-analysis by Stern et al.[16] who showed a lower risk of new respiratory failure in patients receiving corticosteroids. In line with the IPDMA by Briel et al.[5], we did not observe a beneficial effect of corticosteroids on 30-day mortality. Stern et al.[16] did show a beneficial effect of corticosteroids on mortality. However, in that meta-analysis, small studies with an unclear allocation concealment were mainly responsible for that finding.[17–19]

Contrary to our hypothesis, we did not observe a beneficial effect of dexamethasone in patients with severe CAP. The beneficial effects of dexamethasone seemed greater in the non-severe CAP subgroup. In the latter group, no patients receiving dexamethasone were admitted to the ICU and the median LOS was 1.0 day shorter in patients receiving dexamethasone compared with those receiving placebo (although not statistically significant). It is difficult to draw conclusions due to the relatively small number of patients in each subgroup. However, it is still interesting to explore this counterintuitive finding. It could be related to the fact that we used the PSI score to define severe CAP. The PSI score is a good predictor of mortality, yet the PSI score does not necessarily correspond with the level of inflammation. The PSI score is mainly influenced by age and the presence of comorbidities. We therefore performed an additional explorative analysis using the CURB-65 (confusion, urea >7 mmol/L, respiratory rate ≥30 breaths/min, blood pressure <90 mmHg (systolic) or ≤60 mmHg (diastolic), age ≥65 years) score. The CURB-65 score is based on clinical parameters; it does not include comorbidities and is less influenced by age than the PSI score. Indeed, we found the largest LOS reduction in patients aged <65 years with high CURB-65 scores (≥2 points) (Supplementary Figure E2). Furthermore, in our predefined subgroup analysis dexamethasone reduced LOS and the rate of secondary ICU admission in patients with a CRP above the median. We did not find this effect in patients with a CRP below the median. Two post hoc analyses of RCTs investigating corticosteroids in CAP have also noted that patients with a high level of inflammation benefitted most from corticosteroids. Remmelts et al.[20] previously observed that dexamethasone was most effective in patients with a high level of pro-inflammatory cytokines combined with discrepantly low cortisol levels. Urwyler et al.[21] found that only a high level of pro-inflammatory cytokines predicted a positive response to steroids. Consequently, a prediction score based solely on the level of inflammation is of interest as it might aid in identifying the subgroup of patients that would benefit most from dexamethasone.

Regarding safety, the rate of patients readmitted within 30 days of admission was twice as high in the dexamethasone group compared with the placebo group (5% versus 10%; number needed to harm n=20). However, this difference did not reach statistical significance. The rate of hyperglycaemia was higher in the dexamethasone group, which is in line with the pharmacology of corticosteroids and with an earlier trial.[6]

Our study has several strengths. First, it is the second largest multicentre trial assessing the effects of corticosteroids in patients with CAP and it is the first trial to use stratified randomisation to assess the effects of corticosteroids within subgroups based on CAP severity. Second, a short course of oral dexamethasone has several advantages over longer courses of iv administered corticosteroids.

There were several limitations to this study. First, the results cannot be generalised to all patients with CAP. Patients admitted directly to an ICU (i.e. the most critically ill patients) were excluded. Second, the trial was prematurely terminated due to slower inclusion rates than anticipated. The results of the interim review of the study's data at 400 patients showed a shorter LOS compared with our sample size calculation and therefore we do not expect a different outcome for LOS at 600 patients. Furthermore, because 30-day mortality was lower than anticipated and because there was no difference in 30-day mortality between treatment groups at 400 patients, we would not expect different findings if the planned 600 patients would have been included. Last, the number of patients reported to have hyperglycaemia is substantially lower than described by Briel et al.[5] We cannot exclude the possibility of underreporting as the presence of hyperglycaemia was based on voluntarily reporting by research physicians instead of a structured assessment. Glucose was measured on day 4, a time when many patients were already discharged. In hindsight, this might limit an all-inclusive benefit–risk assessment. However, the relative risk was similar to other studies.

The benefits of dexamethasone should be weighed against the risks. A 10% reduction in LOS and reduction in ICU admissions seems to be a considerable benefit for patients. However, this should be weighed against a possible rise in readmissions. The sensitivity analysis using HFDs showed a small (statistically nonsignificant) difference between treatment groups in favour of dexamethasone. It seems that corticosteroid treatment does not benefit all patients with CAP. Therefore, it is important to identify subgroups of patients who benefit most and/or suffer least from corticosteroid treatment. High levels of inflammatory biomarkers such as cytokines, procalcitonin, pro-adrenomedullin and a high neutrophil/lymphocyte ratio have been associated with unfavourable outcomes in CAP.[21–23] In other studies, only measurement of inflammation based on cytokine levels has been shown to predict response to corticosteroids. In the present study we found that dexamethasone had a greater effect in patients with a high CRP. Future research is necessary to determine how CRP and other inflammatory biomarkers can predict response to corticosteroids, preferably using readily available biochemical tests that provide fast results.

FUNDING

This work was supported by St Antonius Hospital, St Antonius Research Fund via an earmarked donation from Verwelius Construction Corporation.

MEMBERS OF THE SANTEON-CAP STUDY GROUP

Willem Jan W. Bos (St Antonius Hospital, Nieuwegein, The Netherlands), Ewoudt M.W. van de Garde (St Antonius Hospital, Nieuwegein, The Netherlands and University of Utrecht, Utrecht, The Netherlands), Jan C. Grutters (St Antonius Hospital Nieuwegein, The Netherlands and University Medical Center Utrecht, Utrecht, The Netherlands), Ger T. Rijkers (Roosevelt Academy, Middelburg, The Netherlands), Douwe H. Biesma (St Antonius Hospital, Nieuwegein, The Netherlands), G. Paul Voorn (St Antonius Hospital, Nieuwegein, The Netherlands), Simone M.C. Spoorenberg (University Medical Center Utrecht, Utrecht, The Netherlands), Stefan M.T. Vestjens (St Antonius Hospital, Nieuwegein, The Netherlands), Esther Wittermans (St Antonius Hospital, Nieuwegein, The Netherlands), Frank W.J.M. Smeenk (Catharina Hospital, Eindhoven, The Netherlands), Arnoud F. Aldenkamp (Catharina Hospital, Eindhoven, The Netherlands), Rob Janssen (Canisius Wilhelmina Hospital, Nijmegen, The Netherlands), Charlotte A. van Ruitenbeek (Canisius Wilhelmina Hospital, Nijmegen, The Netherlands), Willem L. Blok (OLVG, Amsterdam, The Netherlands), Paul Bresser (OLVG, Amsterdam, The Netherlands), Joris W.T. van Enschot (Maxima Medical Center, Veldhoven, The Netherlands) and Hester A.A. Zegers (Hospital Bernhoven, Uden, The Netherlands).

REFERENCES

1. Welte T, Torres A, Nathwani D. Clinical and economic burden of community-acquired pneumonia among adults in Europe. *Thorax*. 2012;67(1):71-79. doi:10.1136/thx.2009.129502
2. Sibila O, Rodrigo-Troyano A, Torres A. Nonantibiotic Adjunctive Therapies for Community-Acquired Pneumonia (Corticosteroids and Beyond): Where Are We with Them? *Semin Respir Crit Care Med*. 2016;37(06):913-922. doi:10.1055/s-0036-1593538
3. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. *Arch Intern Med*. 2007;167(15):1655-1663. doi:10.1001/archinte.167.15.1655
4. Remmelts HHF, Meijvis SCA, Biesma DH, et al. Dexamethasone downregulates the systemic cytokine response in patients with community-acquired pneumonia. *Clin Vaccine Immunol*. 2012;19(9):1532-1538. doi:10.1128/CVI.00423-12
5. Briel M, Spoorenberg SMC, Snijders D, et al. Corticosteroids in Patients Hospitalized With Community-Acquired Pneumonia: Systematic Review and Individual Patient Data Metaanalysis. *Clinical Infectious Diseases*. 2017;66(3):346-354. doi:10.1093/cid/cix801
6. Meijvis SC, Hardeman H, Remmelts HH, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. *The Lancet*. 2011;377(9782):2023-2030. doi:10.1016/S0140-6736(11)60607-7
7. Spoorenberg SMC, Deneer VHM, Grutters JC, et al. Pharmacokinetics of oral vs. intravenous dexamethasone in patients hospitalized with community-acquired pneumonia. *Br J Clin Pharmacol*. 2014;78(1):78-83. doi:10.1111/BCP.12295
8. Chalmers JD. Corticosteroids for community-acquired pneumonia: a critical view of the evidence. *European Respiratory Journal*. 2016;48(4):984 - 986. doi:10.1183/13993003.01329-2016
9. Fine MJ, Singer DE, Hanusa BH, Lave JR, Kapoor WN. Validation of a pneumonia prognostic index using the MedisGroups comparative hospital database. *Am J Med*. 1993;94(2):153-159. doi:10.1016/0002-9343(93)90177-Q
10. Wiersinga W, Bonten M, Boersma W, et al. SWAB/NVALT (Dutch Working Party on Antibiotic Policy and Dutch Association of Chest Physicians) guidelines on the management of community-acquired pneumonia in adults. *Neth J Med*. 2012;70(2):90-101.
11. Wiersinga W, Bonten MJM, Boersma W, et al. Management of community-acquired pneumonia in adults: 2016 guideline update from the Dutch Working Party on Antibiotic Policy (SWAB) and Dutch Association of Chest Physicians (NVALT). *Neth J Med*. 2018;76(1):4-13.
12. Li J, Ma S. *Survival Analysis in Medicine and Genetics*. 1st ed. (Li J, Ma S, eds.). Taylor & Francis group; 2013.
13. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ*. 2010;340:c332. doi:10.1136/bmj.c332
14. Yehya N, Harhay MO, Curley MAQ, Schoenfeld DA, Reeder RW. Reappraisal of Ventilator-Free Days in Critical Care Research. *Am J Respir Crit Care Med*. 2019;200(7):828-836. doi:10.1164/rccm.201810-2050CP
15. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: A multicentre, double-blind, randomised, placebo-controlled trial. *The Lancet*. 2015;385(9977):1511-1518. doi:10.1016/S0140-6736(14)62447-8
16. Stern A, Skalsky K, Avni T, Carrara E, Leibovici L, Paul M. Corticosteroids for pneumonia. *Cochrane Database of Systematic Reviews*. 2017;(12). doi:10.1002/14651858.CD007720.pub3
17. El-Ghamrawy A, Shokeir M, Essmat A. Effects of low-dose hydrocortisone in ICU patients with severe community-acquired pneumonia. *Egypt J Chest Dis Tuberc*. 2006;55:91-99.
18. Nafae R, Ragab M, Amany F, Rashed S. Adjuvant role of corticosteroids in the treatment of community-acquired pneumonia. *Egypt J Chest Dis Tuberc* . 2013;62:439-445.

19. Sabry N, Omar E. Corticosteroids and ICU Course of Community Acquired Pneumonia in Egyptian Settings. *Pharmacol Pharm*. 2011;2(2):73-81. doi:10.4236/pp.2011.22009.

20. Remmelts HHF, Meijvis SCA, Heijligenberg R, et al. Biomarkers define the clinical response to dexamethasone in community-acquired pneumonia. *Journal of Infection*. 2012;65(1):25-31. doi:10.1016/j.jinf.2012.03.008

21. Urwyler SA, Blum CA, Coslovsky M, Mueller B, Schuetz P, Christ-Crain M. Cytokines and Cortisol – predictors of treatment response to corticosteroids in community-acquired pneumonia? *J Intern Med*. 2019; 286(1):75-87 doi:10.1111/joim.12891

22. Viasus D, del Rio-Pertuz G, Simonetti AF, et al. Biomarkers for predicting short-term mortality in community-acquired pneumonia: A systematic review and meta-analysis. *Journal of Infection*. 2016;72:273-282. doi:http://dx.doi.org/10.1016/j.jinf.2016.01.002

23. Curbelo J, Luquero Bueno S, Galván-Román JM, et al. Inflammation biomarkers in blood as mortality predictors in community-acquired pneumonia admitted patients: Importance of comparison with neutrophil count percentage or neutrophil-lymphocyte ratio. *PLoS One*. 2017;12(3):e0173947-e0173947. doi:10.1371/journal.pone.0173947

SUPPLEMENTARY MATERIAL

Supplementary Table E1 Etiological diagnosis for all enrolled patients

	Placebo Group (n=198)	Dexamethasone group (n=203)
Streptococcus pneumoniae	35 (18)[1]	40 (20)[2]
Legionella spp.	15 (8)[3]	12 (6)[4]
Haemophilus influenzae	8 (4)[5]	7 (3)[6]
Mycoplasma pneumoniae	6 (3)	6 (3)
Chlamydia psittaci	4 (2)	2 (1)
Staphylococcus aureus	4 (2)[7]	1 (0)[8]
Influenza A/B virus	9 (5)[9]	8 (4)
Other pathogen*	3 (2)[10]	5 (2)[11]
Other viruses‡	5 (3)	6 (3)
Unidentified	109 (55)	116 (57)

*Other pathogens: *Coxiella burnetti*, *Pneumocystis jiroveci*, *Escheria coli*, group A streptococci, *Haemophilus haemolyticus*, *chlamydia pneumoniae*, *Neisseria meningitidis*
‡Other virusses: Parainfluenza virus, Rhinovirus, Respiratory synctiel virus, human metapneumovirus (hMPV).
[1]Mixed infection with: influenza A virus (n=1), *Moraxella catarrhalis* (n= 1), hMPV (n=1), Rhinovirus (n=2), *H. influenzae* (n = 1), *H. influenza* and Rhinovirus *(n=1).*
[2]Mixed infection with: *S. aureus* (n=1), Influenza type A (n=2), *H. influenza* (n=1), *E. coli* (n=1)
[3]Mixed infection with: hMPV (n=1), Influenza type B (n=1)
[4]Mixed infection with: *S. pneumoniae* (n=1)
[5]Mixed infection with: *S. aureus* (n=2), Influenza type A (n=1)
[6]Mixed infection with: *Klebsiella pneumoniae* and *E. coli* (n=1), Influenza type A virus (n=2)
[7]Mixed infection with: *Pseudomonas aeruginosa* and Rhinovirus (n=1)
[8]Mixed infection with: Rhinovirus (n=1)
[9]Mixed infection with: *Candida albicans* (n=1)
[10]Mixed infection with: Rhinovirus (n=1), *M .pneumoniae* (n=1)
[11]Mixed infection with: Rhinovirus (n=1)

Supplementary Table E2 Initial antibiotic regimen at time of hospital admission

	Dexamethasone group (n= 203)	Placebo group (n= 198)
Penicillin monotherapy*	81 (40)	80 (40)
Cephalosporin monotherapy	31 (15)	28 (14)
Fluoroquinolone, macrolide or doxycycline monotherapy	5 (3)	10 (5)
Penicillin combined with a fluoroquinolone, macrolide or doxycycline	38 (19)	37 (19)
Cephalosporin combined with a fluoroquinolone, macrolide or doxycycline	36 (18)	32 (16)
Other	10 (5)	10 (5)
Unknown	2 (1)	1 (1)

Data are number (%). *Penicillin, amoxicillin or amoxicillin/clavulanic acid.

Supplementary Table E3 Overview of primary and secondary endpoints for the per-protocol population.

Endpoint	**Dexamethasone (n=180)**	**Placebo (n=166)**	**risk ratio (95% CI)**	**p-value**
Length of stay (days)				
All patients	4.5 (4.2 to 4.8)	5.0 (4.6 to 5.4)		0.021*
PSI class I-III	4.0 (3.6 to 4.4)	5.0 (4.5 to 5.5)		0.054*
PSI class IV-V	5.5 (4.4 to 6.6)	6.5 (5.5 to 7.5)		0.16*
Secondary ICU admission				
All patients	4 (2)	12 (7)	RR 0.31 (0.10 to 0.93)	0.027‡
PSI class I-III	0 (0)	6 (6)	-	0.009‡
PSI class IV-V	4 (6)	6 (9)	RR 0.65 (0.19 to 2.18)	0.48‡
30-day mortality				
All patients	3 (2)	7(4)	RR 0.40 (0.10 to 1.50)	0.16‡
PSI class I-III	0(0)	2 (2)	-	0.13‡
PSI class IV-V	3 (5)	5 (8)	RR 0.58 (0.14 to 2.33)	0.44‡

Data are median (95% CI) or number (%). ICU = Intensive care unit. PSI = Pneumonia Severity Index. RR = Risk ratio. *Gehan-Breslow-Wilcoxon test. ‡Chi-squared test. Numbers analysed: PSI I-III placebo (n= 102) and dexamethasone (n=114). PSI IV-V: placebo (n= 64) and dexamethasone (n=66).

Supplementary Table E4 Overview primary and secondary endpoints for subgroup analyses

Endpoint	Dexamethasone	Placebo	risk ratio (95% CI)	p-value
Length of stay (days)				
Initial CRP at admission				
CRP < 210 mg/l	4.5 (4.0 to 5.0)	5.0 (4.6 to 5.4)		0.28*
CRP ≥ 210 mg/l	5.0 (4.4 to 5.6)	5.5 (4.9 to 6.1)		0.046*
Pneumococcal urinary antigen test result				
Positive	5.0 (3.9 to 6.1)	6.0 (5.2 to 6.8)		0.45*
Negative	4.5 (4.1 to 4.9)	5.0 (4.5 to 5.5)		0.034*
Secondary ICU admission				
Initial CRP at admission				
CRP < 210 mg/l	3 (3)	6 (6)	RR 0.54 (0.14 to 2.11)	0.37‡
CRP ≥ 210 mg/l	2 (2)	8 (9)	RR 0.22 (0.05 to 1.01)	0.031‡
Pneumococcal urinary antigen test result				
Positive	0 (0)	0 (0)	-	-
Negative	4 (3)	11 (7)	RR 0.38 (0.12 to 1.17)	0.078‡
30-day mortality				
Initial CRP at admission				
CRP < 210 mg/l	3 (3)	5 (5)	RR 0.65 (0.16 to 2.65)	0.54‡
CRP ≥ 210 mg/l	1 (1)	2 (2)	RR 0.44 (0.04 to 4.77)	0.49‡
Pneumococcal urinary antigen test result				
Positive	0 (0)	1 (4)	-	0.26‡
Negative	4 (3)	5 (3)	RR 0.84 (0.23 to 3.06)	0.79‡

Data are median (95% CI) or number (%). ICU = Intensive care unit. RR = Risk ratio. CRP = C-reactive protein. Numbers analysed (dexamethasone/placebo): CRP < 210 mg/l (96/104), CRP ≥ 210 mg/l (107/94), Positive pneumococcal urinary antigen test result (32/26), negative pneumococcal urinary antigen test result (154/161). *Grehan-Breslow-Wilcoxon test. ‡Chi-squared test.

Supplementary Table E5 Reasons for ICU admission.

Patients	Age	PSI class	Reason for ICU admission
Placebo			
1	42	3	Respiratory failure
2	82	4	Respiratory failure
3	75	3	Respiratory failure
4	81	4	Respiratory failure
5	67	3	Observation after VATS[1] for empyema
6	85	3	Respiratory failure
7	69	2	Observation after VATS for empyema
8	66	3	Respiratory failure
9	59	4	Respiratory failure

Supplementary Table E5 Continued

Patients	Age	PSI class	Reason for ICU admission
10	58	4	Respiratory failure
11	85	4	Sepsis; Hypotension
12	65	4	Respiratory failure
13	56	4	Respiratory failure
14	80	5	Sepsis; Hypotension
Dexamethasone			
1	76	4	Respiratory failure
2	52	4	Respiratory failure
3	85	5	Arrhythmia with hypotension
4	85	4	Respiratory failure
5	80	4	Respiratory failure and pulmonary hemorrhage

[1]Video assisted thoracic surgery

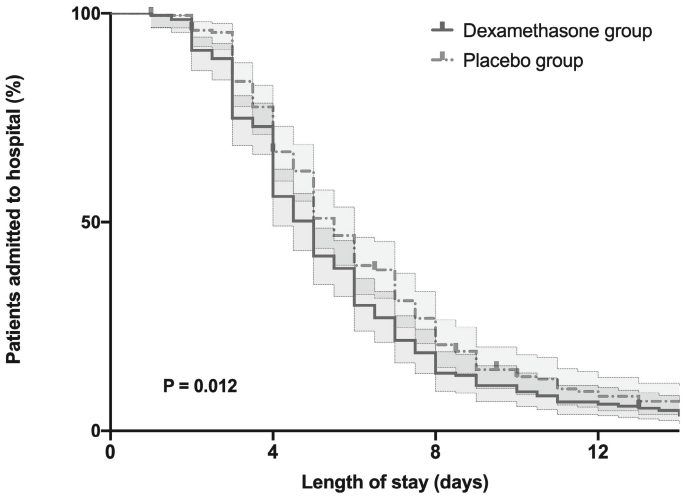
Supplementary Table E6 Cause of death

Patients	Age	PSI risk class	Cause of death
Placebo			
1	82	4	Respiratory failure; Severe legionella pneumonia
2	75	3	Respiratory failure; post-obstruction pneumonia newly diagnosed lung tumor
3	67	3	Died after VATS[1] for empyema
4*	58	4	Sepsis; Respiratory failure
5	85	4	Sepsis
6	77	4	Respiratory failure due to influenza pneumonia and congestive heart failure
7	84	4	Respiratory failure after opting for palliative care
8	81	4	Died 3 days after discharge; unknown cause of death
Dexamethasone			
1*	76	4	Died after ICU discharge due to multiple complications
2	80	4	Respiratory failure; Pulmonary hemorrhage
3	79	3	Strangulated femoral hernia after readmission
4	82	4	Respiratory failure; pulmonary infection and congestive heart failure
5	94	5	Died 10 days after discharge; unknown cause of death

*Died in hospital after 30 days of hospital admission. [1]Video assisted thoracic surgery.

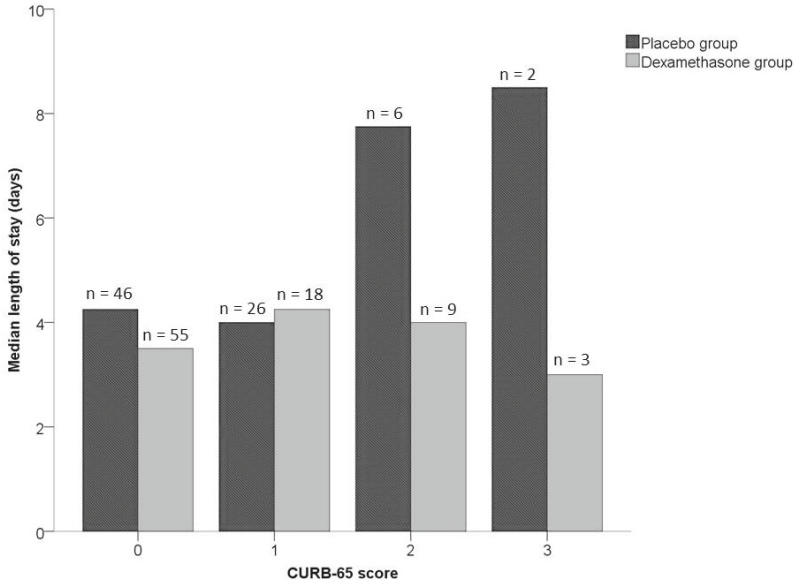
Supplementary Table E7 Reasons for readmission < 30 days of admission

Patients	Age	PSI risk class	Reason for readmission
Placebo			
1	52	3	Antrum gastritis
2	70	3	Mediastinitis
3	44	1	Hospital-acquired pneumonia; urticarial reaction to amoxicillin/clavulanic acid
4	90	4	Urosepsis
5	54	3	Relapse of pulmonary infection
6	71	4	Psychiatric complaints
7	40	1	Bronchiolitis
8	67	3	Relapse of pulmonary infection
9	71	5	Relapse of pulmonary infection
Dexamethasone			
1	79	3	Strangulated femoral hernia
2	82	4	Relapse of pulmonary infection and congestive heart failure
3	69	5	Congestive heart failure
4	74	3	Relapse of pulmonary infection
5	56	2	Altered mental status
6	84	4	Hospital-acquired pneumonia
7	61	2	Angina Pectoris
8	46	1	Relapse of pulmonary infection
9	76	4	Relapse of pulmonary infection
10	61	2	Elective cardioversion for atrial fibrillation
11	85	5	Fever of unknown origin
12	54	2	Urine retention
13	56	2	Relapse of pulmonary infection
14	61	3	Chest pain caused by pleurisy
15	61	5	Ischemic cerebrovascular accident
16	84	4	Fatigue
17	27	1	Relapse of pulmonary infection
18	71	4	Dehydration and altered mental status
19	64	4	Relapse of pulmonary infection
20	85	4	Acute decompensated heart failure



Number at risk				
Dexamethasone group	203	148	38	14
Placebo group	198	152	51	16

Supplementary Figure E1 Kaplan-Meier analysis of the effect of dexamethasone on length of hospital stay in all enrolled patients including ICU patients



Supplementary Figure E2 Length of stay according to CURB-65 score and treatment group in patients under 65 years of age

CHAPTER 3

Neutrophil count, lymphocyte count and neutrophil-to-lymphocyte ratio in relation to response to adjunctive dexamethasone treatment in community-acquired pneumonia

E Wittermans, EMW van de Garde, GP Voorn, AF Aldenkamp, R Janssen, JC Grutters, WJW Bos, The Santeon-CAP study group

EUR J INTERN MED. 2022;96:102-108

ABSTRACT

Background

It is hypothesised that community-acquired pneumonia (CAP) patients with more severe disease or inflammation might benefit more from adjunctive corticosteroid treatment. Neutrophil count, lymphocyte count and neutrophil-lymphocyte ratio (NLR) have been associated with inflammation and disease severity in CAP. We investigated the interaction between these parameters and adjunctive dexamethasone effects on clinical outcomes in CAP.

Methods

We conducted a post hoc analysis of the randomised placebo-controlled Santeon-CAP trial (n = 401), which showed a positive effect of adjunctive oral dexamethasone on length of stay (LOS) in CAP patients. White blood cell (WBC) count, neutrophil count, NLR (highest tertile vs. lowest two tertiles) and lymphocyte count (lowest tertile vs. highest two tertiles) were examined as potential effect modifiers of treatment with dexamethasone on LOS (primary outcome) and ICU admission, 30-day mortality and hospital readmission.

Results

WBC differential counts were available for 354 patients. The effect of dexamethasone on LOS was more pronounced in high WBC count, high neutrophil count or high NLR subgroups (difference in median LOS of 2 days versus zero days in the reference subgroups, p for interaction < 0.05). There was no effect modification for the other outcomes. Patients with low WBC and low neutrophil counts did not benefit from dexamethasone, while hospital readmission rate was higher in those treated with dexamethasone (6% vs. 11%).

Conclusions

WBC count and/or neutrophil count might be easily available biomarkers to guide selection of CAP patients who are more likely to benefit from adjunctive dexamethasone treatment. Future prospective trials are needed to confirm this predictive potential.

INTRODUCTION

The cornerstones of community-acquired pneumonia (CAP) treatment are early diagnosis and timely initiation of appropriate antibiotic treatment.[1] Despite advances in antibiotic treatment, CAP remains a leading cause of morbidity and mortality worldwide.[2] Adjunctive therapies, such as corticosteroids, might improve clinical outcomes.

In CAP, invading pathogens trigger a host immune response essential for controlling and eliminating pathogens in the lung. However, dysregulation of the initial inflammatory response can lead to tissue damage and excessive systemic inflammation resulting in severe disease and ultimately unfavourable clinical outcomes.[3]

Adjunctive treatment with corticosteroids, potent inhibitors of the host immune response, has shown to improve outcomes for CAP patients by reducing length of hospital stay (LOS) and time to clinical stability.[4] In addition, we recently showed that adjunctive corticosteroids reduced ICU admission rate.[5] However, because CAP is a heterogeneous disease, it is unlikely that all patients benefit equally from adjunctive corticosteroid treatment.[6] Furthermore, a higher risk of hospital readmission in patients treated with adjunctive corticosteroids remains a concern.[4,5] Therefore, identifying a subset of patients who are more likely to benefit from corticosteroid treatment is necessary.

It is hypothesised that patients with an excessive inflammatory response and thus more severe disease would benefit most from adjunctive corticosteroid treatment. However, stratification of CAP patients by parameters indicative of more inflammation or more severe disease, such as C-reactive protein levels, pneumonia severity index (PSI) score, inflammatory status based on cytokine levels, initial ICU admission or the presence of systemic inflammatory response criteria, have not yielded a clear-cut definition of a CAP subgroup benefiting from corticosteroid treatment.[4,5,7,8]

White blood cells (WBCs) populations play a key role in the local and systemic inflammatory response in CAP.[3] Neutrophilia is a widely used and recognised infection marker in CAP and more recently, lymphocytopenia has been associated with more severe disease and higher levels of systemic inflammatory cytokines in CAP.[9] In acute respiratory distress syndrome, lymphocyte depletion correlated with severity of lung injury.[10] A combination of both, the neutrophil-lymphocyte count ratio (NLR), has been recognized as an indicator of systemic inflammation and predictor of clinical outcomes in sepsis, cardiovascular and oncological disease.[11–13] In CAP, NLR has also shown to be associated with more severe disease and has been identified as a predictor of mortality.[14,15] So far, no studies have investigated the interaction between WBC differential cell counts and adjunctive corticosteroid treatment on clinical outcomes in patients with CAP.

We performed a post hoc analysis of a randomised trial investigating adjunctive oral dexamethasone in non-ICU patients with CAP to assess if neutrophil count, lymphocyte count and/or NLR modified the response to adjunctive oral dexamethasone treatment in adults hospitalised with CAP.

METHODS

Population and study design

We performed a post hoc analysis of the multicentre Santeon-CAP study (n = 401; NCT01743755).[5] In short, the Santeon-CAP study investigated the effect of adjunctive treatment with 6 mg oral dexamethasone for four days vs. placebo on the primary outcome LOS in non-ICU hospitalised CAP patients. Randomisation was stratified by disease severity defined by PSI risk class (PSI risk class I-III vs. PSI risk class IV-V).[16] In the Santeon-CAP study, dexamethasone reduced LOS by 0.5 days and decreased the risk of secondary ICU admission. CAP was defined as a new opacity on chest x-ray combined with at least two of the following signs and symptoms: cough, sputum production, body temperature > 38.0 °C or < 36.0 °C, findings at chest auscultation consistent with pneumonia, C-reactive protein concentration (CRP) > 15 mg/l, and/or white blood cell count > 10×10^9 cells per litre or < 4×10^9 cells per litre. Immunocompromised patients, patients for whom corticosteroid treatment was indicated or patients who used corticosteroids prior to admission were excluded. Further information on inclusion criteria and study procedures is reported elsewhere.[5] For this post hoc analysis, we included those patients for whom a full WBC differential was available at emergency department presentation.

Data collection

We retrospectively searched the medical records of all patients enrolled in the Santeon-CAP study for the availability of a WBC differential (not part of the original study protocol) at time of presentation to the emergency department. We collected WBC counts, neutrophil counts and lymphocyte counts. NLR was calculated by dividing neutrophil count by lymphocyte count. Baseline patient characteristics, baseline laboratory test results and clinical outcomes were available as part of the original study protocol.

Definition of subgroups and outcomes

Patients were stratified based on WBC count, neutrophil count, lymphocyte count and NLR values. For each parameter, patients were divided in a “high” group and a “low" group. Because there are no earlier studies assessing the relationship between WBC count, neutrophil count, lymphocyte count, and NLR and the effect of adjunctive corticosteroid treatment on clinical outcomes in CAP, there was no clear guidance for choosing cut-off values for stratification into subgroups. Based on the hypothesis that patients with more extreme values would benefit most from corticosteroid treatment, we stratified patients into high or low groups according to tertiles. Thereby selecting

a reference group with more extreme values while minimising the risk of too small numbers in subgroups, as might be the case when using quartiles. Based on the hypothesis that patients with the highest WBC count, neutrophil count, and NLR would have more severe disease and thus would benefit most from dexamethasone, the high subgroup for these parameters was defined as a count or ratio equal to or higher than the third tertile value. The low subgroup was defined as a count or ratio below the third tertile value. For lymphocyte count, we hypothesised that patients with the lowest lymphocyte count would have more severe disease. Therefore, the low lymphocyte subgroup was defined as a lymphocyte count below the first tertile value and the high lymphocyte count subgroup was defined as a lymphocyte count equal to or higher than the first tertile value.

The primary outcome was LOS. LOS was measured in days and was calculated from day of hospital admission to day of hospital discharge or day of in-hospital death. Rules for discharge were that patients needed to be clinically stable (improvement of shortness of breath, absence of hyperthermia or hypothermia, consistent decrease of C-reactive protein concentrations and adequate oral intake and gastrointestinal absorption) and be in a condition to leave the hospital. Secondary outcomes were ICU admission after initial admission to the general ward, all-cause 30-day mortality, and hospital readmission within 30 days of initial hospital admission.

Statistical analysis

Statistical analysis was performed using IBM SPSS 26.0. After stratifying patients into subgroups, differences in baseline characteristics between the high and low subgroups of each parameter were analysed using the Chi-squared test for categorical variables, and a Student's T-test or Mann-Whitney U-test for continuous variables. Multivariate binary logistic regression analysis was performed to further analyse the association between baseline characteristics and WBC count parameter subgroups. The multivariate model was adjusted for baseline characteristics with a statistically significant difference between high and low subgroups upon univariate analysis. Next, time to discharge was plotted in a Kaplan-Meier curve for the placebo and dexamethasone group in each WBC differential subgroup. Finally a Poisson regression model, including treatment allocation, WBC differential parameter subgroup and their interaction as covariates, was used to test for interaction between randomly assigned treatment with dexamethasone and WBC differential parameters on LOS. For secondary categorical outcomes, a binary logistic regression analysis was used. Because LOS is cut short for patients who died in hospital, these patients might incorrectly count as having a shorter LOS. Therefore, a sensitivity analysis was performed for LOS excluding patients who died in hospital.

Unless noted otherwise, data are presented as mean (SD, standard deviation) or median [IQR, interquartile range] for continuous variables, and as count (%) for categorical variables.

RESULTS

Population characteristics

A full blood count differentiation at time of hospital admission was available for 354 out of 401 Santeon-CAP study participants. Patient characteristics are shown in Table 1. There were no differences in baseline characteristics between the placebo group (n = 169) and the dexamethasone group (n = 185). Clinical outcomes (Table 1) showed a trend towards similar results as observed for the total Santeon-CAP study population with a statistically significant difference in LOS and a trend towards a reduction in secondary ICU admissions.

Subgroups based on differential blood count values

For WBC count, neutrophil count, and NLR the high subgroups were defined as a count or ratio ≥ 15.6 10^9 cells/l, ≥ 13.2 10^9 cells/l, and ≥ 15.5, respectively. For lymphocyte count, the cut-off value for the low subgroup was ≤ 0.71 10^9 cells/l. Patient characteristics at baseline for each subgroup are shown in Table 2. Multivariate analysis showed that COPD (OR 1.91 (95% CI (1.07–3.39)), heart rate (OR 1.02 (95% CI 1.01–1.03)), and no antibiotic treatment prior to admission (OR 1.99 (95% CI 1.12–3.53)) were associated with a neutrophil count ≥ 13.2 10^9 cells/l. Similar results were found for WBC count, where COPD (OR 2.15 (95%CI 1.20–3.85)), heart rate (OR 1.02 (95% CI 1.00–1.03)), no antibiotic treatment prior to admission (OR 1.83 (95% CI 1.03–3.24)) and female gender (OR 1.73 (95% CI 1.07–2.80)) were associated a WBC count ≥ 15.6 10^9 cells/l. The high NLR subgroup had a higher mean PSI score and more signs of systemic inflammation compared to the low NLR subgroup (Table 2). On multivariate analysis a NLR ≥ 15.5 was associated with higher body temperature at presentation (OR 1.40 (95%CI 1.11–1.76)), infection with *S. pneumoniae* (OR 2.17 (95%CI 1.19–3.98)), COPD (OR 1.86 95%CI (1.02–3.40)) and no antibiotic treatment prior to admission (OR 2.10 (95% CI 1.12 - 3.75)). The low lymphocyte subgroup also had a higher mean PSI score than the high lymphocyte subgroup. On multivariate analysis a low lymphocyte count ≤ 0.71 10^9 cells/l was only associated with higher body temperature at presentation (OR 1.42 (95% CI 1.14–1.77)).

Except for a lower ICU admission rate in the low lymphocyte count subgroup compared to the high lymphocyte subgroup (10 (9%) vs. 6 (3%); p = 0.010), there was no statistically significant differences in clinical outcomes between WBC differential parameter subgroups for the whole study population (Table 2). Selecting only patients who received placebo, thus excluding any effect of dexamethasone on clinical outcomes, we found that NLR ≥ 15.5 was associated with a significantly longer median LOS compared to NLR < 15.5 (5.0 [4.0–7.0] vs 6.0 [4.0–8.0]; p = 0.023). Similar to the analysis in the whole cohort, ICU admission rate was higher in the low lymphocyte count subgroup compared to the high lymphocyte count subgroup (7 (13%) vs 4 (4%); p = 0.026).

Table 1 Baseline characteristics and clinical outcomes for the whole study population.

	All patients N = 354	**Placebo N = 169**	**Dexamethasone N = 185**	**P***
Baseline characteristics				
Male	209 (59)	101 (60)	108 (58)	0.79
Age (years)	64.7 (15.9)	63.7 (16)	65.6 (15)	0.25
PSI score	80.8 (28.1)	80.5 (28.5)	81.1 (27.8)	0.86
CURB65 score	1 [0-2]	1 [0-2]	1 [0-2]	0.91
Antibiotic treatment prior to hospital admission	101 (29)	51 (30)	50 (27)	0.49
Altered mental status	20 (6)	10 (6)	10 (5)	0.84
Current smoker	87 (25)	39 (24)	48 (27)	0.44
COPD	67 (19)	31 (18)	36 (20)	0.79
Diabetes	74 (21)	37 (22)	37 (20)	0.66
Congestive heart failure	31 (9)	12 (7)	19 (10)	0.29
Liver disease	4 (1)	2 (1)	2 (1)	0.93
Neoplastic disease	14 (4)	6 (4)	8 (4)	0.71
Renal disease	51 (14)	21 (12)	30 (16)	0.31
Heart rate (bpm)	99.5 (20.2)	98.0 (18.8)	100.0 (21.4)	0.60
Body temperature (°C)	38.3 (1.1)	38.3 (1.2)	38.4 (1.1)	0.37
Respiratory rate (breaths/min)	21.7 (6.0)	21.9 (6.0)	21.4 (6.0)	0.49
Oxygen saturation (%)	93.7 (4.1)	93.7 (4.1)	93.6 (4.1)	0.83
C-reactive protein (mg/L)	210 [84-319]	201 [80-309]	215 [91-330]	0.22
Leukocyte count(10^9 cells/l)	13.0 [9.7-17.8]	12.5 [9.4-17.4]	13.7 [10.2-18.2]	0.21
Neutrophil count (10^9 cells/l)	10.7 [7.8-15.1]	10.4 [7.5-14.9]	11.0 [8.0-15.3]	0.30
Lymphocyte count (10^9 cells/l)	0.95 [0.63-1.4]	0.99 [0.63-1.4]	0.94 [0.36-1.3]	0.68
Legionella spp.	24 (7)	13 (8)	11 (6)	0.51
Influenza virus A/B	23 (7)	11 (7)	12 (7)	0.99
Streptococcus pneumoniae	64 (18)	28 (17)	36 (20)	0.48
Clinical outcomes				
LOS (days)	5.0 [4.0-7.0]	5.0 [4.0-8.0]	5.0 [3.0-7.0]	0.029
ICU admission	16 (5)	11 (7)	5 (3)	0.085
30-day mortality	11 (3)	7 (4)	4 (2)	0.28
Readmission <30 days	28 (8)	9 (6)	19 (10)	0.10

Data are presented as mean (SD), median [IQR], or n (%). P-value for Students-T test, Whitney-Mann U or Chi-squared test as appropriate. *P for difference between placebo and dexamethasone group.

Effect modification by subgroup

Although scatterplots show a large spread in WBC count differential parameter values, we observed more placebo patients compared to dexamethasone patients in the upper right quadrant (LOS longer than 3rd quartile and high count/ratio) for WBC count (n = 13 vs n = 10), Neutrophil count (n = 13 vs n = 7) and NLR (n = 20 vs n = 9), and in the lower right quadrant (LOS longer than 3rd quartile and lowest count) for lymphocyte count (n = 21 vs n = 11) (Supplementary Figure 1). Kaplan-Meier curves of time to discharge per subgroup showed shorter time to discharge for patients receiving dexamethasone compared to placebo in the high WBC count, neutrophil count and NLR subgroup and the low lymphocyte subgroup. This was not seen in the other subgroups (Figure 1).

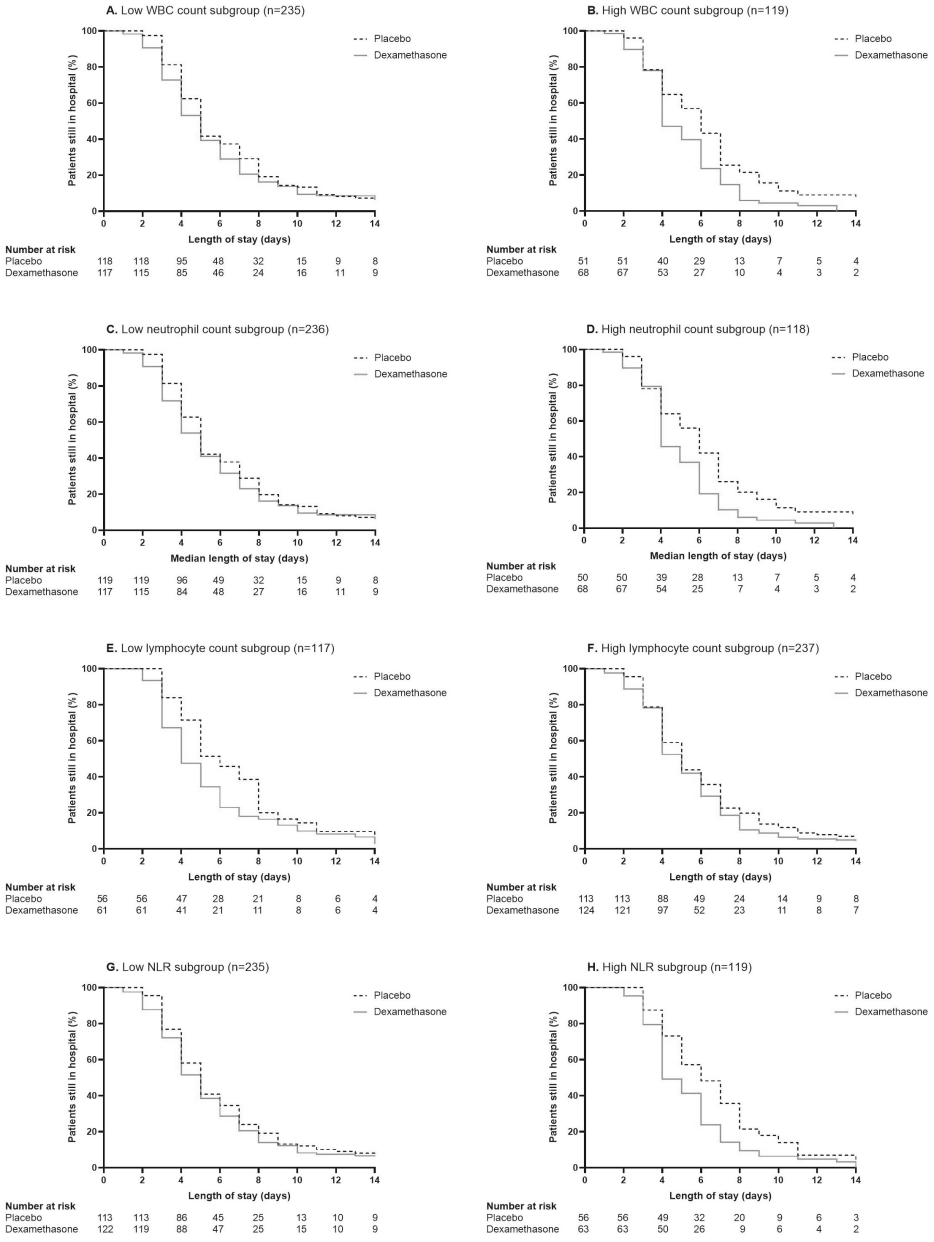


Figure 1 Kaplan Meier curves for time to discharge comparing dexamethasone group and placebo group within each white blood count differential subgroup. 1A Low WBC count subgroup. 1B High WBC count subgroup. 1C Low neutrophil count subgroup. 1D High neutrophil count subgroup. 1E Low lymphocyte count subgroup. 1F High lymphocyte count subgroup. 1G Low neutrophil-lymphocyte ratio subgroup. 1H High neutrophil-lymphocyte ratio subgroup.

Table 2 Comparison of baseline characteristics and clinical outcomes between high and low subgroups for each WBC differential parameter

	White blood cell count			Neutrophil count			Lymphocyte count			Neutrophil/lymphocyte ratio		
	Low (n=235)	High (n=119)	P	Low (n=236)	High (n=118)	P	Low (n=117)	High (n=237)	P	Low (n=235)	High (n=119)	P
Baseline characteristics												
Male	150 (64)	60 (50)	.010	147 (62)	62 (53)	.079	79 (68)	130 (55)	.023	134 (57)	75 (63)	.28
Age (years)	64 (16)	66 (15)	.17	64 (16)	67 (15)	.13	68 (15)	63 (16)	.002	63 (16)	68 (14)	.014
PSI score	81 (28)	81 (27)	.90	80 (28)	82 (27)	.56	89 (26)	77 (28)	<.001	76 (27)	90 (27)	<.001
CURB65 score	1.0 [0-2]	2.0 [1-2]	.12	1.0 [0-2]	2.0 [1-2]	.039	2.0 [1-2]	1.0 [0-2]	<.001	1.0 [0-2]	2.0 [1-2]	<.001
Antibiotics prior to admission	80 (34)	21 (18)	.001	81 (35)	20 (17)	.001	25 (21)	76 (32)	.034	82 (35)	19 (16)	<.001
Altered mental status	13 (6)	7 (6)	.89	12 (5)	8 (7)	.52	8 (7)	12 (5)	.50	8 (3)	12 (10)	.010
Current smoker	48 (21)	39 (34)	.010	50 (22)	37 (33)	.033	26 (23)	61 (27)	.44	52 (23)	35 (30)	.13
COPD	34 (15)	33 (28)	.003	34 (14)	33 (28)	.002	19 (16)	48 (20)	.36	36 (15)	31 (26)	.015
Diabetes	48 (20)	26 (22)	.76	47 (20)	27 (23)	.52	29 (25)	45 (19)	.21	49 (21)	25 (21)	.97
CHD	23 (10)	8 (7)	.34	25 (11)	7 (6)	.084	15 (13)	16 (7)	.057	22 (9)	9 (8)	.57
Liver disease	4 (2)	0 (0)	.15	4 (2)	0 (0)	.16	2 (2)	2 (1)	.47	2 (1)	2 (2)	.49
Neoplastic disease	9 (4)	5 (4)	.87	9 (4)	5 (4)	.85	7 (6)	7 (3)	.17	7 (3)	7 (6)	.19
Renal disease	36 (15)	15 (13)	.49	35 (15)	16 (14)	.75	21 (18)	30 (13)	.18	35 (15)	16 (13)	.71
Heart rate (bpm)	97 (18)	104 (23)	.002	97 (18)	104 (23)	.001	100 (18)	99 (21)	.94	98 (20)	102 (21)	.08
Temperature (°C)	38.4 (1.2)	38.3 (1.1)	.78	38.3 (1.2)	38.4 (1.1)	.47	38.6 (1.1)	38.2 (1.2)	.001	38.2 (1.1)	38.6 (1.1)	.001
Respiratory rate (Breaths/min)	22 (6)	21 (6)	.61	22 (6)	22 (6)	.92	22 (6)	21 (6)	.14	21 (6)	23 (6)	.010
O_2 saturation (%)	94 (4)	94 (4)	.33	94 (4)	94 (4)	.47	93 (4)	94 (4)	.016	94 (4)	93 (4)	.023
CRP (mg/L)	206 [82-313]	234 [88-329]	.26	198 [82-311]	238 [95-335]	.13	210 [63-331]	210 [96-310]	.98	193 [88-300]	253 [81-345]	.051

Table 2 Continued

	White blood cell count			Neutrophil count			Lymphocyte count			Neutrophil/lymphocyte ratio		
	Low (n=235)	High (n=119)	P	Low (n=236)	High (n=118)	P	Low (n=117)	High (n=237)	P	Low (n=235)	High (n=119)	P
Legionella spp.	18 (8)	6 (5)	.36	18 (8)	6 (5)	.37	9 (8)	15 (6)	.63	13 (6)	11 (9)	.19
Influenza virus	19 (8)	4 (3)	.088	18 (8)	5 (4)	.22	11 (9)	12 (5)	.12	17 (7)	6 (5)	.43
S. pneumoniae	34 (15)	30 (25)	.013	36 (15)	28 (24)	.051	29 (25)	35 (15)	.021	32 (14)	32 (27)	.002
Clinical outcomes												
LOS (d)	5.0 [4.0-7.0]	5.0 [4.0-7.0]	.90	5.0 [4.0-7.8]	5.0 [4.0-7.0]	0.59	5.0 [3.5-8.0]	5.0 [4.0-7.0]	0.61	5.0 [3.0-7.0]	5.0 [4.0-7.0]	0.11
ICU admission	12 (5)	4 (3)	.46	12 (5)	4 (3)	0.47	10 (9)	6 (3)	0.010	9 (4)	7 (6)	0.38
30-day mortality	8 (3)	3 (3)	.65	8 (3)	3 (3)	0.67	5 (4)	6 (3)	0.37	8 (3)	3 (3)	0.65
Readmission <30d	20 (9)	8 (7)	.53	20 (9)	8 (7)	0.56	12 (11)	16 (7)	0.24	16 (7)	12 (10)	0.30

Data are presented as mean (SD), median [IQR], or n (%). P-value for Students-T test, Whitney-Mann U or Chi-squared test as appropriate. COPD: chronic obstructive pulmonary disease; CHD: congestive heart failure CRP: C-reactive protein; d: days.

There was a statistically significant interaction between treatment allocation and WBC count, neutrophil count and NLR subgroups on LOS (Table 3). In the high subgroups of these parameters, median LOS was 2 days shorter in patients who received dexamethasone compared to those who received a placebo. In the low subgroups of these parameters, there was no difference in LOS between the placebo and dexamethasone group. The interaction term between lymphocyte count subgroups and treatment allocation was not statistically significant. Nine (2.5%) patients died in hospital. In the sensitivity analysis excluding these patients, results were similar to those of the primary analysis (Table 3).

Table 3 Differences in response to dexamethasone on median length of stay by WBC differential parameter subgroups for the whole cohort and for patients who did not die in hospital.

	Low		**High**		
	Placebo	Dexamethasone	Placebo	Dexamethasone	P*
White blood cell count					
All patients	5.0 [4.0-8.0]	5.0 [3.0-7.0]	6.0 [4.0-8.0]	4.0 [4.0-6.0]	0.002
Patients who did not die in hospital	5.0 [4.0-8.0]	5.0 [3.0-7.0]	6.0 [4.0-7.0]	4.0 [4.0-6.0]	0.035
Neutrophil count					
All patients	5.0 [4.0-8.0]	5.0 [3.0-7.0]	6.0 [4.0-8.0]	4.0 [4.0-6.0]	0.001
Patients who did not die in hospital	5.0 [4.0-8.0]	5.0 [3.0-7.0]	6.0 [4.0-7.0]	4.0 [4.0-6.0]	0.018
Lymphocyte count					
All patients	5.5 [4.0-8.0]	4.0 [3.0-6.0]	5.0 [4.0-7.0]	5.0 [4.0-7.0]	0.52
Patients who did not die in hospital	5.0 [4.0-8.0]	4.0 [3.0-6.0]	5.0 [4.0-7.0]	5.0 [4.0-7.0]	0.15
NLR					
All patients	5.0 [4.0-7.0]	5.0 [3.0-7.0]	6.0 [4.0-8.0]	4.0 [4.0-6.0]	0.007
Patients who did not die in hospital	5.0 [3.3-7.0]	5.0 [3.0-7.0]	6.0 [4.0-8.0]	4.0 [4.0-6.0]	0.031

*p-value for interaction between randomly assigned treatment allocation and low/high subgroup membership.

Because the high neutrophil count, high WBC count and high NLR subgroups were all associated with a history of COPD and no antibiotic treatment prior to hospital admission, we also examined whether antibiotic treatment status prior to admission or COPD status were driving factors behind the observed response to dexamethasone in these subgroups. We therefore tested for effect modification of dexamethasone by COPD status and antibiotic treatment prior to admission on LOS. There was no interaction between treatment allocation and COPD status or between treatment allocation and antibiotic treatment prior to hospital admission on LOS. Results of this analysis are shown in Supplementary Tables 1 and 2. There was also no interaction

between presence of pneumococcal pneumonia and treatment allocation on LOS (p for interaction 0.16).

In the high WBC and neutrophil count subgroups, no patients in the dexamethasone group were admitted to the ICU. Therefore, logistic regression analysis to test the statistical significance of the interaction between WBC and neutrophil count subgroups and treatment allocation for ICU admission was not possible. There was no further statistically significant interaction between WBC differential parameter subgroups and treatment allocation on secondary outcomes (Table 4). Frequency of adverse events per subgroup are shown in Supplementary Table 3.

Table 4 Differences in response to dexamethasone on secondary outcomes by WBC differential parameter subgroups.

	Low		**High**		
	Placebo	Dexamethasone	Placebo	Dexamethasone	P*
White blood cell count					
ICU admission	7 (6)	5 (4)	4 (8)	0 (0)	-
30-day mortality	5 (4)	3 (3)	2 (4)	1 (2)	0.74
Readmission <30 days	7 (6)	13 (11)	2 (4)	6 (9)	0.87
Neutrophil count					
ICU admission	7 (6)	5 (4)	4 (8)	0 (0)	-
30-day mortality	5 (4)	3 (3)	2 (4)	1 (2)	0.72
Readmission <30 days	7 (6)	13 (11)	2 (4)	6 (9)	0.89
Lymphocyte count					
ICU admission	7 (13)	3 (5)	4 (4)	2 (2)	0.85
30-day mortality	4 (7)	1 (2)	3 (3)	3 (2)	0.31
Readmission <30 days	4 (8)	8 (13)	5 (5)	11 (9)	0.93
Neutrophil-lymphocyte ratio					
ICU admission	6 (5)	3 (3)	5 (9)	2 (3)	0.79
30-day mortality	5 (4)	3 (3)	2 (4)	1 (2)	0.88
Readmission <30 days	6 (6)	10 (8)	3 (6)	9 (14)	0.49

*p-value for interaction between randomly assigned treatment allocation and low/high subgroup membership.

DISCUSSION

In this secondary analysis of the Santeon-CAP cohort, we observed that the effect of adjunctive oral dexamethasone treatment on LOS was modified by WBC count ≥ 15.6 10^9 cells/l, neutrophil count ≥ 13.2 10^9 cells/l and NLR ≥ 15.5. In these subgroups dexamethasone reduced LOS by two days compared to no reduction in the reference groups. We did not observe differences in treatment response between subgroups for secondary outcomes.

In line with our hypothesis and similar to other reports, we observed that both patients with a high NLR and a low lymphocyte count had more severe disease.[9,14,15] Furthermore, secondary ICU admission rate was three times higher in patients with a lymphocyte count < 0.71 10^9 cells/l compared to those with a lymphocyte count ≥ 0.71 10^9 cells/l (9% vs 3%, p = 0.010). These findings are similar to Mendez et al.[9] who defined a subgroup of patients with lymphocytopenic CAP (lymphocyte count < 0.724 10^9 cells/l) with more severe disease. Compared to the high NLR or low lymphocyte count subgroup, the high WBC count and high neutrophil count subgroup constituted of a different type of patient. A neutrophil count ≥ 13.2 10^9 cells/l and WBC count ≥ 15.6 10^9 cells/l were both associated with a history of COPD. Yet, regarding PSI score, clinical signs and clinical outcomes, there was no difference between the high and low neutrophil count/WBC count subgroups.

Contrary to our hypothesis and despite the fact that patients with low lymphocyte count showed more severe disease, we did not find a statistically significant interaction between lymphocyte count and adjunctive treatment with dexamethasone for the clinical outcomes studied. Because we did find an interaction between neutrophil count and dexamethasone treatment but not between lymphocyte count and dexamethasone treatment, the effect modification by NLR subgroup is more likely to be driven by the high neutrophil count than by low lymphocyte count.

To our knowledge, this is the first study investigating the interaction between WBC differential parameters and the effect of dexamethasone on clinical outcomes in patients with CAP. Other parameters indicative of more inflammation or more severe disease such as PSI score and CRP have been studied previously. Subgroups analyses by PSI score and CRP were conducted as part of the primary analysis of the Santeon-CAP study.[5] Stratification by PSI score did not yield a subgroup benefitting more from adjunctive dexamethasone. In the subgroups with a CRP concentration above the median, LOS was shorter and ICU admission rate was lower for patients who received dexamethasone compared those who received placebo, this was not seen in patients with a CRP below median. However, in an individual patient data meta-analysis of six trials investigating adjunctive corticosteroid treatment, there was no effect modification by CRP concentration > 188 mg/L. Furthermore, there was also no effect modification

by PSI score on LOS.[4] The uncertain role of PSI score and CRP in identifying patients who benefit from corticosteroid treatment makes it interesting to further explore the role of white blood cell differential parameters.

Neutrophils are the first immune cells to infiltrate the lung in response to microorganisms invading the lung. Neutrophils use several mechanisms to eliminate invading pathogens including the formation of neutrophil extracellular traps (NETs).[3] In a secondary analysis of a randomised trial investigating adjunctive prednisone in CAP, Ebrahimi et al.[17] found that CAP is accompanied by pronounced NET formation and that the degree of NETosis was correlated with peripheral WBC and neutrophil count. Furthermore, the authors found that prednisone modulated NETosis and they noted significant effect modification of the effect of adjunctive prednisone treatment by NET levels on time to clinical stability. Thus it was postulated that the beneficial effects of corticosteroids in CAP might be caused by modulation of NET formation or pre-activation of neutrophils. These findings may be a possible explanation for the fact that, in the present study, the beneficial effect of dexamethasone seemed to be stronger in patients with higher neutrophil counts.

We also found an association between high neutrophil count and history of COPD. Only patients with COPD who did not have clinical signs of an exacerbation COPD at hospital admission were enrolled in the Santeon-CAP study, therefore we do not believe that the positive effects of dexamethasone on LOS in the high neutrophil group were due to treatment of COPD exacerbations. Furthermore, similar to an individual patient data meta-analysis of six trials investigating adjunctive corticosteroids in CAP, we did not find effect modification of the effect of dexamethasone by COPD on LOS.[4] Moreover, we found that high neutrophil count, WBC count and NLR were more frequent in patients without prior outpatient antibiotic treatment. A possible explanation might be that these patients had more fulminant disease and thus were sent to hospital in an earlier stage of disease. Patients pre-treated with antibiotics at home might have had less fulminant disease and might have had some treatment effect leading to a decrease in WBC counts and thus lower WBC counts at admission. This is supported by the fact that mean PSI score (83 (28) vs. 75 (28); p = 0.012) was higher in patients who did not receive antibiotics prior to admission. Nevertheless, we did not find interaction of the effect of dexamethasone on LOS by antibiotic treatment prior to admission.

The aim of this study was to search for subgroups of patients who are more likely to benefit from corticosteroid treatment. When it comes to balancing benefits and harms regarding adjunctive corticosteroid treatment in CAP, risk of hospital readmission is an important concern. In the original analysis of the Santeon-CAP study, readmission rate was twice as high in the dexamethasone group compared to the placebo group (10% vs. 5%; p = 0.051).[5] Briel et al. reported similar findings in their individual patient data meta-analysis of six trials investigating adjunctive corticosteroid treatment in CAP.[4]

In the present study, we did not observe that the effect of adjunctive dexamethasone on hospital readmission rate was modified by WBC differential parameters. For WBC count and neutrophil count, differences in readmission rates between patients treated with dexamethasone and those treated with placebo were similar in both the high and low subgroups. Because a 2-day (33%) reduction in LOS can be quite significant for a patient, the risk of readmission should be weighed against the significant gains of an earlier discharge. An additional finding, which might be equally important in clinical practice, is that in this study low WBC count and low neutrophil count subgroups constituted of a subgroup of patients who did not benefit from corticosteroid treatment but did have a higher risk of hospital readmission due to corticosteroid treatment. We might have identified a subgroup with no benefits but just the harms of corticosteroid treatment. This might be as important as the identification of a subgroup with benefits and not harms of corticosteroids.

There are several limitations to the present study. First and most importantly, this is a secondary analysis of a single study and our results need to be verified in a separate cohort, and would need validation in a prospective study before these findings can be implemented in clinical practice. Second, we could not include all patients from the initial Santeon-CAP study due to missing WBC differential counts thus some selection bias cannot be excluded. However, baseline characteristics were very similar to those of the whole Santeon-CAP population reported in the original analysis.[5] Third, the cut-off point for stratification into subgroups was based on the distribution of our data rather than predefined cut-off points. Since our study is the first to investigate if the effect of adjunctive corticosteroid treatment on clinical outcomes was modified by WBC differential parameters, there were no clear cut-off points available in literature. Furthermore, our patient population consisted of non-ICU patients with CAP. Our results cannot be generalised to patients admitted to the ICU with CAP. Finally, in our population, 30-day mortality rate was lower compared to the population in similar trials investigating corticosteroids in CAP, if there were effect modification for 30-day mortality we might not have enough statistical power to show those differences.[18–20]

Even though further confirmatory research is required, neutrophil count or WBC appear a promising parameter in guiding corticosteroid treatment in non-ICU patients with CAP. This study can be seen as one in many for identifying a subgroup of CAP who should, or should not, be enrolled in future clinical trials. A leukogram is easy to perform and is often already part of the initial patient work-up in the emergency department.

FUNDING

This study was funded by St. Antonius research fund and St Antonius Hospital.

REFERENCES

1. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am J Respir Crit Care Med*. 2019;200(7):e45-e67. doi:10.1164/rccm.201908-1581ST
2. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis*. 2018;18(11):1191-1210. doi:10.1016/S1473-3099(18)30310-4
3. Pechous RD. With friends like these: The complex role of neutrophils in the progression of severe pneumonia. *Front Cell Infect Microbiol*. 2017;7:160. doi:10.3389/fcimb.2017.00160
4. Briel M, Spoorenberg SMC, Snijders D, et al. Corticosteroids in Patients Hospitalized With Community-Acquired Pneumonia: Systematic Review and Individual Patient Data Metaanalysis. *Clin Infect Dis*. 2017;66(3):346-354. doi:10.1093/cid/cix801
5. Wittermans E, Vestjens SM, Spoorenberg SM, et al. Adjunctive treatment with oral dexamethasone in non-ICU patients hospitalised with community-acquired pneumonia: A randomised clinical trial. *Eur Respir J*. 2021;58(2):2002535 doi:10.1183/13993003.02535-2020
6. Chalmers JD. Corticosteroids for community-acquired pneumonia: a critical view of the evidence. *Eur Respir J*. 2016;48(4):984- 986. doi:10.1183/13993003.01329-2016
7. Urwyler SA, Blum CA, Coslovsky M, Mueller B, Schuetz P, Christ-Crain M. Cytokines and Cortisol – predictors of treatment response to corticosteroids in community-acquired pneumonia? *J Intern Med*. 2019;286(1):75-87. doi:10.1111/joim.12891
8. Remmelts HHF, Meijvis SCA, Heijligenberg R, et al. Biomarkers define the clinical response to dexamethasone in community-acquired pneumonia. *J Infect*. 2012;65(1):25-31. doi:10.1016/j.jinf.2012.03.008
9. Méndez R, Menéndez R, Amara-Elori I, et al. Lymphopenic community-acquired pneumonia is associated with a dysregulated immune response and increased severity and mortality. *J Infect*. 2019;78(6):423-431. doi:10.1016/j.jinf.2019.04.006
10. Lee KY. Pneumonia, acute respiratory distress syndrome, and early immune-modulator therapy. *Int J Mol Sci*. 2017;18(2):388. doi:10.3390/ijms18020388
11. Huang Z, Fu Z, Huang W, Huang K. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. *Am J Emerg Med*. 2020;38(3):641-647. doi:10.1016/j.ajem.2019.10.023
12. Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: Experience in patients with cancer. *Crit Rev Oncol Hematol*. 2013;88(1):218-230. doi:10.1016/j.critrevonc.2013.03.010
13. Angkananard T, Anothaisintawee T, McEvoy M, Attia J, Thakkinstian A. Neutrophil Lymphocyte Ratio and Cardiovascular Disease Risk: A Systematic Review and Meta-Analysis. *Biomed Res Int*. 2018;2018:2703518. doi:10.1155/2018/2703518
14. de Jager CPC, Wever PC, Gemen EFA, et al. The Neutrophil-Lymphocyte Count Ratio in Patients with Community-Acquired Pneumonia. *PLoS One*. 2012;7(10):4-11. doi:10.1371/journal.pone.0046561
15. Cataudella E, Giraffa CM, di Marca S, et al. Neutrophil-To-Lymphocyte Ratio: An Emerging Marker Predicting Prognosis in Elderly Adults with Community-Acquired Pneumonia. *J Am Geriatr Soc*. 2017;65(8):1796-1801. doi:10.1111/jgs.14894
16. Fine MJ, Auble TE, Yealy DM, et al. A Prediction Rule to Identify Low-Risk Patients with Community-Acquired Pneumonia. *N Engl J Med*. 1997;336(4):243-250. doi:10.1056/NEJM199701233360402

17. Ebrahimi F, Giaglis S, Hahn S, et al. Markers of neutrophil extracellular traps predict adverse outcome in communityacquired pneumonia: Secondary analysis of a randomised controlled trial. *Eur Respir J*. 2018;51(4):1701389. doi:10.1183/13993003.01389-2017

18. Snijders D, Daniels JMA, de Graaff CS, van der Werf TS, Boersma WG. Efficacy of Corticosteroids in Community-acquired Pneumonia. *Am J Respir Crit Care Med*. 2010;181(9):975-982. doi:10.1164/rccm.200905-0808OC

19. Meijvis SC, Hardeman H, Remmelts HH, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. *The Lancet*. 2011;377(9782):2023-2030. doi:10.1016/S0140-6736(11)60607-7

20. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: A multicentre, double-blind, randomised, placebo-controlled trial. *The Lancet*. 2015;385(9977):1511-1518. doi:10.1016/S0140-6736(14)62447-8

SUPPLEMENTARY MATERIAL

Supplementary Table 1 Effect of dexamethasone vs placebo on median length of stay by COPD status

	No COPD		COPD		
	Placebo N=138	**Dexamethasone N=149**	**Placebo N=31**	**Dexamethasone N=36**	**P***
Median LOS (days)	5.0 [4.0-8.0]	4.0 [3.0-6.0]	6.0 [4.0-7.0]	5.5 [4.0-8.0]	0.83

* P-value for interaction between randomly assigned treatment allocation and COPD status.

Supplementary Table 2 Effect of dexamethasone vs placebo on median length of stay by antibiotic treatment status prior to hospital admission

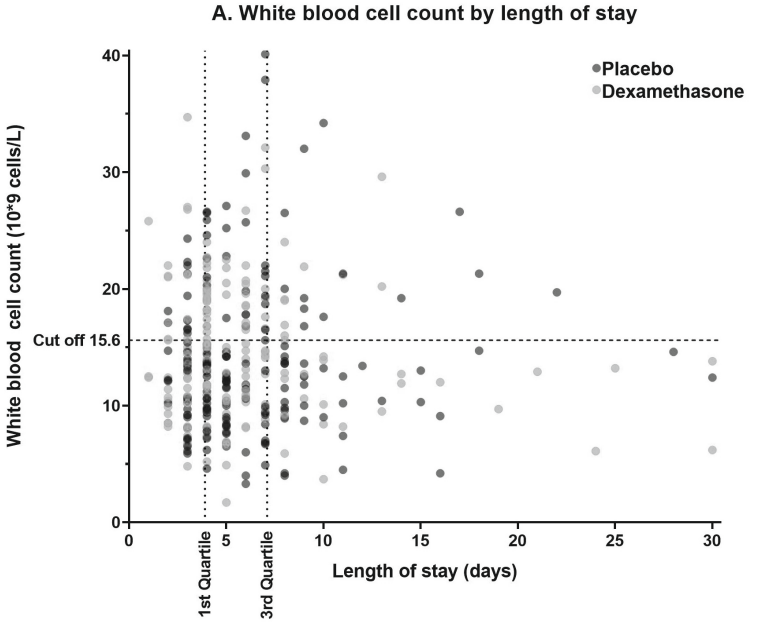
	No antibiotic treatment prior to admission		Antibiotic treatment prior to admission		
	Placebo N=117	**Dexamethasone N=135**	**Placebo N=51**	**Dexamethasone N=50**	**P***
Median LOS (days)	5.0 [4.0-8.0]	5.0 [3.0-7.0]	5.0 [4.0-8.0]	4.0 [3.0-6.0]	0.19

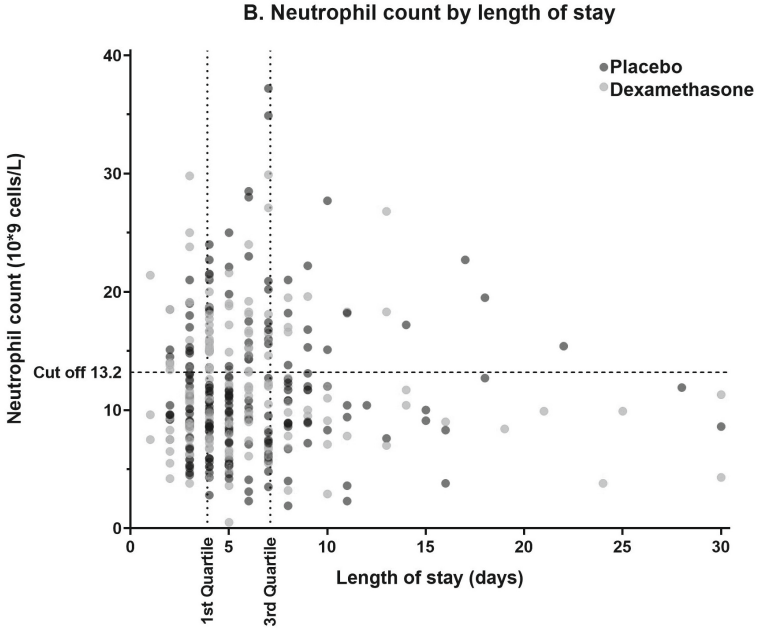
* P-value for interaction between randomly assigned treatment allocation and antibiotic treatment prior to admission

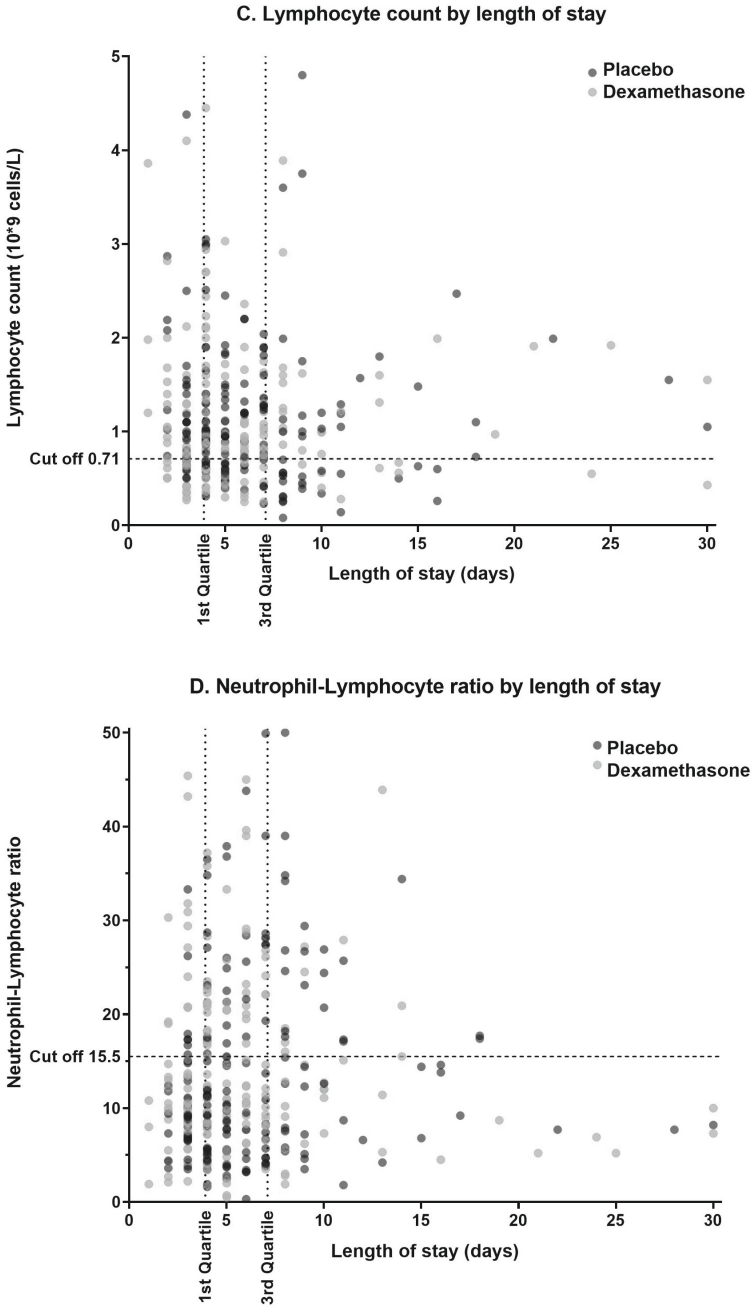
Supplementary Table 3 Incidence of adverse events by treatment allocation and white blood cell differential parameter

	LOW		HIGH	
	Placebo	**Dexamethasone**	**Placebo**	**Dexamethasone**
White blood cell count				
Hyperglycaemia	0 (0)	8 (7)	1 (2)	6 (9)
Neuropsychiatric complaints	5 (4)	6 (5)	0 (0)	3 (4)
Co-infection during hospital stay	4 (3)	0 (0)	0 (0)	0 (0)
Neutrophil count				
Hyperglycaemia	0 (0)	7 (6)	1 (2)	7 (10)
Neuropsychiatric complaints	5 (4)	6 (5)	0 (0)	3 (4)
Co-infection during hospital stay	4 (3)	0 (0)	0 (0)	0 (0)
Lymphocyte count				
Hyperglycaemia	0 (0)	2 (3)	1 (1)	12 (10)
Neuropsychiatric complaints	2 (4)	5 (8)	3 (3)	4 (3)
Co-infection during hospital stay	1 (2)	0 (0)	3 (3)	0 (0)
Neutrophil-lymphocyte ratio				
Hyperglycaemia	0 (0)	10 (8)	1 (2)	4 (6)
Neuropsychiatric complaints	3 (3)	4 (3)	2 (4)	5 (8)
Co-infection during hospital stay	3 (3)	0 (0)	1 (0)	0 (0)

Data are shown as number (%).


A. White blood cell count by length of stay
Placebo
Dexamethasone
White blood cell count (10*9 cells/L)
Cut off 15.6
1st Quartile
3rd Quartile
Length of stay (days)
0
10
20
30
40
0
5
10
15
20
25
30


B. Neutrophil count by length of stay
Placebo
Dexamethasone
Neutrophil count (10*9 cells/L)
Cut off 13.2
1st Quartile
3rd Quartile
Length of stay (days)
0
10
20
30
40
0
5
10
15
20
25
30



Supplementary Figure 1 Scatterplots of each WBC count differential parameter by LOS

1A Length of stay by WBC count. **1B** Length of stay by neutrophil count. **1C** Length of stay by lymphocyte count. **1D** Length of stay by NLR. Horizontal line on the y-axis represents the cut-off value used for stratifying patients into low or high subgroups. The vertical lines on the X-axis represent the 1st and 3rd quartile for length of stay for the whole study population. In the scatterplots, Length of stay was cut off at 30 days, patients with a length of stay >30 are shown at day 30 (n=3).

Supplementary Table 4 Differences in median length of stay between the placebo and dexamethasone group for each tertile for each WBC differential count parameter

	1st tertile		2nd tertile		3rd tertile	
	Placebo	**Dexa-methasone**	**Placebo**	**Dexa-methasone**	**Placebo**	**Dexa-methasone**
WBC count	5.0 [4.0-8.0]	5.0 [3.0-7.0]	5.0 [4.0-8.0]	5.0 [3.5-7.0]	6.0 [4.0-8.0]	4.0 [4.0-6.0]
Neutrophil count	5.0 [4.0-7.0]	5.0 [3.0-7.0]	5.0 [4.0-8.0]	5.0 [3.3-8.0]	6.0 [4.0-8.0]	4.0 [4.0-6.0]
Lymphocyte count	5.5 [4.0-8.0]	4.0 [3.0-6.0]	4.0 [3.0-7.0]	5.0 [4.0-7.0]	6.0 [4.0-7.0]	4.0 [3.8-7.3]
NLR	5.0 [4.0-7.0]	5.0 [4.0-8.0]	4.0 [3.0-7.0]	5.0 [3.0-7.0]	6.0 [4.0-8.0]	4.0 [4.0-6.0]

Data are shown as median [IQR]

CHAPTER 4

Community-acquired pneumonia subgroups and differential response to corticosteroids: a secondary analysis of controlled studies

E Wittermans, PA van der Zee*, H Qi, EMW van de Garde, CA Blum, M Christ-Crain, D Gommers, JC Grutters, GP Voorn, WJW Bos, H Endeman*
**Authors contributed equally*

ERJ OPEN RES. 2022;8(1):00489-2021.

ABSTRACT

Background

Latent class analysis (LCA) has identified subgroups with meaningful treatment implications in acute respiratory distress syndrome. We performed a secondary analysis of three studies to assess whether LCA can identify clinically distinct subgroups in community-acquired pneumonia (CAP) and whether the treatment effect of adjunctive corticosteroids differs between subgroups.

Methods

LCA was performed on baseline clinical and biomarker data from the Ovidius trial (n=304) and the Steroids in Pneumonia (STEP) trial (n=727), both randomised controlled trials investigating adjunctive corticosteroid treatment in CAP, and the observational TripleP cohort (n=201). Analyses were conducted independently in two cohorts (Ovidius–TripleP combined and the STEP trial). In both cohorts, differences in clinical outcomes and response to adjunctive corticosteroid treatment were examined between subgroups identified through LCA.

Results

A two-class model fitted both cohorts best. Class 2 patients had more signs of systemic inflammation compared to class 1. In both cohorts, length of stay was longer and in-hospital mortality rate was higher in class 2. In the Ovidius trial, corticosteroids reduced the median length of stay in class 2 (6.5 versus 9.5 days) but not in class 1 (p-value for interaction=0.02). In the STEP trial, there was no significant interaction for length of stay. We found no significant interaction between class assignment and adjunctive corticosteroid treatment for secondary outcomes.

Conclusions

In two independent cohorts, LCA identified two classes of CAP patients with different clinical characteristics and outcomes. Given the different response to adjunctive corticosteroids in the Ovidius trial, LCA might provide a useful basis to improve patient selection for future trials.

INTRODUCTION

Treatment of community-acquired pneumonia (CAP) is based on early diagnosis and prompt initiation of antibiotic therapy.[1] Despite effective treatment, CAP remains a leading cause of mortality and morbidity worldwide.[2] Adjunctive treatment with corticosteroids might improve clinical outcomes in patients with CAP.[3]

A local immune response is crucial to contain and eliminate the primary infection in CAP.[4] However, an uncontrolled or excessive local immune response could result in systemic inflammation and subsequent multi-organ dysfunction.[5]

Adjunctive treatment with corticosteroids, a potent inhibitor of the immune response, has shown to reduce length of stay (LOS) and time to clinical stability in hospitalised patients with CAP.[3] However, corticosteroids did not lower the mortality rate, and increased the incidence of hospital readmission and hyperglycaemia requiring insulin therapy.[3] Therefore, treatment guidelines do not recommend routine use of corticosteroids in patients with CAP.[1]

In a clinically heterogeneous condition as CAP, it is likely that a subgroup of patients does benefit from corticosteroid treatment.[6] It has been hypothesised that corticosteroid treatment should be given to the subgroup with an excessive systemic inflammation response, whereas patients with a local and controlled immune response should not receive corticosteroid treatment.[7] So far, patients with CAP have been stratified by pneumonia severity index (PSI), initial C-reactive protein concentration, and inflammatory status, but stratification did not result in an unequivocal definition of a subgroup benefiting from corticosteroid therapy and therefore did not result in adjustment of clinical guidelines.[3,8–10]

In other heterogeneous conditions, such as sepsis or acute respiratory distress syndrome, substantial efforts have been made to identify subgroups characterised by different prognoses and responses to treatment.[11] In patients with acute respiratory distress syndrome, a latent class analysis (LCA) was used to identify subgroups with different treatment responses to ventilator and fluid management .[12,13] The identification of patients that are likely to respond to (corticosteroid) treatment, i.e. predictive enrichment, is a step towards personalised medicine and improved patient selection for future clinical trials.[14]

In this secondary analysis of three controlled studies, we attempted to identify CAP subgroups through LCA of baseline clinical and biomarker data from two randomised controlled trials and one prospective cohort study. In addition, we examined whether LCA-based subgroups were associated with different clinical outcomes and a different response to adjunctive corticosteroids.

METHODS

Study population and study design

This is a secondary analysis of demographic, clinical and biomarker data obtained at baseline from patients enrolled in the observational TripleP cohort[15], and two multicentre randomised controlled trials: the Ovidius trial (NCT00471640)[16] and the Steroids in Pneumonia (STEP) trial (NCT00973154).[17] All studies included hospitalised adult patients with CAP (see supplementary material).

In the Ovidius trial, patients with CAP were randomly allocated to receive intravenous dexamethasone 5 mg daily or placebo for 4 days following hospital admission.[16] The STEP trial randomised 727 patients with CAP to either placebo or oral prednisolone 50 mg daily for 7 days in the per protocol analysis.[17] LOS, the primary endpoint in the Ovidius trial and main secondary endpoint in the STEP trial, was significantly reduced in patients assigned to adjunctive treatment with corticosteroids. Details of the original studies are published elsewhere.[16,17]

The Ovidius trial and TripleP study were approved by the Medical Ethics Committee at the St Antonius Hospital. The ethical committees of all participating hospitals and Swissmedic approved the STEP trial.

Methods

Two separate LCAs were performed for the identification of subgroups: one in a combined cohort of TripleP and the Ovidius trial, and one in the STEP trial. The observational TripleP cohort (n=201) and the Ovidius trial (n=304) were combined to obtain a larger sample size. We chose to combine these cohorts as the TripleP cohort preceded the Ovidius trial and reported similar clinical and biomarker data. The Ovidius trial and TripleP study are two mutually exclusive cohorts. The STEP trial (n=727) was analysed independently as different clinical and biomarker data were recorded.

After identification of subgroups by LCA, differences in clinical outcomes between these subgroups and the presence of interaction between treatment allocation and LCA-defined subgroups were assessed separately in both cohorts (Ovidius–TripleP combined and STEP). For the Ovidius–TripleP cohort, only patients who participated in the Ovidius trial were included in the analysis of the interaction between adjunctive corticosteroids. The primary outcome was LOS and secondary outcomes were intensive care unit (ICU) admission, in-hospital mortality, 30-day mortality and 30-day hospital readmission.

Statistical analysis

Baseline characteristics of the Ovidius–TripleP combined and STEP cohorts were presented as count (%) for categorical variables, and mean (standard deviation) or median (interquartile range, IQR) for continuous variables, after testing for normal

distribution. Baseline characteristics of both cohorts were compared using an independent samples t-test, Mann–Whitney U test or Chi-squared test, as appropriate.

The DepmixS4 package in R 4.0.0 (R core team, 2020) was used to conduct the LCA. Baseline clinical and biomarker data obtained at hospital admission were used as class-defining variables in the LCA. A full list of class-defining variables included in the LCA for each cohort is shown in the supplementary material. Assignment of patients to classes was performed independently of clinical outcomes. LCA was first conducted in the Ovidius–TripleP cohort, and was repeated independently in the STEP cohort. Missing data were accommodated by estimating model parameters based on the full information maximum likelihood.[18]

We fitted models with latent classes ranging from two to five classes. To determine the best-fitting model, we used the following criteria: 1) clinical interpretability, i.e. whether identified classes corresponded to clinically coherent clusters of clinical and biomarker data; 2) the number of patients assigned to the smallest class, where a model with small class size is statistically less meaningful; and 3) the Bayesian information criterion, where a lower number corresponds with improved model fit. For clinical interpretability, all continuous variables in the LCA were rescaled to a z-scale with a mean of zero and standard deviation of 1. Subsequently, clinical interpretability was assessed by two authors independently (PZ and HE). Discrepancies were resolved by consensus and, if necessary, a third author was consulted.

Once the number of classes was determined, patients were assigned to the class with maximum probability of class assignment based on the LCA model. The probability of a patient being assigned to a specific class is a weighted average of the N class-specific probabilities in LCA, so each patient has probabilities assigned to all classes, respectively. For example, a patient with a probability of 90% to be assigned to class 1 and 10% probability to be assigned to class 2 was assigned to class 1. Subsequently, the association between class assignment and baseline characteristics or clinical outcomes was tested using Chi-squared, Mann–Whitney U or independent samples t-test, as appropriate. Finally, for the Ovidius trial and STEP cohorts, we tested the interaction between randomly assigned treatment and class on clinical outcomes with the Poisson regression model for LOS and Chi-squared test for categorical outcomes. A p-value <0.05 was deemed statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics of both cohorts are presented in Table 1 and Supplemental Table E1. In short, patients in the Ovidius–TripleP cohort were younger, had fewer comorbidities and had higher levels of inflammatory biomarkers as compared to

patients in the STEP cohort. LOS was longer in the Ovidius–TripleP cohort as compared to the STEP cohort (8.5; 6.0–13.0 days versus 7.0; 4.0–10.0 days, p-value <0.001). Secondary outcomes were similar between both cohorts.

Table 1 Baseline characteristics

	Ovidius–TripleP cohort (n=505)	**STEP cohort (n=727)**
Demographic data		
Age (years)	67 (51–78)	73 (60–83)
Male	295 (58.4)	452 (62.2)
Caucasian	491 (97.2)	712 (97.9)
Duration of symptoms (days)	4 (2–7)	4 (2–7)
Antibiotics at home	130 (25.7)	164 (22.6)
Corticosteroids at home	34 (6.7)	14 (1.9)
Comorbidities		
Nursing home resident	19 (3.8)	0 (0.0)
Cerebrovascular accident	46 (9.1)	67 (9.2)
Malignancy	45 (8.9)	70 (9.6)
Liver disease	2 (0.4)	28 (3.9)
Renal disease	40 (7.9)	218 (30.0)
Congestive heart failure	68 (13.5)	134 (18.4)
Chronic obstructive pulmonary disease	98 (19.4)	122 (16.8)
Diabetes mellitus	77 (15.2)	139 (19.1)
Current smoker	81 (16.0)	188 (25.9)
Pneumonia severity index score	87 (63–114)	90 (64–113)
Outcome		
Length of stay (days)	8.5 (6.0–13.0)	7.0 (4.0–10.0)
ICU admission	38 (7.5)	39 (5.4)
In-hospital mortality	24 (4.8)	24 (3.3)
30-day mortality	26 (5.1)	28 (3.9)
Readmission	37 (7.3)	39 (5.4)

Data are n (%), mean (standard deviation) or median (interquartile range). ICU: intensive care unit; STEP: Steroids in Pneumonia.

Latent class modelling: identification of number of classes

We fitted latent class models ranging from two to five classes (Table 2). First, we examined clinical interpretability by plotting class-defining variables for all models and assessed whether identified classes corresponded to clinically coherent subgroups (Figure 1 and Supplemental Figure E1). In both the Ovidius–TripleP cohort and the STEP cohort, a two-class model resulted in two coherent and distinct clinical classes.

Addition of a third, fourth or fifth class resulted in further subdivision of patients assigned to class 2 in the two-class model, without adding an additional coherent or distinct clinical class. Subsequently, we explored the number of patients per subgroup in all models (Table 2). The addition of a third class to the two-class model resulted in a smaller third class of 58 patients in the Ovidius–TripleP cohort and 72 patients in the STEP cohort. We observed a further decline in the number of patients in the smallest class in a four- or five-class model. Lastly, the Bayesian information criterion was lowest in the five-class model in both the Ovidius–TripleP cohort and the STEP cohort, suggesting a better fit for the five-class model. Even though a data-driven approach suggested more than two classes, a three-class model did not result in an evident third clinical entity. Thus, clinical interpretability of the two-class models in conjunction with the relatively small number of patients in the three-, four- or five-class models led us to proceed with the two-class models for both cohorts. We will refer to the classes as class 1 and class 2 in the remainder of the manuscript. For the three-class model we show clinical characteristics for each class in the supplementary material.

Table 2 Fit statistics for latent class models from two to five class models

Number of classes	BIC	Number of patients per class				
		1	2	3	4	5
Ovidius-TripleP cohort						
2	124577.2	411	94			
3	120741.9	153	58	294		
4	120507.3	61	112	296	36	
5	118372.7	33	25	94	108	245
STEP cohort						
2	116815.7	574	153			
3	106770.5	99	556	72		
4	71445.1	24	125	466	112	
5	70684.5	132	18	44	434	99

BIC: Bayesian information criterion; STEP: Steroids in Pneumonia.

Patients were assigned to the class for which the probability of belonging to that class was the highest. Thus, all patients in both cohorts were assigned to either class 1 or class 2. In the Ovidius–TripleP cohort, 411 patients were assigned to class 1 and 94 to class 2. In the STEP cohort, 574 and 153 patients were assigned to class 1 and class 2, respectively. Probabilities of class assignment for the two-class model are presented in supplemental Figure E2. The average probability of a patient belonging to the class to which it was assigned was 99.4% for class 1 and 98.6% class 2 in the Ovidius–TripleP cohort, and 98.7% for class 1 and 99.1% for class 2 in the STEP cohort. This indicated a good model fit and robust class assignment.

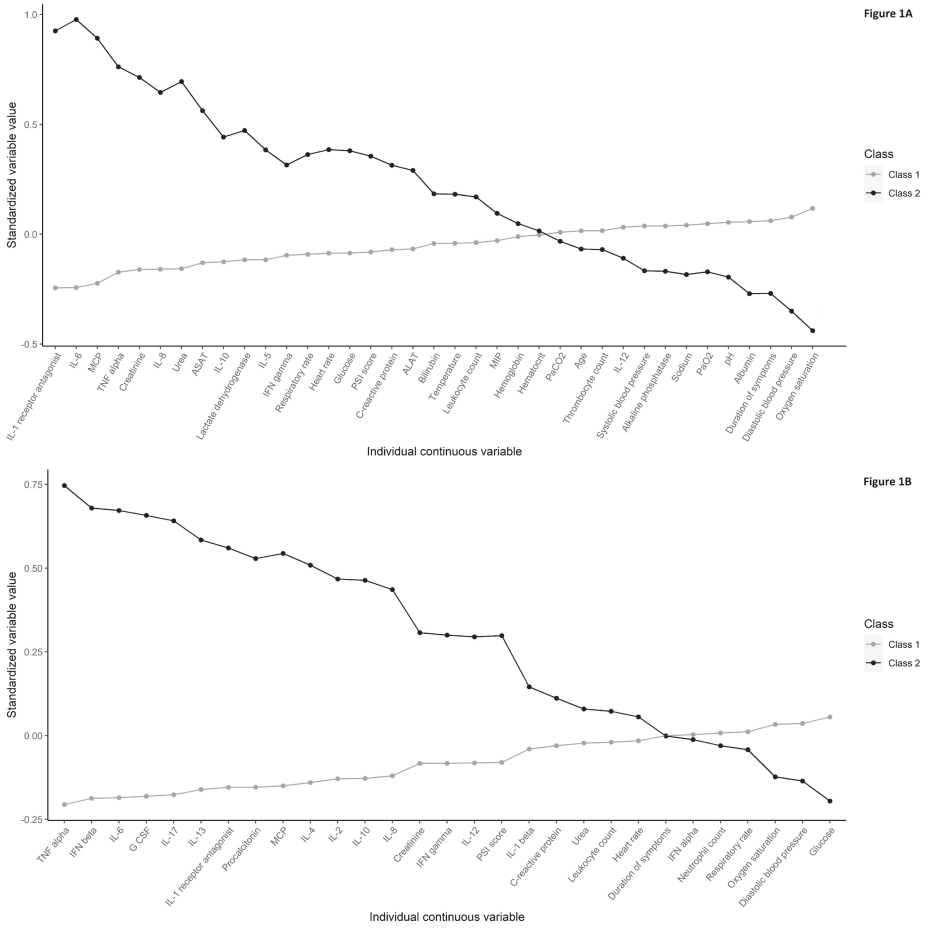


Figure 1 Continuous variables (standardised) by class assignment for the a) Ovidius–TripleP cohort and b) Steroids in Pneumonia (STEP) cohort. Differences between the standardised values of each variable by class (y-axis) for the variable shown on the x-axis. The variables are sorted by degree of separation between classes: from the maximum positive separation on the left (where the standardised value of class 2 is higher than the standardised value of class 1) to the maximum negative separation on the right (where the standardised value of class 2 to is lower than the standardised value of class 1). The crossover of the lines indicates that the standardised value for this variable was the same for classes 1 and 2 (i.e. no difference between class 1 and class 2 for this variable). Therefore, variables near the intersection of both lines are similar in both classes and thus are not class-defining. The method of variable standardisation is described in the methods section. If the standardised value of a certain variable is 1 for a class, it means that the mean value for that variable within that class was one standard deviation higher than the mean value for that variable in the whole cohort. ALAT: alanine transaminase; ASAT: aspartate transaminase; G-CSF: granulocyte colony-stimulating factor; IFN: interferon; IL: interleukin; MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; PaCO2: arterial carbon dioxide tension; PaO2: arterial oxygen tension; PSI: pneumonia severity index; TNF: tumour necrosis factor.

Class characteristics

Differences between class 1 and class 2 in the Ovidius–TripleP cohort are shown in Figure 1a and Table 3. The most noteworthy and clinically relevant differences were that patients in class 2 had higher plasma concentration of interleukin (IL)-1 receptor antagonist, IL-6, monocyte chemoattractant protein and tumour necrosis factor-α (TNF-α) compared to class 1. Furthermore, patients assigned to class 2 seemed to have more severe illness seeing as they had lower oxygen saturation, lower diastolic blood pressure and had a higher PSI score at admission.

Table 3 Values of variables at baseline stratified by class in the Ovidius–TripleP cohort

Variable	Class 1 (n=411)	Class 2 (n=94)	Missing n (%)
Age (years)	67 (51–79)	67 (53–76)	0 (0)
Alanine transaminase (U/L)	28 (16–44)	28 (19–55)	152 (30.1)
Albumin (g/L)	37 (33–40)	36 (33–38)	339 (67.0)
Alkaline phosphatase (U/L)	90 (70–130)	90 (61–113)	167 (33.1)
Altered mental status ¶	47 (11.4)	10 (10.6)	11 (2.2)
Aspartate transaminase (U/L)	34 (23–51)	38 (25–78)#	153 (30.3)
Bilirubin (µmol/L)	12 (9–16)	16 (12–24)#	199 (39.4)
C-reactive protein (mg/L)	196 (94–300)	294 (107–389)#	9 (1.8)
Cortisol (nmol/L)¶	226.0 (148.0–159.1)	446.8 (322.4–691.4)#	23 (4.6)
Corticosteroids at home¶	30 (7.5)	4 (4.4)	15 (3.0)
Creatinine (µmol/L)	84 (70–106)	111 (91–157)#	10 (2.0)
Diastolic blood pressure (mmHg)	75 (68–83)	70 (60– 80)#	11 (2.2)
Duration of symptoms (days)	4 (3–7)	3 (2–5)#	16 (3.2)
Glucose (mmol/L)	7.0 (6.0–8.3)	7.5 (6.2–9.8)#	39 (7.7)
Heart rate (beats/min)	95 (82–109)	110 (87–118)#	9 (1.8)
Haematocrit (L/L)	0.40 (0.36–0.43)	0.39 (0.37–0.43)	17 (3.4)
Haemoglobin (mmol/L)	8.3 (7.6–9.0)	8.3 (7.8–9.0)	10 (2.0)
Interferon-γ (pg/mL)	202.1 (16.8–288.3)	217.8 (10.0–354.9)	213 (42.2)
Interleukin-1 receptor antagonist (pg/mL)	102.8 (18.0–448.4)	1042.5 (204.2–4309.2)#	79 (15.6)
Interleukin-6 (pg/mL)	51.0 (18.0–156.3)	749.7 (101.2–2209.7)#	63 (12.5)
Interleukin-5 (pg/mL)	0.54 (0.24–0.77)	0.46 (0.26–0.61)	333 (65.9)
Interleukin-8 (p/mL)	14.8 (8.1–29.3)	59.5 (32.1–152.2)#	56 (11.1)
Interleukin-10 (pg/mL)	3.4 (1.4–9.0)	15.9 (5.8–79.7)#	94 (18.6)
Interleukin-12 (pg/mL)	7.3 (4.1–10.5)	8.3 (5.6–11.5)	337 (66.7)
Lactate dehydrogenase (U/L)	328 (252–480)	435 (313–604)#	212 (42.0)
Legionella species¶	14 (3.4)	6 (6.4)	0 (0)

Table 3 Continued

Variable	Class 1 (n=411)	Class 2 (n=94)	Missing n (%)
Leukocyte count (10^9 cells/L)	13.5 (9.5–17.7)	14.9 (10.8–20.1)	9 (1.8)
Macrophage inflammatory protein (pg/mL)	6.1 (3.7–8.5)	6.8 (4.6–10.4)	236 (47)
Male¶	236 (57.4)	59 (62.8)	0 (0)
Monocyte chemoattractant protein (pg/mL)	274.2 (74.7–536.6)	918.4 (242.9–2463.3)#	46 (9.1)
Oxygen saturation (%)	94 (92–97)	94 (88–96)#	107 (21.2)
Oxygen therapy¶	70 (17.0)	30 (31.9)#	312 (61.8)
P_{aO2} (kPa)	8.80 (7.80–10.38)	8.40 (7.10–9.90)#	124 (24.6)
P_{aCO2} (kPa)	4.40 (4.10–4.90)	4.40 (4.00–4.85)	124 (24.6)
pH	7.47 (7.44–7.50)	7.46 (7.42–7.49)	124 (24.6)
Pleural effusion¶	61 (14.8)	21 (22.3)	9 (1.8)
Pneumonia severity index score	84 (60–111)	102 (73–126)#	0 (0)
Respiratory rate (breaths/min)	22 (18–30)	25 (20–30)#	104 (20.6)
Sodium (mmol/L)	135 (132–137)	133 (129–137)#	9 (1.8)
Streptococcus pneumoniae ¶	85 (20.7)	39 (41.5)#	0 (0)
Systolic blood pressure (mmHg)	131 (120–146)	126 (112–145)	11 (2.2)
Temperature (°C)	38.2 (37.4–39.0)	38.5 (37.4–39.3)	9 (1.8)
Thrombocyte count (10^9 cells/L)	253 (200–317)	237 (177–327)	9 (1.8)
Tumour necrosis factor-α (pg/mL)	5.9 (3.1–10.2)	12.4 (6.1–29.6)#	224 (44.4)
Urea (mmol/L)	6.4 (4.6– 9.5)	9.8 (6.3–15.2)#	17 (3.4)

Data are shown as median (interquartile range) or n (%). #: statistically significant difference between class 1 and class 2. ¶: non-class-defining variables (variable not included in latent class analysis). Missing data is n (%) for the whole cohort.

Differences between class 1 and class 2 in the STEP cohort are shown in Figure 1b and Table 4. In the STEP cohort, the most noteworthy and clinically relevant differences between classes were higher plasma concentrations of TNF-α, interferon-β, IL-6, granulocyte colony stimulating factor and IL-17 in class 2 compared to class 1. Patients in class 2 also had a higher PSI score compared to class 1. However, there was no difference in oxygen saturation or diastolic blood pressure.

Table 4 Values of variables at baseline stratified by class in the STEP cohort

Variable	Class 1 (n=574)	Class 2 (n=153)	Missing n (%)
Altered mental status¶	33 (5.7)	13 (8.5)	0 (0)
C-reactive protein (mg/L)	155 (74–247)	171 (93–268)	7 (1)
Creatinine (µmol/L)	86 (68–109)	98 (72–132)#	6 (0.8)
Diastolic blood pressure (mmHg)	70 (60–78)	66 (59–75)	4 (0.6)
Duration of symptoms (days)	4 (2–7)	4 (2–7)	17 (2.3)
Glucose (mmol/L)	6.4 (5.5–7.7)	6.0 (5.5–7.3)	179 (24.6)
Granulocyte colony stimulating factor (pg/mL)	7.0 (7.0–8.7)	21.1 (9.3–59.3)#	55 (7.6)
Heart rate (beats/min)	83 (72–95)	84 (71–101)	4 (0.6)
Interferon-α (pg/mL)	0.24 (0.24–0.33)	0.56 (0.30–1.02)#	55 (7.6)
Interferon-β (pg/mL)	22.7 (14.5–34.0)	41.3 (22.0–74.1)#	55 (7.6)
Interferon-γ (pg/mL)	2.8 (2.8–2.8)	2.8 (2.8–4.6)#	55 (7.6)
Interleukin-1β (pg/mL)	1.0 (1.0–1.0)	1.0 (1.0–2.8)#	55 (7.6)
Interleukin-1 receptor antagonist (pg/mL)	33.0 (33.0–551.5)	1280.1 (33.0–6244.1)#	55 (7.6)
Interleukin-2 (pg/mL)	4.4 (4.4–4.4)	4.4 (4.4–4.4)#	55 (7.6)
Interleukin-4 (pg/mL)	5.5 (5.5–5.5)	5.5 (5.5–24.4)#	55 (7.6)
Interleukin-6 (pg/mL)	40.6 (14.6–102.5)	172.0 (59.7–748.4)#	55 (7.6)
Interleukin-8 (pg/mL)	3.9 (1.9–9.7)	19.8 (6.6–46.1)#	55 (7.6)
Interleukin-10 (pg/mL)	0.9 (0.7–1.4)	2.2 (1.3–4.8)#	55 (7.6)
Interleukin-12 (pg/mL)	1.1 (1.1–1.4)	2.2 (1.3–3.7)#	55 (7.6)
Interleukin-13 (pg/mL)	1.3 (1.3–1.3)	2.4 (1.3–8.8)#	55 (7.6)
Interleukin-17 (pg/mL)	0.57 (0.57–0.57)	0.87 (0.57–1.86)#	55 (7.6)
Legionella species¶	11 (1.9)	3 (2.0)	102 (14.0)
Leukocyte count (10^9 cells/L)	11.9 (8.7–15.6)	12.2 (9.2–15.8)	4 (0.6)
Male¶	345 (60.1)	107 (69.9)#	0 (0)
Monocyte chemoattractant protein (pg/mL)	39.8 (25.5–70.1)	66.6 (37.2–242.9)#	55 (7.6)
Neutrophil count (10^9 cells/L)	9.8 (6.9–13.2)	10.2 (7.4–13.3)	64 (9.7)
Oxygen saturation (%)	95 (92–96)	94 (92–96)	25 (3.4)
Oxygen therapy¶	298 (51.9)	79 (51.6)	6 (0.8)
Pleural effusion¶	65 (11.3)	18 (11.8)	0 (0)
Pneumonia severity index score	88 (63–111)	98 (74–131)#	0 (0)
Procalcitonin (ng/mL)	0.39 (0.16–1.68)	1.14 (0.28–10.35)#	133 (18.3)
Respiratory rate (breaths/min)	20 (18–24)	20 (17–24)	136 (18.7)
Streptococcus pneumoniae ¶	75 (13.1)	31 (20.3)#	104 (14.3)
Tumour necrosis factor-α (pg/mL)	1.8 (1.8–1.9)	2.7 (1.8–4.0)#	55 (7.6)
Urea (mmol/L)	6.6 (4.8–10.0)	7.9 (5.4–13.4)#	37 (5.1)

Data are shown as median (interquartile range) or n (%). #: statistically significant difference between class 1 and class 2. ¶: non-class-defining variables (variable not included in latent class analysis). Missing data is n (%) for the whole cohort.

Class prediction with a small number of variables

In order to determine whether classes could be identified based on a reduced number of variables, we tested a three-variable model including variables available for both cohorts and differing most between classes (IL-6, TNF-α and oxygen saturation at hospital admission). An area under the curve (AUC) was calculated to evaluate this reduced model compared to the full model. The AUC was 0.78 and 0.65, respectively, for the Ovidius–TripleP cohort and the STEP cohort. Contingency tables comparing class membership between reduced and full model are shown in the supplementary material (Table E2).

Association between class and clinical outcomes

Subsequently, we assessed clinical outcomes in both classes (Table 5). In the Ovidius–TripleP cohort, patients in class 2 had a significantly longer LOS (10.5; 6.5–16.0 days versus 8.0; 6.0–12.0 days, p-value <0.01) and higher rate of ICU admissions. In-hospital mortality and 30-day mortality rates were significantly higher in class 2. Similar results were observed in the STEP cohort, as patients in class 2 had a longer LOS (7.0; 5.0–12.0 days versus 7.0; 4.0–10.0 days, p-value <0.01), and a higher in-hospital mortality rate (Table 5).

Effect of corticosteroids on outcome stratified by class

Lastly, we used the data from the Ovidius trial and the STEP cohort to determine whether classes responded differently to randomly assigned adjunctive treatment with corticosteroids (Table 6). In the Ovidius trial, dexamethasone reduced LOS in patients assigned to class 2 (6.5; 5.5–10.0 days versus 9.5; 5.0–14.5 days), whereas LOS was similar between treatment groups in class 1 (p-value for interaction 0.02). In the STEP cohort, there was no significant interaction for LOS between class assignment and adjunctive treatment with corticosteroids. In both cohorts, we found no significant interaction for secondary outcomes between class assignment and adjunctive treatment with corticosteroids.

Table 5 Association between class assignment and clinical outcomes

Ovidius–TripleP cohort			
Clinical outcome	**Class 1 (n=411)**	**Class 2 (n=94)**	**p-value**
Length of stay (days)	8.0 (6.0–12.0)	10.5 (6.5–16.0)	<0.01
ICU admission	16 (3.9%)	22 (23.4%)	<0.01
In-hospital mortality	14 (3.4%)	10 (10.6%)	0.01
30-day mortality	15 (3.6%)	11 (11.7%)	<0.01
Readmission	29 (7.1%)	8 (8.5%)	0.79
STEP cohort			
Clinical outcome	**Class 1 (n=574)**	**Class 2 (n=153)**	**p-value**
Length of stay (days)	7.0 (4.0–10.0)	7.0 (5.0–12.0)	<0.01
ICU admission	28 (4.9%)	11 (7.2%)	0.35
In-hospital mortality	13 (2.3%)	11 (7.2%)	<0.01
30-day mortality	18 (3.1%)	10 (6.5%)	0.09
Readmission	30 (5.2%)	9 (5.9%)	0.91

Data are n (%) or median (interquartile range). ICU: intensive care unit.

Table 6 Differential response to adjunctive corticosteroid treatment by latent class assignment

Ovidius trial					
	Class 1 (n=251)		**Class 2 (n=52)**		
	Corticosteroid (n=124)	**Placebo (n=128)**	**Corticosteroid (n=27)**	**Placebo (n=25)**	**P***
Length of stay (days)	6.5 (5.0–8.5)	7.5 (5.5–10.5)	6.5 (5.5–10.0)	9.5 (5.0–14.5)	0.02
ICU admission	4 (3.2)	4 (3.1)	3 (11.1)	6 (24.0)	0.64
In-hospital mortality	7 (5.6)	3 (2.3)	1 (3.7)	5 (20.0)	0.12
30-day mortality	7 (5.6)	4 (3.1)	2 (7.4)	5 (20.0)	0.33
Readmission	6 (4.8)	4 (3.1)	1 (3.7)	3 (12.0)	0.56
STEP cohort					
	Class 1 (n=574)		**Class 2 (n=153)**		
	Corticosteroid (n=285)	**Placebo (n=289)**	**Corticosteroid (n=77)**	**Placebo (n=76)**	**P***
Length of stay (days)	6.0 (4.0–9.0)	7.0 (5.0–10.0)	7.0 (4.0–11.0)	8.0 (5.0–13.3)	0.46
ICU admission	11 (3.9)	17 (5.9)	6 (7.8)	5 (6.6)	0.61
In-hospital mortality	8 (2.8)	5 (1.7)	5 (6.5)	6 (7.9)	0.71
30-day mortality	11 (3.9)	7 (2.4)	4 (5.2)	6 (7.9)	0.50
Readmission	21 (7.4)	9 (3.1)	5 (6.5)	4 (5.3)	0.69

Data are n (%) or median (interquartile range). *p-value: for interaction between class assignment and corticosteroid treatment. ICU: intensive care unit.

DISCUSSION

In this secondary analysis of three controlled studies, LCA identified two distinct classes of CAP patients with different biomarker profiles, clinical characteristics and clinical outcomes. Classes were identified in two independent cohorts, despite multiple significant differences in baseline characteristics between cohorts. In the Ovidius trial, adjunctive treatment with corticosteroids reduced LOS only in patients assigned to class 2. We found no differential treatment response for LOS in the STEP cohort or for secondary outcomes in both cohorts.

In both cohorts, class 2 was characterised by higher concentrations of inflammatory biomarkers, creatinine and higher PSI scores. Additionally, patients assigned to class 2 in the Ovidius–TripleP cohort had lower oxygen saturation, lower diastolic blood pressure and higher incidence of oxygen therapy. In contrast, patients in class 1 were characterised by lower concentrations of inflammatory plasma biomarkers and lower PSI scores. Furthermore, in the Ovidius–TripleP cohort, cortisol was also higher in class 2 compared to class 1; we assume this is explained by the that fact patients with more inflammation have a higher activation of the hypothalamic–pituitary–adrenal axis and thus higher cortisol levels than patients with lower levels of systemic inflammation because they are more severely ill. Moreover, in both cohorts, LOS was longer, and incidence of ICU admissions and mortality rates were higher in class 2. Thus, patients in class 2 had a stronger systemic inflammatory response, whereas patients in class 1 had fewer signs of systemic inflammation. Patients in class 2 were more likely to benefit from the anti-inflammatory effects of corticosteroids, whereas the patients assigned to class 1 were less likely to benefit from the anti-inflammatory effects, at a similar risk of adverse effects.

Corticosteroids reduced LOS in patients with CAP in the Ovidius trial and in the STEP trial.[16,17] An individual patient data meta-analysis enrolling data from six randomised controlled trials comparing corticosteroids with placebo in 1506 patients with CAP, including the Ovidius trial and STEP trial, confirmed that adjunctive treatment with corticosteroids reduced LOS.[3] In this meta-analysis, however, the authors could not identify patient subgroups more likely to benefit from corticosteroids based on PSI score (PSI class 1–3 versus PSI class 4–5), initial C-reactive protein concentration (cut-off 188 mg/L), initial ICU admission, or systemic inflammatory response syndrome criteria. However, in a clinically heterogeneous condition as CAP, it is unlikely that all patients benefit equally from corticosteroids.[9,14]

In the Ovidius trial, we found that patients assigned to class 2 who were treated with corticosteroids showed a significant reduction in LOS, whereas corticosteroids did not reduce LOS in patients assigned to class 1. These results suggest that the subgroup of CAP patients with signs of a systemic inflammatory response benefit

from corticosteroids and patients with a less pronounced systemic inflammatory response do not. However, these results could not be verified in the STEP cohort, even though PSI score was similar between both cohorts. A possible explanation is that LCAs were performed separately in the Ovidius–TripleP cohort and the STEP cohort and included a different set of class-defining variables for each cohort (Figure 1) because available biomarkers differed between both cohorts. Thus, the LCA models were not identical in both cohorts. Furthermore, concentrations of inflammatory biomarkers were higher at baseline in the Ovidius cohort compared to the STEP cohort, indicating a more pronounced inflammatory response in the Ovidius cohort that corticosteroids could inhibit. The reduced three variable model – consisting of IL-6, TNF-α and oxygen saturation – showed that the AUC for class assignment was higher in the Ovidius–TripleP cohort as compared to the STEP cohort. This also suggests that the Ovidius–TripleP cohort relies more on inflammatory response. Adding to the above, in the STEP cohort, disease severity defined by PSI score was mainly influenced by higher age and more comorbidities, whereas in the Ovidius cohort PSI score was mainly influenced by clinical characteristics and biomarker data indicative of more severe disease. Consequently, clinical variables at baseline did not differ between class 1 and class 2 in the STEP cohort, whereas clinical variables at baseline did differ between classes in the Ovidius cohort. Other explanations might be the difference in corticosteroid therapy (dexamethasone versus prednisolone) or the shorter LOS in the STEP cohort (median 8.5; 6.0–13.0 days in Ovidius cohort versus 7.0; 4.0–10.0 days in STEP cohort) making potential differences between classes in the STEP cohort more difficult to detect.

Inflammatory biomarkers contributed more to the determination of classes than clinical data, including C-reactive protein, procalcitonin or leukocyte count. These results suggest that the inflammatory biomarkers were able to identify aspects of CAP pathophysiology that otherwise remained hidden in routinely collected clinical data.

This study has several limitations. First, LCA model selection and interpretation often involves a level of subjectivity.[19] We decided to select a two-class model instead of more classes based on clinical interpretability and the number of patients assigned to the smallest class. Hypothetically, a third class or even a fourth class could have been forced in by generating a smaller cluster of patients with a more extreme set of variables. However, a three-or-more-class model did not result in additional groups with more extreme variables, but in mixed classes without a coherent clinical pattern. Second, we assumed patients in class 2 to have a systemic inflammatory response and patients in class 1 to have a more controlled inflammatory response based on distribution of inflammatory biomarkers in plasma. We did not measure the pulmonary response and therefore do not know whether inflammation is indeed contained locally in patients assigned to class 1. We refrained from using terms as hyperinflammatory or hypoinflammatory, previously proposed in subgroups of patients with acute respiratory distress syndrome, as all patients are admitted because of CAP, which can hardly be

considered a hypoinflammatory condition.[20,21] Third, this is a secondary analysis which requires prospective validation before definitive conclusions regarding patient subgroup identification and adjunctive corticosteroid treatment can be drawn. Fourth, LOS was calculated from day of hospital admission to day of discharge or day of in-hospital death. Thus, LOS was underestimated in patients that died during hospital admission. However, in both cohorts, in-hospital mortality rate was higher in class 2 as compared to class 1. If reported LOS were an underestimation, this would mainly be the case in class 2 and the difference in LOS between classes would be even larger than reported. Fifth, the clinical and biomarker data used in this analysis was limited to the data available for both cohorts and to data obtained at time of hospital admission. As the aim of data collection for the original studies was to calculate the PSI score, clinical data used in the LCA resembled the PSI score to some extent and PSI score differed significantly between class 1 and class 2 in both cohorts. However, the classes identified by LCA were largely based on biomarker data and thus captured different subgroups of patients than classes based on PSI score only. Lastly, because data was obtained at time of hospital admission, it is unknown whether identified classes remained stable later during the course of CAP.

To our knowledge, this is the first study that identified CAP subgroups through LCA. Because the present study is a proof-of-concept study, our results are not directly applicable for daily clinical practice. Future studies should include validation of our findings in a third independent cohort, after which a clinically useful model with a limited number of variables should be developed to ensure applicability. Lastly, validation of these clinical models in predicting response to treatment should be assessed in prospective studies.

In conclusion, we identified two classes of CAP patients with different clinical characteristics, inflammatory profiles and clinical outcomes in two independent cohorts. Furthermore, in the Ovidius trial, adjunctive treatment with corticosteroids reduced LOS only in the patients assigned to class 2 and not in the patients assigned to class 1. Given the different response to adjunctive treatment in subgroups in the Ovidius cohort, identification of subgroups might provide a useful basis for improved patient selection in future clinical trials.

FUNDING

St Antonius Research Fund

REFERENCES

1. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am J Respir Crit Care Med*. 2019;200(7):e45-e67. doi:10.1164/rccm.201908-1581ST
2. Troeger C, Blacker B, Khalil IA, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis*. 2018;18(11):1191-1210. doi:10.1016/S1473-3099(18)30310-4
3. Briel M, Spoorenberg SMC, Snijders D, et al. Corticosteroids in Patients Hospitalized With Community-Acquired Pneumonia: Systematic Review and Individual Patient Data Metaanalysis. *Clin Infect Dis*. 2017;66(3):346-354. doi:10.1093/cid/cix801
4. Bermejo-Martin JF, Almansa R, Martin-Fernandez M, Menendez R, Torres A. Immunological profiling to assess disease severity and prognosis in community-acquired pneumonia. *Lancet Respir Med*. 2017;5(12):e35-e36. doi:10.1016/S2213-2600(17)30444-7
5. Rhen T, Cidlowski JA. Antiinflammatory Action of Glucocorticoids — New Mechanisms for Old Drugs. *https://doi.org/101056/NEJMra050541*. 2005;353(16):1711-1723. doi:10.1056/nejmra050541
6. Chalmers JD. Corticosteroids for community-acquired pneumonia: a critical view of the evidence. *Eur Respir J*. 2016;48(4):984 - 986. doi:10.1183/13993003.01329-2016
7. Meijvis SCA, van de Garde EMW, Rijkers GT, Bos WJW. Treatment with anti-inflammatory drugs in community-acquired pneumonia. *J Intern Med*. 2012;272(1):25-35. doi:10.1111/j.1365-2796.2012.02554.x
8. Torres A, Sibila O, Ferrer M, et al. Effect of Corticosteroids on Treatment Failure Among Hospitalized Patients With Severe Community-Acquired Pneumonia and High Inflammatory Response: A Randomized Clinical Trial. *JAMA*. 2015;313(7):677-686. doi:10.1001/jama.2015.88
9. Remmelts HHF, Meijvis SCA, Heijligenberg R, et al. Biomarkers define the clinical response to dexamethasone in community-acquired pneumonia. *J Infect*. 2012;65(1):25-31. doi:10.1016/j.jinf.2012.03.008
10. Urwyler SA, Blum CA, Coslovsky M, Mueller B, Schuetz P, Christ-Crain M. Cytokines and Cortisol – predictors of treatment response to corticosteroids in community-acquired pneumonia? *J Intern Med*. 2019;286(1): 75-87. doi:10.1111/joim.12891
11. Scicluna BP, van Vught LA, Zwinderman AH, et al. Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study. *Lancet Respir Med*. 2017;5(10):816-826. doi:10.1016/S2213-2600(17)30294-1
12. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med*. 2014;2(8):611-620. doi:10.1016/S2213-2600(14)70097-9
13. Famous KR, Delucchi K, Ware LB, et al. Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy. *Am J Respir Crit Care Med*. 2017;195(3):331-338. doi:10.1164/rccm.201603-0645OC
14. Prescott HC, Calfee CS, Thompson BT, Angus DC, Liu VX. Toward Smarter Lumping and Smarter Splitting: Rethinking Strategies for Sepsis and Acute Respiratory Distress Syndrome Clinical Trial Design. *Am J Respir Crit Care Med*. 2016;194(2):147-155. doi:10.1164/rccm.201512-2544CP
15. Endeman H, Meijvis SCA, Rijkers GT, et al. Systemic cytokine response in patients with community-acquired pneumonia. *Eur Respir J*. 2011;37(6):1431-1438. doi:10.1183/09031936.00074410

16. Meijvis SC, Hardeman H, Remmelts HH, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. *The Lancet*. 2011;377(9782):2023-2030. doi:10.1016/S0140-6736(11)60607-7

17. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: A multicentre, double-blind, randomised, placebo-controlled trial. *The Lancet*. 2015;385(9977):1511-1518. doi:10.1016/S0140-6736(14)62447-8

18. Cham H, Reshetnyak E, Rosenfeld B, Breitbart W. Full Information Maximum Likelihood Estimation for Latent Variable Interactions With Incomplete Indicators. *Multivariate Behav Res*. 2017;52(1):12-30. doi:10.1080/00273171.2016.1245600

19. al Sallakh MA, Rodgers SE, Lyons RA, Sheikh A, Davies GA. Identifying patients with asthma-chronic obstructive pulmonary disease overlap syndrome using latent class analysis of electronic health record data: a study protocol. *NPJ Prim Care Respir Med*. 2018;28(1):22. doi:10.1038/s41533-018-0088-4

20. Sinha P, Calfee CS. Phenotypes in acute respiratory distress syndrome. *Curr Opin Crit Care*. 2019;25(1):12-20. doi:10.1097/MCC.0000000000000571

21. Calfee CS, Delucchi KL, Sinha P, et al. Acute respiratory distress syndrome subphenotypes and differential response to simvastatin: secondary analysis of a randomised controlled trial. *Lancet Respir Med*. 2018;6(9):691-698. doi:10.1016/S2213-2600(18)30177-2

SUPPLEMENTARY MATERIAL

METHODS

Definition of CAP

CAP was defined as a new pulmonary infiltrate on chest x-ray, accompanied by at least one of the following criteria: cough, sputum, temperature >38°C (or <35°C), auscultatory findings consistent with pneumonia, C-reactive protein >15 mg/L, leukocyte count >10x10^9 cells/L or <4x10^9 cells/L, or >10% bands in leucocyte differentiation.[1,2]

Systemic biomarkers

Systemic concentrations of inflammatory biomarkers were measured in plasma samples obtained on the day of hospital admission before administration of any study medication. Samples were stored at -80°C. Analysis was performed using multiplex multi-analyte profiling (Millipore, Billerica, USA), as described previously.[3,4] Different biomarker panels were used in the Ovidius-TripleP cohort and the STEP cohort (Table 1).

REFERENCES:

1. Fine MJ, Singer DE, Hanusa BH, Lave JR, Kapoor WN. Validation of a pneumonia prognostic index using the MedisGroups Comparative Hospital Database. *Am J Med.* 1993;94(2):153-159.
2. Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, Campbell GD, Dean N, File T, Fine MJ, Gross PA, Martinez F, Marrie TJ, Plouffe JF, Ramirez J, Sarosi GA, Torres A, Wilson R, Yu VL, American Thoracic S. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. *Am J Respir Crit Care Med.* 2001;163(7):1730-1754.
3. Endeman H, Meijvis SC, Rijkers GT, van Velzen-Blad H, van Moorsel CH, Grutters JC, Biesma DH. Systemic cytokine response in patients with community-acquired pneumonia. *European Respiratory Journal.* 2011;37(6):1431-1438.
4. Urwyler SA, Blum CA, Coslovsky M, Mueller B, Schuetz P, Christ-Crain M. Cytokines and Cortisol - predictors of treatment response to corticosteroids in community-acquired pneumonia? *J Intern Med.* 2019;286(1):75-87.

VARIABLES INCLUDED IN THE LCA MODEL

Class defining variables included in the LCA of the Ovidius-TripleP cohort

Age	Urea	CRP	Interleukin-6
Systolic blood pressure	Albumin	Thrombocyte count	Interleukin-8
Diastolic blood pressure	ALAT	Hemoglobin	Interleukin-10
Symptom duration	ASAT	Hematocrit	Interleukin-12
Oxygen saturation	Alkaline phosphatase (U/L)	pH	Monocyte chemoattractant protein
Body temperature	LDH	PaO2	Macrophage inflammatory protein
Heart rate	Bilirubin	PaCO2	Tumour necrosis factor α
Respiratory rate	Glucose	Interleukin-1 receptor antagonist	Interferon gamma
PSI score	Sodium	Interleukin-5	Interleukin-12
Creatinine			

Class defining variables included in the LCA of the STEP cohort

Diastolic blood pressure	Glucose	Interleukin-4	Monocyte chemoattractant protein
Symptom duration	CRP	Interleukin-6	Tumour necrosis factor alpha
Oxygen saturation	Procalcitonin	Interleukin-8	Interferon alpha
Heart rate	Neutrophil count	Interleukin-10	Interferon beta
Respiratory rate	White blood cell count	Interleukin-12	Interferon gamma
PSI score	Interleukin-1 beta	Interleukin-13	
Creatinine	Interleukin-1 receptor antagonist	Interleukin-17	
Urea	Interleukin-2	Granulocyte-colony stimulating factor	

SUPPLEMENTARY FIGURES

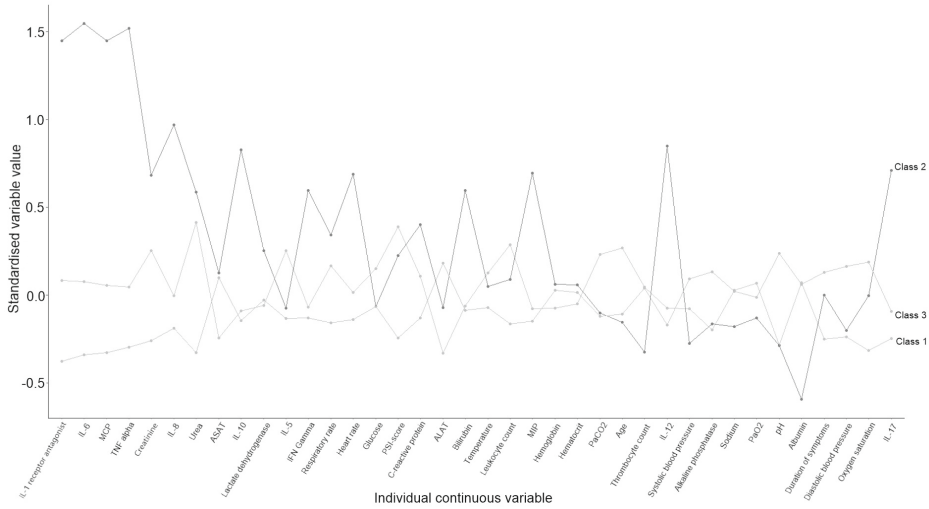


Figure E1a-1 Three-class model

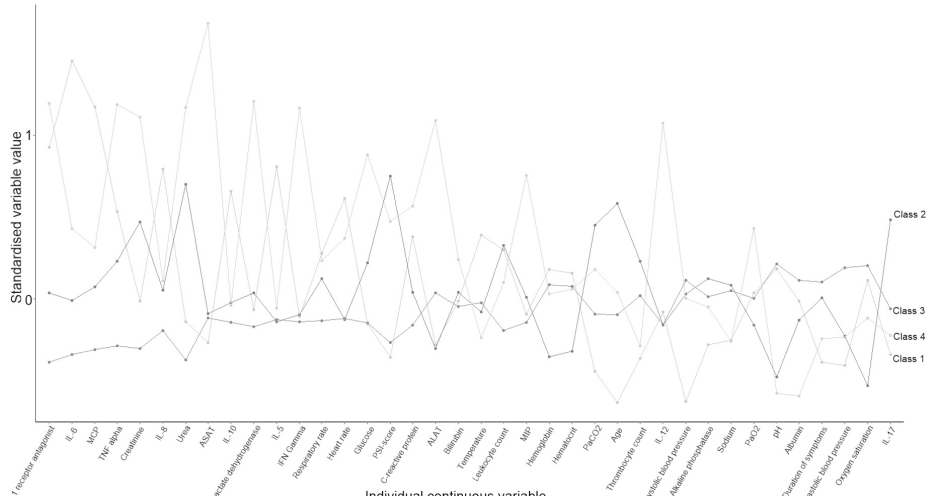


Figure E1a-2 Four-class model

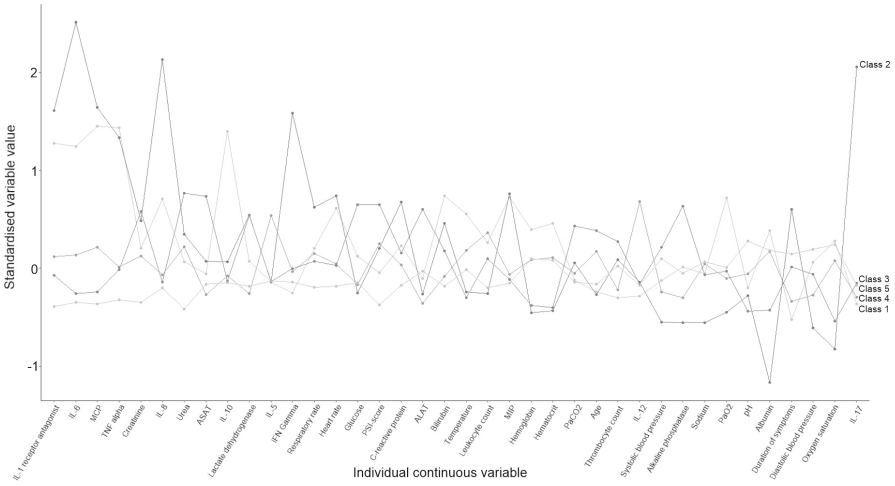


Figure E1a-3 Five-class model

Supplementary Figure E1a Continuous variables by class assignment in a three, four, or five-class model in the Ovidius-TripleP cohort.

On the Y-axis differences in the standardised values of each variable by subgroup are shown. The individual continuous variables are shown along the x-axis. Variables are sorted by degree of separation between classes.
Abbreviations: IL= interleukin; MCP= Monocyte chemoattractant protein; TNF=Tumour necrosis factor; , ASAT= Aspartate transaminase ; IFN= Interferon; PSI= Pneumonia Severity index; ALAT=Alanine transaminase; MIP= Macrophage inflammatory protein.

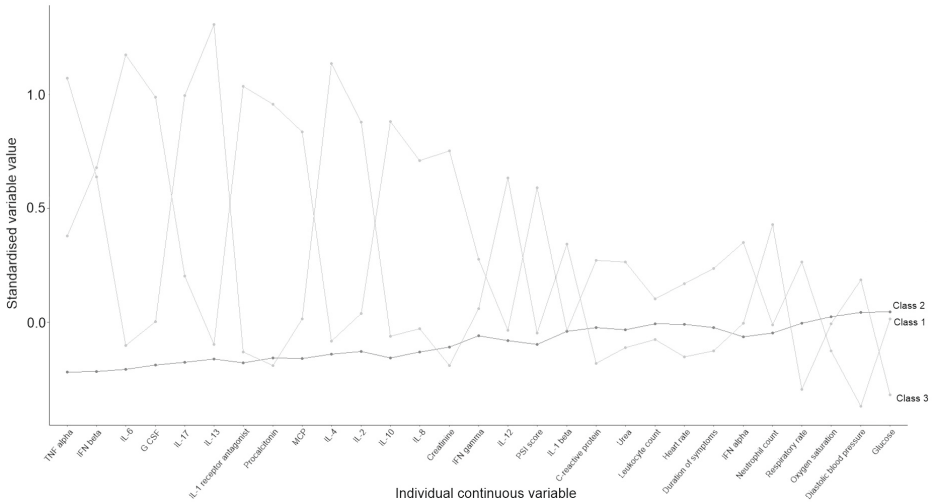


Figure E1b-1 Three-class model

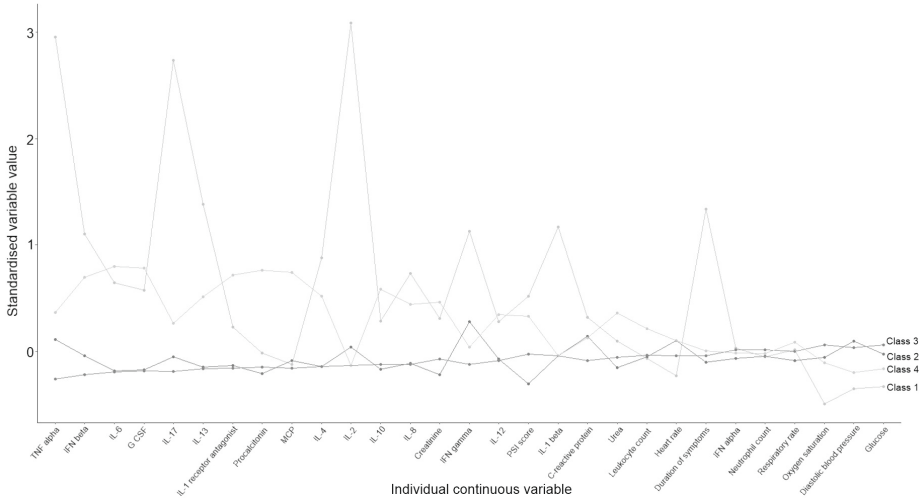


Figure E1b-2 Four-class model

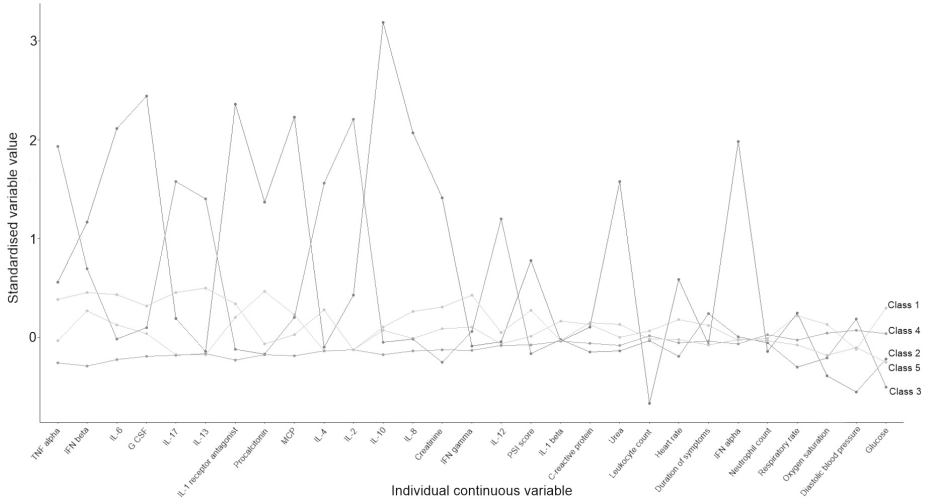


Figure E1b-3 Five-class model

Figure E1b Continuous variables by class assignment in a three, four, or five-class model in the STEP cohort.

On the Y-axis differences in the standardised values of each variable by subgroup are shown. The individual continuous variables are shown along the x-axis. Variables are sorted by degree of separation between classes.
Abbreviations: IL= interleukin; MCP= Monocyte chemoattractant protein; TNF=Tumour necrosis factor; IFN= Interferon; PSI= Pneumonia Severity index; G-CSF= Granulocyte colony-stimulating factor.

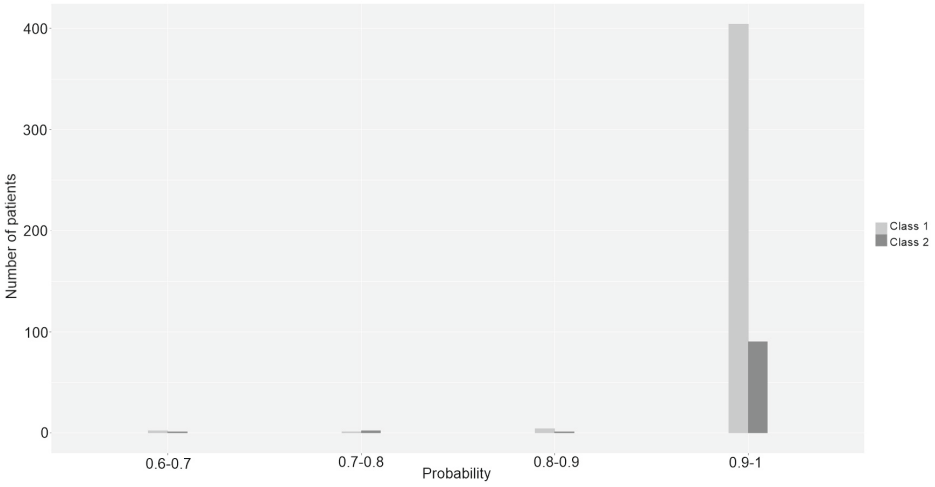


Figure E2a

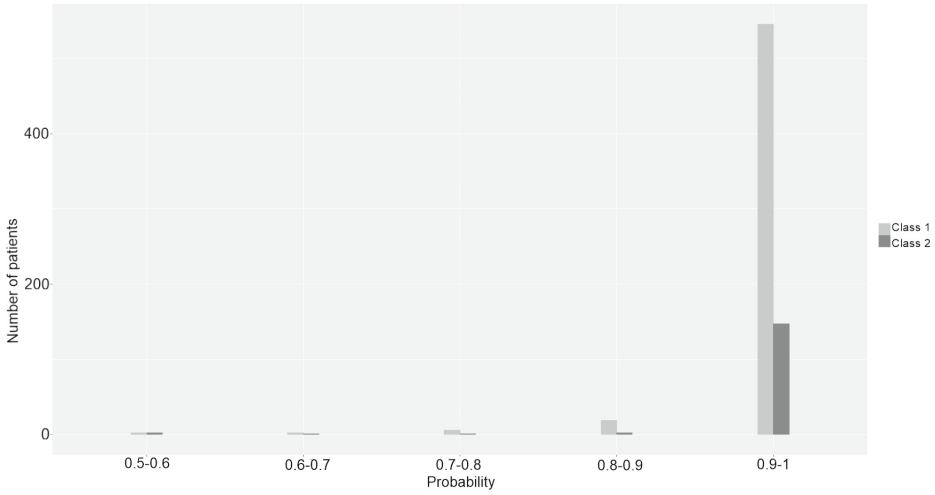


Figure E2b

Figure E2 Probability of class assignment in a two-class model for the Ovidius-TripleP cohort (Figure E2a) and the STEP cohort (Figure E2b).

In the figures above the probability of class assignment is shown on the x-axis and the number of patients on the y-axis. This figure shows that the majority of patients had a chance of 90-100% of being assigned to the correct class. For subsequent analyses, patients were assigned to the class with the highest probability of assignment.

SUPPLEMENTARY TABLES

Table E1 Baseline characteristics Ovidius-TripleP cohort and STEP cohort

	Ovidius-TripleP cohort (n = 505)	**STEP cohort (n = 727)**
Demographic data		
Age (years)	67 (51-78)	73 (60-83)
Male	295 (58.4)	452 (62.2)
Caucasian	491 (97.2)	712 (97.9)
Duration of symptoms (days)	4 (2-7)	4 (2-7)
Antibiotics at home	130 (25.7)	164 (22.6)
Corticosteroids at home	34 (6.7)	14 (1.9)
Comorbidities		
Nursing home resident	19 (3.8)	0 (0.0)
Cerebrovascular accident	46 (9.1)	67 (9.2)
Malignancy	45 (8.9)	70 (9.6)
Liver disease	2 (0.4)	28 (3.9)
Renal disease	40 (7.9)	218 (30.0)
Congestive heart failure	68 (13.5)	134 (18.4)
Chronic obstructive pulmonary disease	98 (19.4)	122 (16.8)
Diabetes mellitus	77 (15.2)	139 (19.1)
Current smoker	81 (16.0)	188 (25.9)
Clinical data		
Altered mental status*	57 (11.3)	46 (6.3)
Pleural effusion	82 (16.2)	86 (11.8)
Systolic blood pressure (mmHg)	130 (118-146)	124 (110-140)
Diastolic blood pressure (mmHg)	75 (66-82)	69 (60-78)
Heart rate (beats per minute)	97 (84-111)	83 (72-96)
Respiratory rate (breaths per minute)	24 (20-30)	20 (18-24)
Temperature (°C)	38.2 (37.4-39.0)	37.6 (37.0-38.2)
Oxygen saturation (%)	94 (91-97)	94 (92-96)
Oxygen therapy	100 (19.8)	377 (51.9)
Oxygen therapy (L/min)	1 (0-4)	2 (2-4)
Pneumonia severity index score	87 (63-114)	90 (64-113)
Routine laboratory data		
Leukocyte count (10^9 cells per L)	13.8 (9.7-18.4)	12.0 (8.8-15.6)
Neutrophil count (10^9 cells per L)	-	9.9 (6.9-13.3)
Thrombocyte count (10^9 cells per L)	250 (197-318)	-
C-reactive protein (mg/L)	210 (95-317)	160 (79-249)

Table E1 Continued

	Ovidius-TripleP cohort (n = 505)	**STEP cohort (n = 727)**
Procalcitonin (ng/mL)	-	0.46 (0.17-2.50)
Hematocrit (L/L)	0.40 (0.37-0.43)	-
Hemoglobin (mmol/L)	8.3 (7.6-9.0)	-
Urea (mmol/L)	6.8 (4.8-10.2)	6.9 (4.9-10.4)
Creatinine (µmol/L)	90 (71-112)	88 (69-113)
Sodium (mmol/L)	134 (131-137)	137 (134-139)
Glucose (mmol/L)	7.1 (6.0-8.6)	7.3 (6.3-8.9)
pH	7.47 (7.44-7.49)	-
PaO_2 (kPa)	8.7 (7.7-10.3)	-
$PaCO_2$ (kPa)	4.4 (4.1-4.9)	-
Alkaline phosphatase (U/L)	90 (68-127)	-
Aspartate transaminase (U/L)	35 (23-52)	-
Alanine transaminase (U/L)	28 (17-45)	-
Lactate dehydrogenase (U/L)	351 (255-518)	-
Bilirubin (µmol/L)	13 (9-17)	-
Albumin (g/L)	37 (33-39)	32 (28-36)
Biomarker data		
Interleukin-1 receptor antagonist (pg/mL)	163.8 (25.1-694.7)	33.0 (33.0-1126.5)
Interleukin-1 beta (pg/mL)	-	1.0 (1.0-1.0)
Interleukin-2 (pg/mL)	-	4.4 (4.4-4.4)
Interleukin-4 (pg/mL)	-	5.5 (5.5-5.5)
Interleukin-5 (pg/mL)	0.5 (0.3-0.7)	-
Interleukin-6 (pg/mL)	72.0 (22.5-248.7)	52.0 (19.0-142.8)
Interleukin-8 (pg/mL)	18.9 (9.1-42.6)	5.0 (2.0-13.0)
Interleukin-10 (pg/mL)	4.5 (1.6-14.2)	1.0 (0.7-1.9)
Interleukin-12 (pg/mL)	7.4 (4.3-10.8)	1.2 (1.1-1.7)
Interleukin-13 (pg/mL)	-	1.3 (1.3-1.3)
Interleukin-17 (pg/mL)	-	0.6 (0.6-0.6)
Tumor necrosis factor alpha (pg/mL)	6.7 (3.6-12.4)	1.7 (1.7-2.3)
Interferon alpha (pg/mL)	-	0.3 (0.3-0.4)
Interferon beta (pg/mL)	-	24.0 (15.0-41.0)
Interferon gamma (pg/mL)	205.9 (12.8-298.6)	2.8 (2.8-2.8)
Monocyte chemoattractant protein (pg/mL)	317.6 (88.5-654.2)	43.0 (27.0-84.8)
Macrophage inflammatory protein (pg/mL)	6.3 (3.9-8.8)	-
Granulocyte colony stimulating factor (pg/mL)	-	7.0 (7.0-13.0)

Table E1 Continued

	Ovidius-TripleP cohort (n = 505)	STEP cohort (n = 727)
Causative microorganism		
S. pneumoniae	124 (24.6)	106 (14.6)
H. influenzae	27 (5.3)	-
Legionella species	20 (4.0)	13 (1.8)
C. burnetii	28 (5.5)	-
Other	96 (19.0)	-
None identified	210 (41.6)	-
Outcome		
Length of stay (days)	8.5 (6.0-13.0)	7.0 (4.0-10.0)
ICU admission	38 (7.5)	39 (5.4)
In-hospital mortality	24 (4.8)	24 (3.3)
30-day mortality	26 (5.1)	28 (3.9)
Readmission	37 (7.3)	39 (5.4)

Data are n (%), mean (SD), or median (IQR). * Defined as a state of awareness that differed from the normal awareness of a conscious person, scored by the attending physician.

Table E2 Contingency tables comparing class membership in the reduced model and the full model for Ovidius-TripleP cohort and STEP cohort

		Full model	
Ovidius-TripleP		**Class 1**	**Class 2**
Reduced model	Class 1	343	26
	Class 2	68	68
		Full model	
STEP		**Class 1**	**Class 2**
Reduced model	Class 1	515	90
	Class 2	59	63

Data are n.

Table E3 Values of variables at baseline stratified by class in the Ovidius-TripleP cohort for a three-class model

Variable	Class 1 (n=153)	Class 2 (n=58)	Class 3 (n=294)
Temperature (°C)	38.4 [37.4 - 39.1]	38.3 [37.4 - 39.2]	38.1 [37.4 - 39.0]
Leukocyte count (10^9 cells per L)	15.7 [11.1- 20.6]	13.6 [9.2- 18.5]	12.6 [9.4- 16.6]
C-reactive protein (mg/L)	235 [90 - 352]	297 [110- 428]	190 [97 - 271]
Age (years)	72 [60- 81]	66 [41- 76]	63 [50 - 76]
Systolic blood pressure (mmHg)	126 [112- 146]	127 [112 - 143]	134 [120 - 147]
Diastolic blood pressure (mmHg)	70 [62 - 79]	70 [60 - 80]	77 [70 - 85]
Heart rate (beats per minute)	100 [84 - 113]	110 [99 - 126]	94 [82 - 107]
Respiratory rate (breaths per minute)	25 [20 - 30]	25 [20 - 30]	20 [18 - 30]
Hematocrit (L/L)	0.39 [0.36- 0.43]	0.40 [0.37- 0.43]	0.40 [0.37- 0.43]
Urea (mmol/L)	9.0 [6.3 – 13.7]	9.8 [6.4- 15.3]	5.7 [4.3 - 8.4]
Sodium (mmol/L)	134 [131 - 137]	133 [130 - 137]	135 [132 - 137]
Glucose (mmol/L)	7.3 [6.1 - 9.1]	7.4 [6.2- 8.6]	7.0 [6.0 - 8.3]
PaO_2 (kPa)	8.70 [7.50 - 10.80]	8.40 [7.68- 9.50]	8.90 [7.90-10.22]
$PaCO_2$ (kPa)	4.40 [4.10 - 5.10]	4.55 [4.00 - 4.93]	4.40 [4.00 - 4.73]
Creatinine (µmol/L)	99 [81 - 134]	107 [83 - 139]	82 [68 - 100]
Alkaline phosphatase (U/L)	86 [64 - 115]	80 [61 - 110]	96 [71 - 137]
Aspartate transaminase (U/L)	32 [24- 43]	47 [24 - 81]	35 [23 - 60]
Alanine transaminase (U/L)	22 [15 - 33]	28 [20 - 45]	32 [18 - 58]
Lactate dehydrogenase (U/L)	370 [265 - 489]	435 [304 - 547]	326 [248- 502]
Bilirubin (µmol/L)	13 [9 - 16]	18 [14 - 26]	12 [9 - 17]
Albumin (g/L)	37 [33 - 40]	35 [31 - 37]	37 [34 - 39]
Hemoglobin (mmol/L)	8.2 [7.5- 9.0]	8.3 [7.8 - 9.0]	8.4 [7.6 - 9.1]
Thrombocyte count (10^9 cells per L)	261 [197 - 315]	228 [177 - 292]	250 [201 - 324]
Oxygen saturation (%)	93 [90 - 97]	94 [91 - 96]	95 [92 - 97]
Duration of symptoms (days)	3 [2 - 5]	4 [2 - 6]	5 [3 - 7]
Interleukin-1 receptor antagonist (pg/mL)	387.9 [72.9- 1538.6]	1937.5 [628.4- 5823.8]	56.4 [11.4- 242.2]
Interleukin-6 (pg/mL)	220.6 [73.1 - 697.7]	1427.2 [258.1 - 2922.7]	35.6 [15.0 - 81.7]
Interleukin-8 (pg/mL)	37.2 [19.5 - 60.9]	113.6 [42.6 - 267.0]	11.5 [6.6 - 19.1]
Interleukin-10 (pg/mL)	11.1 [3.8- 28.9]	55.6 [10.9- 179.6]	2.2 [1.1- 4.8]
Pneumonia severity index score	106 [76 - 129]	95 [70 - 123]	77 [56 - 102]

Table E3 Continued

Variable	Class 1 (n=153)	Class 2 (n=58)	Class 3 (n=294)
Tumor necrosis factor alpha (pg/mL)	9.9 [6.5- 16.2]	32.2 [11.1- 49.0]	5.1 [2.6- 7.7]
Interferon gamma (pg/mL)	239.1 [21.2- 312.5]	195.0 [8.5- 406.7]	182.9 [17.1- 266.9]
Monocyte chemoattractant protein (pg/mL)	462.4 [143.9- 1122.0]	1957.5 [327.3- 3124.5]	226.9 [56.3- 425.0]
Macrophage inflammatory protein (pg/mL)	7.2 [4.9- 9.3]	7.2 [5.2- 12.2]	5.4 [3.4- 7.2]
Interleukin-12 (pg/mL)	9.3 [5.1 - 12.3]	8.5 [5.6 - 11.7]	6.5 [3.8- 10.0]
Interleukin-5 (pg/mL)	0.54 [0.32- 0.81]	0.42 [0.22- 0.60]	0.52 [0.23- 0.67]
pH	7.45 [7.42 - 7.48]	7.45 [7.42 - 7.48]	7.48 [7.45 - 7.50]
Cortisol (nmol/L)	328.6 [225.7 - 540.3]	526.7 [339.3 - 774.7]	195.8 [133.6 - 305.2]
Altered mental status	26 (17.0)	4 (6.9)	27 (9.2)
Pleural effusion	29 (19.0)	15 (25.9)	38 (12.9)
Oxygen therapy	43 (28.1)	18 (31.0)	39 (13.3)
Female	67 (43.8)	23 (39.7)	120 (40.8)

Data are n (%) or mean (SD).

Table E4 Values of variables at baseline stratified by class in the STEP cohort for a three-class model

Variable	Class 1 (n=99)	Class 2 (n=556)	Class 3 (n=72)
C-reactive protein (mg/L)	190 [72 - 294]	168 [81 - 250]	127 [67 - 210]
Diastolic blood pressure (mmHg)	65 [57 - 72]	70 [60 - 78]	69 [60 - 80]
Heart rate (beats per minute)	88 [72 - 104]	84 [73 - 95]	82 [70 - 95]
Respiratory rate (breaths per minute)	22 [18 - 26]	20 [18 - 24]	20 [16 - 24]
Urea (mmol/L)	9.3 [6.4 - 14.8]	6.6 [4.8 - 9.8]	7.0 [4.5 - 9.9]
Glucose (mmol/L)	6.5 [5.6 - 7.7]	6.5 [5.7 - 7.8]	5.8 [5.2 - 6.5]
Creatinine (µmol/L)	109 [85 - 177]	86 [67 - 108]	84 [70 - 106]
Leukocyte count (10^9 cells per L)	11.5 [7.4 - 17.1]	12.0 [8.7 - 15.9]	12.1 [9.3 - 14.6]
Oxygen saturation (%)	94 [92 - 97]	95 [92 - 96]	94 [92 - 96]
Pneumonia severity index score	106 [78 - 141]	89 [63 - 111]	82 [63 - 105]
Duration of symptoms (days)	4 [2 - 7]	4 [2 - 7]	4 [2 - 6]
Granulocyte colony stimulating factor (pg/mL)	33.0 [13.0 – 114.3]	7.0 [7.0 – 8.0]	14.0 [7.0 – 22.5]
Interferon alpha (pg/mL)	0.67 [0.39 - 1.24]	0.25 [0.25 - 0.30]	0.51 [0.27 - 1.10]
Interferon beta (pg/mL)	58.0 [34.0 - 106.5]	22.0 [14.0 – 33.0]	30.0 [17.0 – 55.0]
Interferon gamma (pg/mL)	2.8 [2.8 - 3.8]	2.8 [2.8 - 2.8]	2.8 [2.8 - 4.2]
Interleukin-1 beta (pg/mL)	1.0 [1.0 - 1.3]	1.0 [1.0 – 1.0]	1.0 [1.0 - 3.5]

Table E4 Continued

Variable	Class 1 (n=99)	Class 2 (n=556)	Class 3 (n=72)
Interleukin-1 receptor antagonist (pg/mL)	5375.0 [1466.0 - 11687.3]	33.0 [33.0 – 495.0]	33.0 [33.0 – 733.0]
Interleukin-10 (pg/mL)	3.2 [2.1 - 13.1]	0.9 [0.6 - 1.3]	1.5 [1.0 - 2.7]
Interleukin-12 (pg/mL)	1.8 [1.1 - 2.8]	1.1 [1.1 - 1.4]	2.0 [1.2 - 4.5]
Interleukin-13 (pg/mL)	1.3 [1.3 - 2.5]	1.3 [1.3 - 1.3]	4.0 [1.3 - 13.3]
Interleukin-17 (pg/mL)	0.6 [0.6 - 1.4]	0.6 [0.6 - 0.6]	0.8 [0.6 - 1.7]
Interleukin-2 (pg/mL)	4.4 [4.4 - 4.4]	4.4 [4.4 - 4.4]	4.4 [4.4 - 4.4]
Interleukin-4 (pg/mL)	5.5 [5.5 - 6.9]	5.5 [5.5 - 5.5]	9.0 [5.5 – 32.6]
Interleukin-6 (pg/mL)	540.5 [125.5 - 1422.5]	41.0 [15.0 – 97.0]	73.0 [28.5 - 170.5]
Interleukin-8 (pg/mL)	39.0 [17.8 – 81.0]	4.0 [2.0 – 9.0]	7.0 [4.0 - 16.5]
Monocyte chemoattractant protein (pg/mL)	168.0 [71.3 - 400.3]	39.0 [25.0 – 66.0]	45.0 [27.0 - 74.5]
Tumor necrosis factor alpha (pg/mL)	2.8 [1.7 - 3.9]	1.7 [1.7 - 1.8]	2.5 [1.7 - 3.5]
Procalcitonin (ng/mL)	3.00 [0.60 - 26.36]	0.38 [0.16 - 1.88]	0.39 [0.16 - 1.14]
Neutrophil count (10^9 cells per L)	10.8 [6.6 - 15.4]	9.8 [6.9 - 13.3]	10.1 [7.6 - 12.1]
Altered mental status	8 (8.1)	31 (5.6)	7 (9.7)
Pleural effusion	8 (8.1)	58 (10.4)	17 (23.6)
Oxygen therapy	60 (60.6)	264 (47.5)	53 (73.6)
Female	31 (31.3)	206 (37.1)	38 (52.8)

Data are n (%) or mean (SD).

Table E5 Association between class assignment and clinical outcomes for a three-class model for both cohorts

Ovidius-TripleP cohort				
Clinical outcome	**Class 1 (n = 153)**	**Class 2 (n = 58)**	**Class 3 (n = 294)**	**p-value**
Length of stay (days)	9.0 (7.0-14.0)	10.3 (6.0-23.8)	8.0 (5.5-11.5)	<0.01
ICU admission	12 (7.8)	14 (24.1)	12 (4.1)	<0.01
In-hospital mortality	11 (2.7)	6 (10.3)	7 (2.4)	<0.01
30-day mortality	13 (8.5)	6 (10.3)	7 (2.4)	<0.01
Readmission	11 (7.2)	4 (6.9)	22 (7.5)	0.98
STEP cohort				
Clinical outcome	**Class 1 (n = 99)**	**Class 2 (n = 556)**	**Class 3 (n = 72)**	**p-value**
Length of stay (days)	8.0 (5.0-13.0)	7.0 (4.0-10.0)	7.0 (5.0-10.3)	<0.01
ICU admission	12 (12.1)	26 (4.7)	1 (1.4)	<0.01
In-hospital mortality	11 (11.1)	11 (2.0)	2 (2.8)	<0.01
30-day mortality	10 (10.1)	16 (2.9)	2 (2.8)	<0.01
Readmission	8 (8.1)	27 (4.9)	4 (5.6)	0.42

Data are N (%) or median (IQR). ICU intensive care unit.

CHAPTER 5

Latent class analysis-based subgroups and response to corticosteroids in hospitalised community-acquired pneumonia patients: a validation study

E Wittermans, PA van der Zee*, H Qi, JC Grutters, GP Voorn, WJW Bos, EMW van de Garde, H Endeman*
**Authors contributed equally*

ERJ OPEN RES 2023;9(2):00577-2022

To the editor:

Latent class analysis (LCA), a statistical method to identify 'hidden' subgroups within a population, has identified clinically distinct subgroups with treatment implications in acute respiratory distress syndrome and COVID-19. [1–3] We recently showed that LCA could also identify two clinically distinct subgroups in community-acquired pneumonia (CAP).[4] In two independent cohorts, [5,6] LCA identified a subgroup with more excessive systemic inflammation and worse prognosis (class 2), and a subgroup with less systemic inflammation and better prognosis (class 1). In one of the two cohorts, the Ovidius cohort, we also observed a greater effect of adjunctive dexamethasone on length of stay (LOS) in class 2 compared to class 1. The aim of the present study was to validate the existence of LCA defined subgroups in a third, more recent CAP cohort. And if subgroups prove robust, to validate the finding from the Ovidius cohort that subgroups respond differently to adjunctive corticosteroids.

We conducted a LCA of data from the Santeon-CAP trial (N = 401), a Dutch multicentre placebo-controlled randomised trial investigating the effect of a 4-day course of 6 mg oral dexamethasone on LOS in non-intensive care unit (ICU) patients hospitalised with CAP (NCT 01743755). All patients received study medication within 24 h of hospital admission. Further details on study population characteristics, aetiologies, inclusion and exclusion criteria and specifics of the intervention can be found in the original publication of the Santeon-CAP study.[7] Clinical and laboratory parameters on admission were available as part of the original study protocol. Concentrations of five systemic cytokines were measured in stored (at -80°C) blood samples collected at admission (prior to randomisation) using a Luminex multiplex assay (R&D Systems, Minneapolis, USA).

LCA was conducted using the DepmixS4 package in R 4.0.0 (R core team, 2020). We aimed to replicate the LCA model from the Ovidius cohort. Where available, the same class-defining variables were used. Thirteen out of 37 variables used in the Ovidius LCA were not available for the Santeon-CAP population: pH, arterial pO2 and pCO2, duration of symptoms, glucose, lactate dehydrogenase, alkaline phosphatase, bilirubin, glucose, interleukin (IL)-5, IL-10, IL-12 and interferon-g. Class-defining variables included in the current LCA are shown on the X-axis of Figure 1. Missing data were accommodated by estimating model parameters based on the full information maximum likelihood.[8] For the LCA, we used the same procedures as in our previous study.[4] In short, we fitted models with two to five latent classes and subsequently identified the best fitting model (or put differently the optimal number of classes) using the following criteria 1) clinical interpretability, 2) number of patients in the smallest class, and 3) model fit based on the Bayesian Information Criterion (BIC). After determining the optimal number of classes, patients were assigned to the class with the maximum probability of class assignment based on the LCA model. Next, median LOS and 30-day mortality and ICU admission rates were compared between classes. These outcomes were chosen as these were

the predefined outcomes of the Santeon-CAP study. To test for differences between subgroups a Chi-squared test was used for categorical outcomes and a Mann-Whitney U was used for LOS. Last, we tested for interaction between class assignment and treatment allocation using a Poisson regression model for LOS, and Chi-squared test for categorical outcomes.

After plotting class-defining variables for all models, the plot of a two-class model showed two clinically coherent and distinct classes (Figure 1). Addition of more classes did not result in an additional clinically distinct subgroup. BIC was lowest in a four-class model (81236.98 compared to 83105.67 in the two-class model), indicating better model fit. Yet, addition of a third or fourth class did not result in an extra clinically distinct subgroup. So, although a data driven approach would suggest selection of a model with >2 classes, we chose to prioritise clinical interpretability and proceeded with a two-class model. Three hundred seventeen patients were assigned to Class 1 and 84 patients were assigned to Class 2. Average probability of class assignment was 99.9% for class 1 and 99.3% for class 2, indicating good model fit and robust class assignment.

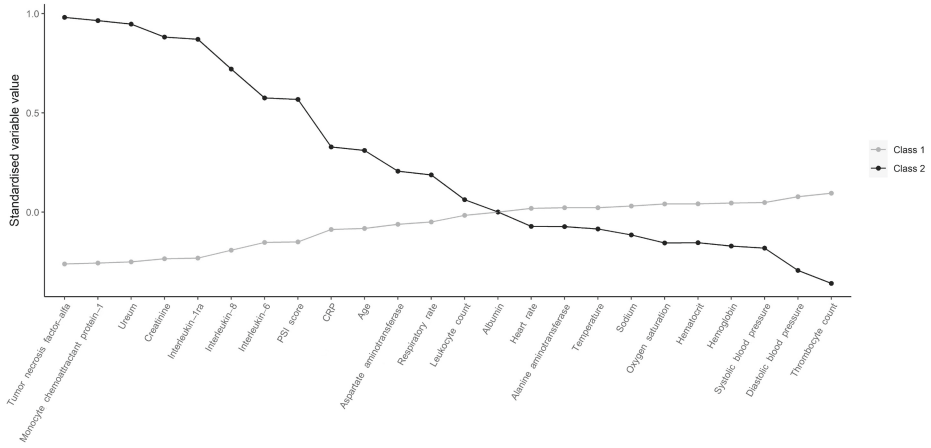


Figure 1 shows the standardised value (y-axis) for each variable (x-axis) by class. A standardised value of 1 for a class indicates that the mean value for that variable within that class was one standard deviation higher than the mean value for that variable in the whole cohort. Variables are sorted by the degree of separation between classes: from the maximum positive separation on the left (where the standardised value of class 2 is higher than the standardised value of class 1) to the maximum negative separation on the right (where the standardised value of class 2 to is lower than the standardised value of class 1). Thus the variables at the far left and far right of the x-axis are the variables that distinguish most between both classes. Variables in the middle of the x-axis differ least between classes or are the same in both classes (where the lines for both classes intersect).

Class 2 patients had higher systemic concentrations of all inflammatory cytokines, higher creatinine levels and lower diastolic blood pressure compared to class 1 patients (Figure 1). In class 2, median LOS was longer (6.0 (IQR 4.0-9.0) vs 5.0 (IQR 3.5-7.0) days; p = ≤0.01), and ICU admission rate (9.5% vs 3.5%; p =0.04) and 30-day mortality rate (8.3% vs 1.3%; p =0.01) were higher. There was no difference in response to adjunctive dexamethasone treatment between classes; median LOS for dexamethasone vs placebo was 4.5 (IQR 3.0-6.5) vs 5.0 (IQR 3.5 – 7.0) days for class 1 and 5.8 (IQR 4.0 – 7.5) vs 7.5 (IQR 5.0 – 9.8) days in class 2 (p-value for interaction 0.38). ICU admission rate for dexamethasone vs placebo was 1.9% vs 5.1% in class 1 and 4.8% vs 14.3% in class 2 (p for interaction 1.00). 30-day mortality rate was 0.6% vs 1.9% in class 1 and 7.1% vs 9.5% in class 2 (p-value for interaction 1.00).

Similar to our previous study, we identified two clinically distinct CAP subgroups: one subgroup with signs of excessive systemic inflammation and worse clinical outcomes (class 2), and one subgroup with less systemic inflammation and better clinical outcomes (class 1). This indicates that subgroups identified by our LCA model of baseline clinical and inflammatory parameters are robust. Yet, in the present study, we could not replicate our previous finding of greater response to corticosteroids in class 2 compared to class 1 despite a similar population with non-ICU patients, similar disease severity, and similar dexamethasone dose as in the Ovidius trial.[9]

In line with other studies, patients with the highest inflammatory biomarker concentrations (class 2) had worse outcomes.[10] From a biological perspective it would make sense that the effect of corticosteroids would be larger in patients in class 2.[11] Yet in the present study, the effect of dexamethasone did not differ between classes. For this, we propose several hypotheses. First, it may be due to too small sample size in class 2 (n = 84) combined with a relatively short median LOS in the Santeon-CAP cohort. This may have led to insufficient statistical power to show a difference in dexamethasone effect on LOS between classes. Second, it has been demonstrated that the host response can show signs of concurrent hyperinflammation (high plasma biomarker concentrations) and immune suppression (reduced cytokine production capacity of blood leukocytes) in CAP. [12] One could hypothesise that corticosteroid treatment in patients with concurrent immune suppression would not be beneficial.

Another hypothesis explaining the absence of differential effect of corticosteroids between classes is that only high levels of certain inflammatory mediators contribute to lung injury and sepsis while other mediators are essential for combating infection. Corticosteroids downregulate numerous inflammatory mediators and thus may also inhibit essential parts of the inflammatory response. Further research is needed to investigate whether targeted immunomodulation would be more appropriate. In sepsis, corticosteroid resistance is an issue; it has been proposed that this might contribute to the conflicting results in corticosteroid trials in patients with sepsis.[13] Yet, whether

corticosteroid resistance plays a role in CAP, and specifically in our population of patients with moderate disease, is unclear.

Nonetheless, we consistently showed that LCA can identify patients with poor prognosis. The main limitations of the present study are the small number of patients in class 2 and the fact that not all class-defining variables used in the Ovidius study were available for the Santeon-CAP study. However, variables that differentiated most between class 1 and class 2 in the Ovidius cohort, were included in the present study. Furthermore, inflammation is a dynamic process. Inflammatory parameters measured at admission only provide a 'snapshot' of this process. It could be possible that patients with similar inflammatory values on admission are in different phases of the inflammatory response. Relative to admission, timing of the initiation of dexamethasone was the same for all patients, yet relative to the phase of the inflammatory response timing could have differed between patients. Lastly, the Santeon-CAP study only included non-ICU patients; thus, these results might not be generalisable to ICU patients.

In conclusion, in patients with CAP, LCA can identify robust prognostic subgroups based on clinical and inflammatory parameters. Yet, these subgroups have not proven robust in predicting response to adjunctive dexamethasone treatment.

FUNDING

St. Antonius Research Fund

REFERENCES

1. Sinha P, Furfaro D, Cummings MJ, et al. Latent Class Analysis Reveals COVID-19-related ARDS Subgroups with Differential Responses to Corticosteroids. *Am J Respir Crit Care Med*. 2021;204(11):1274-1285. doi:10.1164/rccm.202105-1302OC
2. Calfee CS, Delucchi KL, Sinha P, et al. Acute respiratory distress syndrome subphenotypes and differential response to simvastatin: secondary analysis of a randomised controlled trial. *Lancet Respir Med*. 2018;6(9):691-698. doi:10.1016/S2213-2600(18)30177-2
3. Sinha P, Delucchi KL, Thompson BT, McAuley DF, Matthay MA, Calfee CS. Latent class analysis of ARDS subphenotypes: a secondary analysis of the statins for acutely injured lungs from sepsis (SAILS) study. *Intensive Care Med*. 2018;44(11):1859-1869. doi:10.1007/S00134-018-5378-3
4. Wittermans E, van der Zee PA, Qi H, et al. Community-acquired pneumonia subgroups and differential response to corticosteroids: a secondary analysis of controlled studies. *ERJ Open Res*. 2022;8(1):00489-02021. doi:10.1183/23120541.00489-2021
5. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: A multicentre, double-blind, randomised, placebo-controlled trial. *The Lancet*. 2015;385(9977):1511-1518. doi:10.1016/S0140-6736(14)62447-8
6. Meijvis SC, Hardeman H, Remmelts HH, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. *The Lancet*. 2011;377(9782):2023-2030. doi:10.1016/S0140-6736(11)60607-7
7. Wittermans E, Vestjens SM, Spoorenberg SM, et al. Adjunctive treatment with oral dexamethasone in non-ICU patients hospitalised with community-acquired pneumonia: A randomised clinical trial. *Eur Respir J*. 2021;58(2):2002535. doi:10.1183/13993003.02535-2020
8. Cham H, Reshetnyak E, Rosenfeld B, Breitbart W. Full Information Maximum Likelihood Estimation for Latent Variable Interactions With Incomplete Indicators. *Multivariate Behav Res*. 2017;52(1):12-30. doi:10.1080/00273171.2016.1245600
9. Spoorenberg SMC, Deneer VHM, Grutters JC, et al. Pharmacokinetics of oral vs. intravenous dexamethasone in patients hospitalized with community-acquired pneumonia. *Br J Clin Pharmacol*. 2014;78(1):78-83. doi:10.1111/BCP.12295
10. Ramírez P, Ferrer M, Martí V, et al. Inflammatory biomarkers and prediction for intensive care unit admission in severe community-acquired pneumonia. *Crit Care Med*. 2011;39(10):2211-2217. doi:10.1097/CCM.0B013E3182257445
11. Remmelts HHF, Meijvis SCA, Biesma DH, et al. Dexamethasone downregulates the systemic cytokine response in patients with community-acquired pneumonia. *Clin Vaccine Immunol*. 2012;19(9):1532-1538. doi:10.1128/CVI.00423-12
12. Brands X, Haak BW, Klarenbeek AM, et al. Concurrent Immune Suppression and Hyperinflammation in Patients With Community-Acquired Pneumonia. *Front Immunol*. 2020;11:796. doi:10.3389/FIMMU.2020.00796
13. Vandewalle J, Libert C. Glucocorticoids in Sepsis: To Be or Not to Be. *Front Immunol*. 2020;11:1318. doi:10.3389/fimmu.2020.01318

CHAPTER 6

Overweight and obesity are not associated with worse clinical outcomes in COVID-19 patients treated with fixed-dose 6 mg dexamethasone

E Wittermans, JC Grutters, HS Moeniralam, G Ocak, GP Voorn, WJW Bos, EMW van de Garde

INT J OBES (LOND). 2022;46(11):2000-2005.

ABSTRACT

Objective

A fixed 6 mg dexamethasone dose for 10 days is the standard treatment for all hospitalised COVID-19 patients who require supplemental oxygen. Yet, the pharmacokinetic properties of dexamethasone can lead to diminishing systemic dexamethasone exposure with increasing body mass index (BMI). The present study examines whether this translates to overweight and obesity being associated with worse clinical outcomes, defined as ICU admission or in hospital death, in COVID-19 patients treated with fixed-dose dexamethasone.

Methods

We conducted a single centre retrospective cohort study in COVID-19 patients who were admitted to a non-ICU ward and were treated with dexamethasone (6 mg once daily for a maximum of ten days) between June 2020 and January 2021. Univariable and multivariable logistic regression analyses were conducted to assess the association between BMI-categories and an unfavourable clinical course (ICU admission and/or in hospital death). Analyses were adjusted for age, comorbidities, inflammatory status, and oxygen requirement at admission. For reference, similar analyses were repeated in a cohort of patients hospitalised before dexamethasone was introduced (March 2020 through May 2020).

Results

In patients treated with dexamethasone (n= 385) an unfavourable clinical course was most prevalent in patients with normal weight (BMI < 25) compared to patients with overweight (BMI 25–30) and patients with obesity (BMI ≥ 30) with percentages of 33, 26 and 21% respectively. In multivariable analyses, there was no association between BMI-category and an unfavourable clinical course (respectively with aORs of 0.81 (0.43–1.53) and 0.61 (0.30–1.27) with normal weight as reference). In the reference cohort (n = 249) the opposite was observed with an unfavourable clinical course being most prevalent in patients with overweight (39% vs 28%; aOR 2.17 (0.99–4.76)). In both cohorts, CRP level at admission was higher and lymphocyte count was lower in patients with normal weight compared to patients with obesity.

Conclusions

Overweight and obesity are not associated with an unfavourable clinical course in COVID-19 patients admitted to a non-ICU ward and treated with 6 mg dexamethasone once daily.

INTRODUCTION

The RECOVERY TRIAL and the WHO REACT meta-analysis showed that corticosteroids reduced mortality and the need for mechanical ventilation in patients with severe COVID-19.[1,2] After these publications, a course of 6 mg dexamethasone for 10 days was added to the WHO and Dutch national COVID-19 guidelines as standard treatment for hospitalised COVID-19 patients requiring supplemental oxygen.[3,4] The rationale being that severe COVID-19 (defined as an oxygen saturation <94% on ambient air), is caused by dysregulation of the host immune response. This dysregulation can lead to inflammatory organ injury and subsequently unfavourable clinical outcomes.[5] Dexamethasone and other corticosteroids are potent non-specific inhibitors of the immune system.[6] Therefore, they are thought to attenuate the dysregulated immune response in severe COVID-19 and thereby prevent (further) organ damage. Yet even with widespread use of dexamethasone, COVID-19 related morbidity and mortality remain high, and COVID-19 is still a severe burden on healthcare systems.[7–9] A high body mass index (BMI) has been identified as an important risk factor for ICU admission and mortality in hospitalised COVID-19 patients.[10,11] It is therefore important to identify opportunities for improvement of treatment for COVID-19 patients with overweight and obesity. Considering the lipophilic nature of dexamethasone and its relatively large volume of distribution, one might argue that a fixed dose of dexamethasone is less effective in individuals with obesity compared to individuals with normal weight because of lower serum blood levels.[12–14] Besides pharmacokinetic differences, obesity has also been linked to higher inflammatory states in COVID-19 further adding to a potential diminishing relative effectiveness of dexamethasone with increasing body mass index.[15] To the best of our knowledge, none of the dexamethasone trials in COVID-19 patients have conducted subgroup analyses based on BMI. It remains unclear if individuals with overweight or obesity respond differently to the currently recommended fixed dose of dexamethasone 6 mg compared to individuals with normal weight.

The aim of the present study was to examine whether overweight and obesity are associated with worse clinical outcomes (ICU admission and/or in hospital death) in non-ICU patients treated with fixed-dose dexamethasone for COVID-19 compared to patients with normal weight.

METHODS

Study design and study population

We conducted a retrospective cohort study including hospitalised adults (≥18 years of age) with confirmed COVID-19 in a 750-bed teaching hospital (St. Antonius Hospital, Nieuwegein, the Netherlands). We identified two cohorts. First, a cohort with patients who were admitted between June 1st 2020 and January 17th 2021, and were treated with dexamethasone 6 mg in accordance with local and national guidelines.[3] As of June 2020, guidelines stated that treatment with dexamethasone 6 mg once daily (oral or intravenously) should be given to all hospitalised patients with an oxygen saturation <94% on ambient air for 10 days or until hospital discharge. Patients who were admitted to the ICU on the date of emergency department presentation, patients who were transferred to our hospital from a different hospital and patients with missing BMI data were excluded.

The second cohort consisted of patients who were admitted prior to the implementation of the dexamethasone protocol (from March 1st 2020 thru May 31st 2020) and did not receive corticosteroid treatment but did require supplemental oxygen, thus patients who would have had an indication for corticosteroid treatment according to the guidelines implemented after June 1st 2020. Also in this cohort, we excluded patients who were directly admitted to the ICU and patients who were transferred from another hospital. In the remainder of the manuscript, we will refer to the cohorts as the dexamethasone cohort and the historical cohort.

The study was reviewed by the Medical Ethics Committee at the St. Antonius Hospital (No. W21.127), and the need for informed consent was waived due to the retrospective nature of the study and anonymous handling of data.

Data collection

All data analysed in this study were extracted from the hospital's COVID-19 database. This database contains clinical and outcome data for all patients admitted to our hospital with COVID-19. Data was available from time of hospital admission to time of hospital discharge. For patients who were admitted multiple times with COVID-19, we only included the data from the first hospital admission. All records in the database were manually checked to ensure that COVID-19 was the main reason for hospital admission and that patients either had a positive PCR test for COVID-19 prior to hospital admission or upon hospital admission. All variables except Charlson Comorbidity Index (CCI) were directly available from the COVID-19 database. The CCI was calculated using ICD-10 codes from the COVID-19 database for which the coding algorithm by Quan et al.[16] was used.

Data analysis

The primary study outcome was an unfavourable clinical course, which was defined as ICU admission and/or in hospital death. We explored the association between BMI, inflammatory state at admission and an unfavourable clinical course. Lymphocyte count and C-reactive protein (CRP) concentration were used as indicators of inflammation as a lower lymphocyte count and higher CRP concentration have both been associated with a higher level of systemic inflammation in COVID-19 patients.[17,18] Analyses were primarily conducted in the dexamethasone cohort. The historical cohort was used as a reference cohort to put the findings in the dexamethasone cohort into perspective of a non-treated population.

Statistical analyses were conducted using SPSS 26.0. Overall, continuous variables are shown as mean (SD) or median [IQR] depending on distribution. Categorical variables are shown as n (%). Patients were divided into three subgroups based on BMI: patients with normal weight (BMI < 25 kg/m^2), patients with overweight (BMI 25–29.9 kg/m^2), and patients with obesity (BMI > 30 kg/m^2). First, differences in inflammatory state at admission between BMI subgroups was explored. We calculated the median lymphocyte count and median CRP concentration for each BMI subgroup. A Kruskall-Wallis test was used to compare medians. Next, we calculated percentages of patients with an unfavourable clinical course within each BMI subgroup. Subsequently, logistic regression analysis was done with unfavourable clinical course as dependent variable. Associations with BMI-categories were adjusted for age, CCI, CRP level, lymphocyte count and oxygen requirement at admission. CRP was stratified using a cut-off of 75 mg/L this was based on the cut-off used by other COVID-19 studies as a criterion for a heightened inflammatory response.[19] Lymphocyte count was stratified using a cut-off of 0.70 10^9 cells/L (indicating more severe lymphocytopenia). Due to lack of a homogenous cut-off value in literature, this value was determined by ROC analysis of lymphocyte count and an unfavourable clinical course. Oxygen requirement was based on the method of oxygen delivery at admission and was grouped into three groups: (1) Nasal cannula (<6 L O_2) or mask (2) Venturi mask or nasal cannula (≥6 L O_2) and (3) Non-rebreathing mask or high flow nasal cannula (Optiflow).

RESULTS

Patient selection and baseline characteristics for both cohorts

Between June 2020 and January 2021, 715 patients with confirmed COVID-19 were admitted to our hospital of which 385 met the inclusion criteria for the dexamethasone cohort (Figure 1). Median duration of corticosteroid therapy was 6 days (IQR 4.0–10.0 days). Prior to June 2020, 391 patients with confirmed COVID-19 were admitted to our hospital of which 249 met the inclusion criteria for the historical cohort (Figure 1). Baseline characteristics for both cohorts are shown in Table 1. Demographics and baseline clinical characteristics of the historical cohort were similar to the dexamethasone cohort.

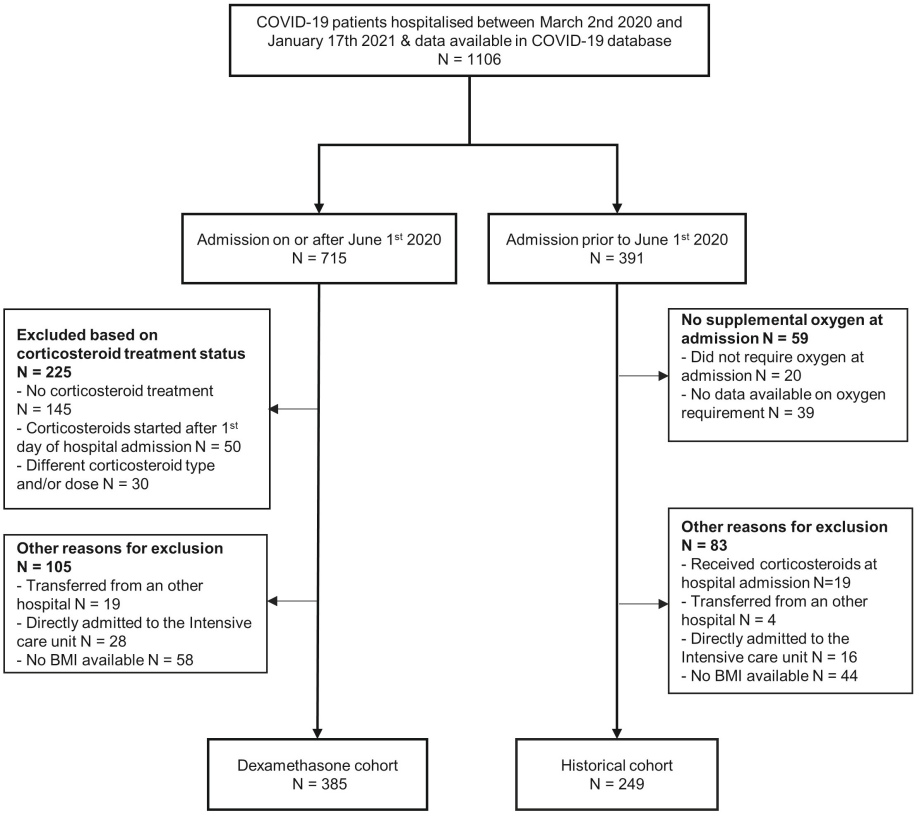


Figure 1 Flowchart showing the process of patient selection for the dexamethasone cohort and the historical cohort.

Table 1 Population characteristics

	Dexamethasone cohort N = 385	**Historical cohort N = 249**
Demographics		
Age (years)	67 (13)	64 (16)
Male	235 (61.0)	162 (65.1)
Body mass index mean (kg/m^2)	28.6 (5.1)	28.5 (5.2)
Body mass index <25 kg/m^2	86 (22.3)	58 (23.3)
Body mass index 25-29.9 kg/m^2	176 (45.7)	109 (43.8)
Body mass index ≥ 30 kg/m^2	123 (31.9)	82 (32.9)
Comorbidities		
Hypertension	113 (29.4)	70 (28.1)
Diabetes Mellitus	92 (23.9)	57 (22.9)
Chronic pulmonary disease	59 (15.3)	27 (10.8)
Charlson comorbidity index score ≥ 3	80 (20.8)	46 (18.5)

Table 1 Continued

	Dexamethasone cohort N = 385	Historical cohort N = 249
Measurement at admission		
Oxygen saturation (%)	94 [92 - 96]	95 [93-97]
Respiratory rate (breaths/min)*	23 (6)	23 (6)
Heartrate (bpm)	92 (18)	93 (17)
Systolic blood pressure (mmHg)	136 (22)	136 (23)
Diastolic blood pressure (mmHg)*	75 (12)	78 (14)
Body temperature (°C)	37.8 (1.9)	37.9 (1.1)
Presence of ≥ 2 SIRS criteria*	193 (50.1)	151 (60.6)
Method of oxygen delivery*		
None	3 (0.8)	-
Nasal Cannula <6L O2	333 (86.5)	203 (81.5)
Mask	11(2.9)	18 (7.2)
Venturi mask	2 (0.5)	1 (0.4)
Nasal cannula ≥ 6L O2	8 (2.1)	1 (0.4)
Non rebreathing mask	23 (6.0)	24 (9.6)
Optiflow/airvo	1 (0.3)	1 (0.4)
Laboratory results at admission*		
C-reactive protein concentration (mg/L)	82 [46 - 134]	79 [39 - 139]
White blood cell count (10^9 cells/L)	6.8 [5.0 – 9.1]	6.9 [5.3 – 9.3]
Lymphocyte count (10^9 cells/L)	0.84 [0.62 - 1.18]	0.85 [0.59 – 1.20]
Estimated glomular filtration rate (EPI)	76 [54 - 90]	78 [54 - 90]

Data are shown as n (%), mean (SD) or median [IQR]. *Missing data for dexamethasone cohort n (%): respiratory rate 1 (0.3%), diastolic blood pressure 1 (0.3%), SIRS criteria 2 (0.5%), Method of oxygen delivery 4 (1.0%), C-reactive protein 7 (1.8%), White blood cell count 6 (1.6%), lymphocyte count 16 (4.2%), Kidney function 6 (1.6%). Missing data for historical cohort n (%): Lymphocyte count 11 (4.4%), White blood cell count 2 (0.8%), eGFR 3 (1.2%), SIRS 1 (0.4%)

Clinical outcomes

In the dexamethasone cohort 65 (16.9%) patients were admitted to the ICU, and 46 (11.9%) patients died in hospital. Eleven patients died after ICU admission. A total of 100 (26.0%) patients met the combined outcome of ICU admission and/or in hospital death. In the historical cohort, 43 (17.3%) patients were admitted to the ICU and 57 (22.9%) died in hospital. Eighteen patients died after ICU admission. A total of 82 (32.9%) patients met the combined outcome of ICU admission and/or in hospital death.

Body mass index, inflammatory state at admission and clinical course

In the dexamethasone cohort, median CRP concentration was lowest in patients with obesity and highest in patients with normal weight. For lymphocyte count the

opposite was observed, patients with obesity had higher lymphocyte counts compared to patients with normal weight. Although not statistically significant, numerically, the same trend was seen in the historical cohort (Table 2).

Table 2 Inflammatory parameters at admission by body mass index category

	Dexamethasone cohort				Historical cohort			
	CRP	**P**	**Lymphocyte count**	**P**	**CRP**	**P**	**Lymphocyte count**	**P**
BMI < 25 kg/m^2	97 [54-150]	0.018	0.68 [0.52-0.96]	<0.001	96 [62-133]	0.25	0.74 [0.47-1.21]	0.075
BMI 25 to 29.9 kg/m^2	81 [52-146]		0.85 [0.64-1.18]		82 [39-144]		0.85 [0.59-1.16]	
BMI ≥ 30 kg/m^2	69 [37-118]		0.89 [0.70 -1.33]		61 [35-138]		0.97 [0.69-1.34]	

Data are shown as Median [IQR]. *P*-values(Kruskall-wallis) represent difference in median CRP or Lymphocyte count between BMI categories within each cohort.

In the dexamethasone cohort, the percentage of ICU admission and/or in hospital death was higher in patients with normal weight than in patients with overweight and patients with obesity (33% vs 26% vs 21%; p for trend = 0.07), though this difference between groups did not reach statistical significance (Figure 2). The unadjusted odds ratio for an unfavourable clinical course was 0.73 (95% CI 0.41–1.29; p = 0.28) for patients with overweight compared to patients with normal weight, and 0.56 (95% CI 0.30–1.04; p = 0.07) for patients with obesity compared to patients with normal weight. In the multivariable model, there was also no association between BMI and an unfavourable clinical course after adjusting for age, CCI, inflammatory status and oxygen requirement (Table 3). In the historical cohort, the rate of ICU admission and/or in hospital death was higher in patients with overweight (39%), compared to patients with normal weight (28%) or patients with obesity (29%) (Figure 2). The unadjusted odds ratio for an unfavourable clinical course was 1.65 (95% CI 0.82–3.29; p = 0.16) for patients with overweight compared to patients with normal weight, and 1.09 (95% CI 0.52–2.29; p = 0.83) for patients with obesity compared to patients with normal weight. In the multivariable model, there was no association between BMI subgroups and ICU admission and/or in hospital death (Table 3).

Regarding inflammatory state at admission, in the multivariable model both the presence of a CRP concentration ≥75 mg/L and lymphocyte count <0.70 10^9 cells/L at admission were associated with an unfavourable clinical course in the dexamethasone cohort but not in the historical cohort (Table 3).

Table 3 Multivariable analysis of the association between BMI subgroups, C-reactive protein, lymphocyte count and ICU admission and/or in hospital death

	Dexamethasone cohort		**Historical cohort**	
	Odds ratio (95% CI)	**P**	**Odds ratio (95% CI)**	**P**
Body mass index				
Body mass index <25 kg/m^2 (ref)				
Body mass index 25 to 29.9 kg/m^2	0.81 (0.43 – 1.53)	0.52	2.17 (0.99 – 4.76)	0.054
Body mass index ≥ 30 kg/m^2	0.61 (0.30 – 1.27)	0.19	1.79 (0.75 – 4.23)	0.19
C-reactive protein concentration				
CRP <75 mg/L (ref)				
CRP ≥75 mg/L	1.73 (1.01 – 2.96)	0.046	1.69 [0.91 – 3.17]	0.10
Lymphocyte count				
Lymphocyte count ≥0.7 10^9 cells/L (ref)				
Lymphocyte count <0.7 10^9 cells/L	1.95 (1.15 – 3.31)	0.013	0.78 [0.41 – 1.50]	0.46

Analysis was adjusted for age, CCI and oxygen requirement upon admission. Numbers analysed: dexamethasone cohort 363; Historical cohort 238. CRP: C-reactive protein.

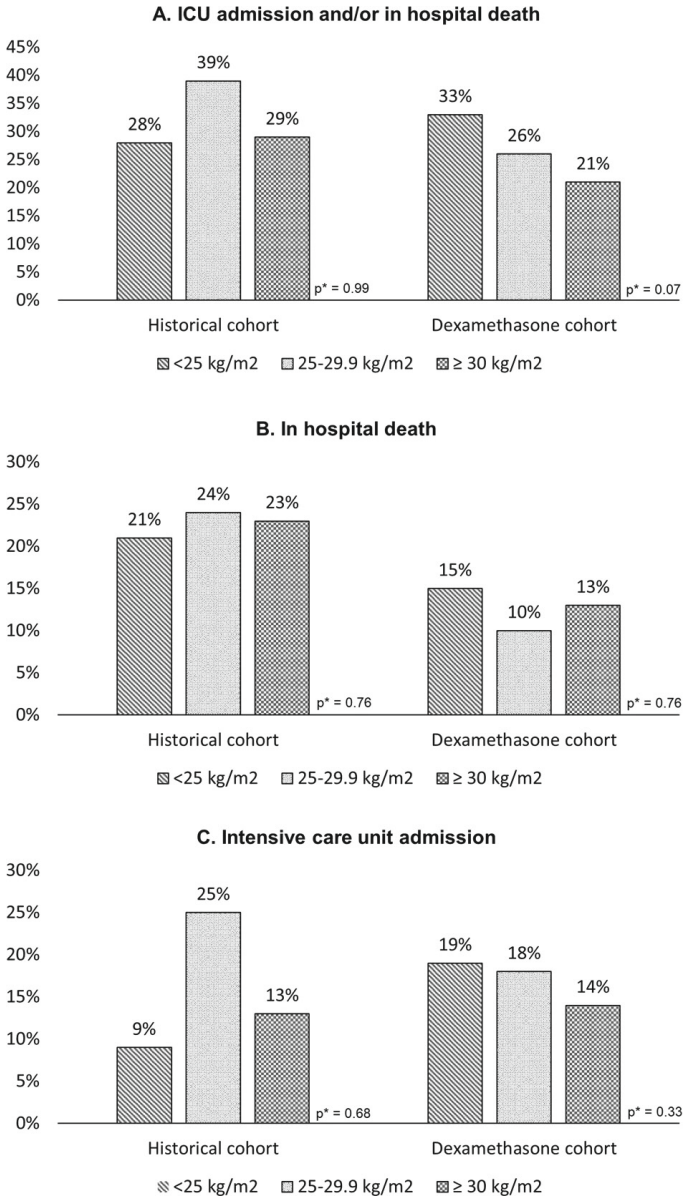


Figure 2 Clinical outcomes by BMI subgroup and cohort **A** shows the percentage of patients with an unfavourable clinical course during admission for each BMI subgroup by cohort. **B** shows the percentage of patients who died in hospital for each BMI subgroup by cohort. **C** shows the percentage of patients who required intensive care unit admission for each BMI subgroup by cohort. Numbers analysed; Historical cohort BMI < 25 kg/m^2 N = 58, BMI 25–29.9 kg/m^2 N = 109, ≥30 kg/m^2 N = 82. Dexamethasone cohort BMI < 25 kg/m^2 N = 86, BMI 25–29.9 kg/m^2 N = 176, ≥30 kg/m^2 N = 123. *Chi-square for trend.

DISCUSSION

This study showed that in COVID-19 patients admitted to a non-ICU ward and treated with dexamethasone, patients with overweight and patients with obesity do not experience worse clinical outcome compared to patients with normal weight. Interestingly, the percentage of secondary ICU admission and/or in hospital mortality was higher in patients with normal weight compared to patients with obesity. Yet, both univariable and multivariable analysis did not show a statistically significant difference in odds of an unfavourable clinical course between BMI subgroups. Furthermore, we observed lower inflammatory status at admission in patients with overweight and those with obesity.

When designing the study, we hypothesised that patients with obesity and patients with overweight would benefit less from a fixed 6 mg dexamethasone dose compared to normal weight patients. Yet, we could not confirm this hypothesis. For this we propose two possible explanations. First this could mean that the systemic dexamethasone exposure from a fixed-dose is not affected or only minimally affected by total body weight. Thus that, contrary to our hypothesis, the systemic exposure of dexamethasone is similar in individuals with and individuals without obesity. However, this would contradict what we know from community-acquired pneumonia patients. In a pharmacokinetic study of dexamethasone in community-acquired pneumonia patients a volume of distribution of 1 L/kg was observed. This indicates a linear decrease in peak serum dexamethasone concentrations with increase in total body weight.[14] Unfortunately, we were not able to measure dexamethasone serum concentrations in the present study to explore this further. But there is a Swiss trial underway that compares dexamethasone pharmacokinetics between COVID-19 patients with normal weight and COVID-19 patients with obesity which might shed more light on the matter.[20]

A second, and more likely, explanation for why we did not observe worse clinical outcomes in patients with obesity may lie in differences in baseline inflammatory state. Our finding of a lower inflammatory status in patients with obesity was unexpected. In general, obesity is linked to higher expressions of CRP and pro-inflammatory cytokines such as TNF-a, and interleukin-6.[21] In an analysis of 781 hospitalised COVID-19 patients McNeill et al. also showed that initial CRP was higher in individuals with obesity than in individuals without obesity.[15] In the present study, higher CRP and lower lymphocyte counts were associated with worse outcome, but it were the patients with normal weight who had the highest baseline inflammatory status. Possibly, the lower state of inflammation in our patients with obesity means that these patients require lower serum peak dexamethasone concentrations compared to patients with normal weight as there is less inflammation to dampen. This would result in a null effect difference between patients with obesity and those with normal weight. The fact that we observed worse outcomes for patients with overweight not treated with dexamethasone compared to patients with healthy weight not treated with dexamethasone could support this hypothesis.

In general, it remains uncertain what dexamethasone dose is optimal for COVID-19 patients, especially for non-ICU patients. Several studies are underway that compare the effectiveness of high vs low dose dexamethasone for COVID-19 patients, however results have not yet been published.[22,23] To our knowledge, as of yet there is only one published randomised controlled trial that has compared low (6 mg) vs high (12 mg) dose dexamethasone. This study did not show a statistically significant difference in days alive without life support and 28-day mortality between patients treated with 12 mg dexamethasone and those treated with 6 mg dexamethasone.[24] However, a secondary Bayesian analysis of the same trial showed higher probability of benefit in patients treated with 12 mg compared to 6 mg.[25] Because, this study only included patients requiring ≥10 L of oxygen or those on mechanical ventilation and there was no baseline information available on BMI (only body weight), it is difficult to relate the results of this trial to the findings in the present study.

The data in the present study represent the time between the start of the SARS-CoV-2 outbreak in the Netherlands in March 2020 and January 2021. Overall, we found that the prognosis for COVID-19 patients improved during this period, as shown by the lower overall mortality rate in the dexamethasone cohort compared to the historical cohort (which coincided with the first wave of the pandemic). Although the introduction of standard corticosteroid treatment was a major improvement of care for COVID-19 patients, it should be noted that other improvements of care were also made between March 2020 and January 2021. An important example is awareness about the increased risk of thromboembolic complications in COVID-19 patients.[26] Furthermore other medications such as remdesivir and tocilizumab became available.[27,28] However, in our hospital tocilizumab was only prescribed in ICU patients and remdesivir was only prescribed during a short two-week period. Considering this, we do not expect that these medications influenced clinical outcomes in our cohort with non-ICU patients.

The main strength of the present study is our well-defined cohort of patients not admitted to the ICU. Though, ICU admission rate is high in COVID-19, the majority of hospitalised COVID-19 patients are still admitted to a regular ward.[29] Optimising non-ICU treatment might help in reducing secondary ICU admissions which is better for both the patient and the health care system. The present study also has several limitations. First, this was a retrospective study in which we had to rely on available data from the COVID-19 database. Because we only included patients for whom a BMI was available, we cannot exclude the possibility that some selection bias may exist due to missing BMI data in the COVID-19 database. Second, it is important to note that due to increasing pressure on the Dutch health care system during the second and third COVID-19 wave, a national system was implemented to equally distribute COVID-19 patients between Dutch hospitals. We cannot exclude the possibility that a patient died or was admitted to the ICU after being transferred to a different hospital. However, only 21 (5.5%) patients in the dexamethasone cohort were transferred to a different hospital

and patients had to be clinically stable to be transferred. Last, the number of patients in the historical cohort was quite small which may have led to insufficient statistical power in this cohort.

In conclusion, overweight and obesity both are not associated with secondary ICU admission and/or in hospital death in patients admitted to a non-ICU ward and treated with dexamethasone 6 mg once daily.

REFERENCES

1. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Eng J Med*. 2021;384(8):693-704. doi:10.1056/nejmoa2021436
2. Sterne JAC, Murthy S, Diaz JV, et al. Association between Administration of Systemic Corticosteroids and Mortality among Critically Ill Patients with COVID-19: A Meta-analysis. *JAMA*. 2020;324(13):1330-1341. doi:10.1001/jama.2020.17023
3. Medicamenteuze behandeling voor patiënten met COVID-19 (infectie met SARS–CoV-2) | SWAB. Accessed December 16, 2021. https://swab.nl/nl/covid-19
4. Corticosteroids for COVID-19. Accessed December 16, 2021. https://www.who.int/publications/i/item/WHO-2019-nCoV-Corticosteroids-2020.1
5. Hsu RJ, Yu WC, Peng GR, et al. The Role of Cytokines and Chemokines in Severe Acute Respiratory Syndrome Coronavirus 2 Infections. *Front Immunol*. 2022;13:832394. doi:10.3389/fimmu.2022.832394
6. Rhen T, Cidlowski JA. Antiinflammatory Action of Glucocorticoids — New Mechanisms for Old Drugs. *N Eng J Med*. 2005;353(16):1711-1723. doi:10.1056/nejmra050541
7. Wolfisberg S, Gregoriano C, Struja T, et al. Comparison of characteristics, predictors and outcomes between the first and second COVID-19 waves in a tertiary care centre in Switzerland: an observational analysis. *Swiss Medical Weekly* 2021;151:w20569. doi:10.4414/smw.2021.20569
8. Cusinato M, Gates J, Jajbhay D, Planche T, Ong YE. Increased risk of death in COVID-19 hospital admissions during the second wave as compared to the first epidemic wave: a prospective, single-centre cohort study in London, UK. *Infection*. 2022:50(2):457-465. doi:10.1007/s15010-021-01719-1
9. Carbonell R, Urgelés S, Rodríguez A, et al. Mortality comparison between the first and second/third waves among 3,795 critical COVID-19 patients with pneumonia admitted to the ICU: A multicentre retrospective cohort study. *The Lancet Reg Heal - Eur*. 2021;11:100243. doi:10.1016/j.lanepe.2021.100243
10. Poly TN, Islam MM, Yang HC, et al. Obesity and Mortality Among Patients Diagnosed With COVID-19: A Systematic Review and Meta-Analysis. *Front Med*. 2021;8:620044. doi:10.3389/fmed.2021.620044
11. Dessie ZG, Zewotir T. Mortality-related risk factors of COVID-19: a systematic review and meta-analysis of 42 studies and 423,117 patients. *BMC Infect Dis*. 2021;21(1):855. doi:10.1186/S12879-021-06536-3
12. Loew D, Schuster O, Graul EH. Dose-dependent pharmacokinetics of dexamethasone. *Eur J of Clin Pharmacol*. 1986;30(2):225-230. doi:10.1007/bf00614309
13. Tsuei SE, Moore RG, Ashley JJ, McBride WG. Disposition of synthetic glucocorticoids. I. Pharmacokinetics of dexamethasone in healthy adults. *J Pharmacokinet Biopharm*. 1979;7(3):249-264. doi:10.1007/bf01060016
14. Spoorenberg SMC, Deneer VHM, Grutters JC, et al. Pharmacokinetics of oral vs. intravenous dexamethasone in patients hospitalized with community-acquired pneumonia. *Br J Clin Pharmacol*. 2014;78(1):78-83. doi:10.1111/bcp.12295
15. McNeill JN, Lau ES, Paniagua SM, et al. The role of obesity in inflammatory markers in COVID-19 patients. *Obes Res Clin Pract*. 2021;15(1):96-99. doi:10.1016/j.orcp.2020.12.004
16. Quan H, Sundararajan V, Halfon P, Fong A. Coding algorithms for defining comorbidities in. *Med Care*. 2005;43(11):1130-1139.
17. Smilowitz NR, Kunichoff D, Garshick M, et al. C-reactive protein and clinical outcomes in patients with COVID-19. *Eur Heart J*. 2021;42(23):2270. doi:10.1093/eurheartj/ehaa1103

18. Mazzoni A, Salvati L, Maggi L, Annunziato F, Cosmi L. Hallmarks of immune response in COVID-19: Exploring dysregulation and exhaustion. *Semin Immunol*. Published online October 2021:101508. doi:10.1016/j.smim.2021.101508

19. Abani O, Abbas A, Abbas F, et al. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet*. 2021;397(10285):1637-1645. doi:10.1016/S0140-6736(21)00676-0

20. Pharmacokinetics of Dexamethasone in COVID-19 Obese Patients. ClinicalTrials.gov. Accessed December 17, 2021. https://clinicaltrials.gov/ct2/show/NCT04996784

21. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Res Clin Pract*. 2005;69(1):29-35. doi:10.1016/j.diabres.2004.11.007

22. Maláska J, Stašek J, Duška F, et al. Effect of dexamethasone in patients with ARDS and COVID-19 – prospective, multi-centre, open-label, parallel-group, randomised controlled trial (REMED trial): A structured summary of a study protocol for a randomised controlled trial. *Trials*. 2021;22(1):172. doi:10.1186/S13063-021-05116-9

23. Low or High Dose of Dexamethasone in Patients With Respiratory Failure by COVID-19. ClinicalTrials.gov. Accessed December 15, 2021. https://clinicaltrials.gov/ct2/show/NCT04726098.

24. Russell L, Uhre KR, Lindgaard ALS, et al. Effect of 12 mg vs 6 mg of Dexamethasone on the Number of Days Alive Without Life Support in Adults With COVID-19 and Severe Hypoxemia. *JAMA*. 2021;326(18):1807-1817. doi:10.1001/jama.2021.18295

25. Granholm A, Munch MW, Myatra SN, et al. Dexamethasone 12 mg versus 6 mg for patients with COVID-19 and severe hypoxaemia: a pre-planned, secondary Bayesian analysis of the COVID STEROID 2 trial. *Intensive Care Med*. 2021;48(1):45-55. doi:10.1007/s00134-021-06573-1

26. Bikdeli B, Madhavan M v., Jimenez D, et al. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. *J Am Coll Cardiol*. 2020;75(23):2950-2973. doi:10.1016/J.JACC.2020.04.031

27. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 — Final Report. *N Eng J Med*. 2020;383(19):1813-1826. doi:10.1056/nejmoa2007764

28. Salama C, Han J, Yau L, et al. Tocilizumab in Patients Hospitalized with Covid-19 Pneumonia. *N Eng J Med*. 2021;384(1):20-30. doi:10.1056/nejmoa2030340

29. Abate SM, Ali SA, Mantfardo B, Basu B. Rate of Intensive Care Unit admission and outcomes among patients with coronavirus: A systematic review and Meta-analysis. *PLoS One*. 2020;15(7):e0235653. doi:10.1371/journal.pone.0235653

CHAPTER 7

The extent of microbiological testing is associated with alteration of antibiotic therapy in adults with community-acquired pneumonia

E Wittermans, SMT Vestjens*, WJW Bos, JC Grutters, EMW van de Garde, BJM Vlaminckx*
**Authors contributed equally*

EUR J CLIN MICROBIOL INFECT DIS. 2019;38(7):1359-1366.

ABSTRACT

The aim of this study was to explore the relationship between the extent of microbiological testing and the frequency of antibiotic alteration in adults hospitalised with community-acquired pneumonia (CAP). We retrospectively studied 283 immunocompetent patients hospitalised with CAP. Information on microbiological testing and prescribed antibiotics was obtained. Patients were grouped according to the number of different microbiological tests performed within the first 2 days of admission (0–5 tests). Alteration rates were compared between these groups. Antimicrobial alteration was defined as a switch at day 3 of hospital stay to (1) a narrower spectrum antibiotics, or (2) a different class of antibiotics, or (3) a switch from dual therapy to monotherapy (4) or discontinuation of antibiotic treatment because the indication for antibiotic treatment did no longer exist. For each additional test performed, a stepwise increase in percentage of patients with altered antibiotic regimen ranging from 0 to 59% ($p = 0.001$) was found. Multivariate logistic regression analyses showed that performing PCR assay for atypical pathogens was most strongly associated with any alteration of antibiotic treatment (OR 2.6 (95% CI 1.4–4.9)) and with changes in atypical coverage specifically (OR 3.1 (95% CI 1.6–6.0). The extent of microbiological testing was positively associated with antibiotic alteration in adults hospitalised with CAP. Antibiotic treatment was most likely to be altered in patients in whom PCR assay for atypical pathogens was performed.

INTRODUCTION

Antimicrobial stewardship aims at encouraging appropriate antibiotic use, which should not only be effective but also limits toxic effects, induction of resistance and microbial selection.[1] This is of particular concern in the treatment of community-acquired pneumonia (CAP), which is one of the most common infectious diseases.[2]

Studies have shown that inappropriate therapy is associated with unfavourable outcomes.[3,4] One of the quality indicators of appropriate antibiotic use is alteration of antimicrobial treatment based on microbiological test results.[1] This may lead to reduced selective pressure for resistance and improved outcomes.[5–8]

Timely and adequate alteration of empiric antibiotics is only possible when actionable microbiological test results are available. However, in day-to-day clinical practice, no causative pathogen is found in over 60% of patients hospitalised with CAP. This is partially due to the limited yield of conventional diagnostics.[9] Newer and more rapid testing methods like urinary antigen tests (UAT) and PCR assays have been introduced in the past years.[10] It has been shown in a research setting that combining traditional sputum and blood cultures with these newer diagnostic tests can increase diagnostic yield up to 67% in patients with CAP.[11–13]

It is assumed that extensive microbiological testing results in an increased diagnostic yield and thereby facilitates more frequent alteration of antibiotic therapy. The aim of this study was to explore the relationship between the extent of microbiological testing and alteration of antibiotic therapy in adults hospitalised with CAP. The secondary objective was to assess the association between the extent of microbiological testing and clinical outcomes.

METHODS

Study design and patients

Adult patients who were hospitalised with CAP at the St. Antonius Hospital (an 850-bed non-academic teaching hospital in the Netherlands) between January 2013 and January 2017 were assessed.

CAP was defined as a new pulmonary infiltrate on chest X-ray in combination with two of the following findings: cough, sputum production, findings at auscultation indicative of pneumonia, body temperature > 38 °C or < 35 °C, C-reactive protein concentration > 15 mg/L and a white blood cell count > 10×10^9 cells/L or a leftward shift. Immunocompromised patients, either due to acquired or congenital immunodeficiencies or due to the use of immunosuppressive medication within 6 months of admission, were excluded, as were patients participating in a placebo-controlled trial evaluating

the effectiveness of adjunctive dexamethasone therapy in patients admitted with CAP (NCT01743755) for whom the diagnostic procedures were specified by the trial protocol. Furthermore, we excluded patients with empyema at admission, patients who were directly admitted to the intensive care unit and patients who died within 24 h of emergency room (ER) presentation. Eligibility for inclusion was based on radiology reports, laboratory results and patient history and physical examination as reported by the treating physician on the day of ER presentation. The study was approved by the Medical Ethics Committee of the St. Antonius Hospital (Nieuwegein).

Data collection

Patient medical records were checked to confirm that inclusion criteria were met, to collect data on any antibiotic use prior to hospital admission, to identify patients with a history of COPD and to determine the CURB-65 score (one point for each of the following criteria: confusion, urea > 7 mmol/L, respiratory rate > 30/min, blood pressure < 90 mmHg systolic or < 60 diastolic, age over 65 years) at time of hospital admission (day 1).[14]

Microbiological tests performed on day 1 and day 2 were selected for analyses using the General Laboratory Information Management System (GLIMS). The following five microbiological tests were included: (1) PCR assays on throat or nasal swabs for detection of respiratory viruses including influenza A; influenza B; parainfluenza viruses 1, 2 and 3; respiratory syncytial viruses type A and B; human metapneumovirus and rhinovirus; (2) PCR assays on throat swabs or on sputum samples for detection of atypical respiratory pathogens including *Coxiella burnetii*, *Legionella* species, *Chlamydophila psittaci* and *Mycoplasma pneumoniae*; (3) sputum cultures; (4) blood cultures and (5) UAT for the detection of *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae* (BinaxNOW®).

Information on prescribed antibiotics was obtained using the Farmadatabase, a database in which all drugs prescribed during admission are registered.[15] All antibiotic prescriptions between January 2013 and January 2017 were extracted. Antibiotics prescribed during hospital admission were identified by matching admission dates to the date that the patient was screened for trial participation. A similar procedure was used to obtain data on all microbiological tests performed from GLIMS. All data were anonymised before analyses were performed.

Outcomes measures

The primary outcome was the percentage of patients whose initial antibiotic regimen had been altered by day 3 of hospital admission. Diagnostic yield was determined according to the number of microbiological tests performed. As secondary outcomes 30-day mortality, secondary intensive care unit (ICU) admission and length of hospital stay (LOS) were determined. Secondary ICU admission was defined as admission to the ICU after 24 h of hospital admission.

Data analyses

Alteration was defined as one of the following changes in antibiotic regimen: (1) switch to narrower spectrum antibiotics, or (2) switch to a different class of antibiotics, or (3) switch from dual therapy to monotherapy or (4) discontinuation of antibiotic treatment because the indication for antibiotic treatment did no longer exist. During the period in which patients were enrolled, the Dutch national guideline on the management of CAP advised to guide empirical antibiotic treatment according to the severity of disease. The antimicrobial spectrum varied from amoxicillin monotherapy for mild CAP to dual therapy with a cephalosporin plus atypical coverage for severe CAP (e.g. erythromycin or ciprofloxacin).[16]

To explore the association between the extent of microbiological testing and alteration of antibiotic treatment, patients were first divided into six groups according to the number of different microbiological tests performed within the first 2 days of hospital admission. The first group consisting of patients in whom no diagnostic tests were performed (0-test group), up to the last group consisting of patients in whom all five different tests were performed (5-test group). Subsequently, antibiotic regimens on the day of hospital admission and antibiotic regimens at day 3 of hospital admission were divided into six groups according to antibiotic classification: (1) a small spectrum penicillin with or without β-lactamase inhibitor, (2) a cephalosporin, (3) dual therapy combining a small spectrum penicillin with coverage of atypical pathogens (e.g. macrolide or fluoroquinolone), (4) dual therapy combining a cephalosporin with coverage of atypical pathogens, (5) monotherapy covering atypical pathogens and (6) other antibiotic classes or other combinations of antibiotic classes. Patients with altered antibiotic regimens by day 3 of admission were identified. The number and percentage of patients with altered antibiotic regimens on day 3 were calculated for each diagnostic test group.

Furthermore, we calculated the percentage of patients with at least one positive microbiological test result for the whole study population and for each of the diagnostic groups separately (0–5 tests). The diagnostic yield was compared between groups to determine its relationship with the extent of microbiological testing. A positive microbiological test was defined as (1) a positive PCR assay for respiratory viruses or atypical pathogens or a positive UAT, or (2) a pathogen identified by blood culture except for contamination as noted in the microbiology report or (3) a clinically relevant pathogen identified by sputum culture (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*). To explore the relationship between the number of microbiological tests performed by the end of day 2 and 30-day mortality and secondary ICU admission, patients were divided into two groups: one group in which limited microbiological testing was performed (0–2 tests) and one group in which extensive testing was performed (3–5 tests). For both

groups, the number and percentage of patients who died within 30 days of admission or were admitted to the ICU was calculated.

Overall descriptives are stated as number (%) for categorical data and mean (standard deviation (SD)) or median (interquartile range [IQR]) for continuous data. Categorical data was compared using Chi-squared tests or Fischer's exact tests, and continuous data was compared using an independent samples t-test or a Mann–Whitney U test as deemed appropriate. A p-value < 0.05 was considered significant.

Multivariable logistic regression analyses were performed to assess the association of each individual microbiological test with the outcomes: (1) any alteration of antibiotic therapy and (2) alterations in atypical coverage (discontinuation of or a switch to atypical coverage) adjusted for pneumonia severity (CURB-65 score).

RESULTS

Patient selection and baseline characteristics

A total of 390 patients with CAP were screened, of which 283 patients were found eligible for inclusion. The flowchart with reasons for exclusion is shown in Figure 1.

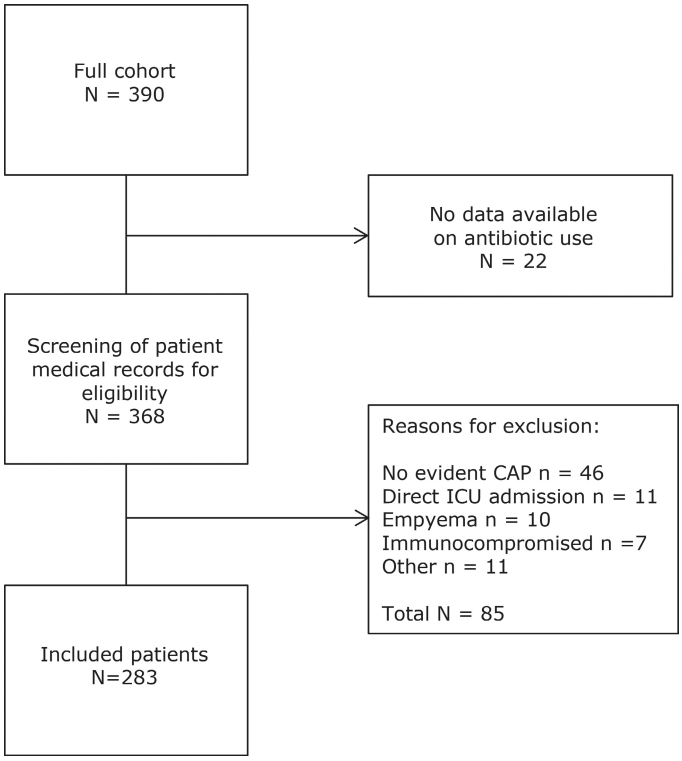


Figure 1 Patient selection flowchart

In Table 1, baseline characteristics are shown. The median CURB-65 score was 1 [IQR 1–2]. Antibiotics were prescribed to 32% of patients prior to admission. Baseline characteristics per group (0-test group to 5-test group) are shown in Supplementary Table 1.

Table 1 Baseline characteristics

Characteristic	
Median age, y [IQR]	70 [57–81]
Male, N (%)	151 (53)
History of COPD, N (%)	29 (10)
Antibiotic use prior to admission, N (%)	90 (32)
CURB-65 score, N (%)	
0	57 (20)
1	87 (31)
2	77 (27)
3	52 (18)
4	8 (3)
5	2 (1)
Initial antibiotic regimen, N (%)	
Small spectrum penicillin	130 (46)
Cephalosporin	35 (12)
Small spectrum penicillin with coverage of atypical pathogens	45 (16)
Cephalosporin with coverage of atypical pathogens	53 (19)
Antibiotics for atypical pathogens	11 (4)
Other	9 (3)

Microbiological testing

Blood cultures were performed in 224 (79%) patients, sputum culture in 109 (39%) patients, UAT in 231 (82%) patients and PCR for atypical pathogens in 70 (25%), and PCR for respiratory viruses was performed in 192 (68%) patients.

A pathogen was identified in 104 (37%) patients. There was a clear trend towards a higher pathogen identification rate in patients that did not use antibiotics prior to admission compared to those who did (40% vs. 29%, respectively, $p = 0.06$). As shown in Figure 2, there was a stepwise increase in the pathogen identification rate for each additional test performed ($p < 0.001$, Chi-squared test for trend). In descending order, the diagnostic yield of individual tests, if performed, was 33% for sputum cultures; 21% for PCR assay for respiratory viruses; 15% for UAT; 11% for PCR for atypical pathogens and 8% for blood cultures. The most frequently identified pathogen was *S. pneumonia* (17%) followed by *H. influenzae* (5%), influenza A virus (6%), *S. aureus* (3%) and *M. pneumoniae* (2%).

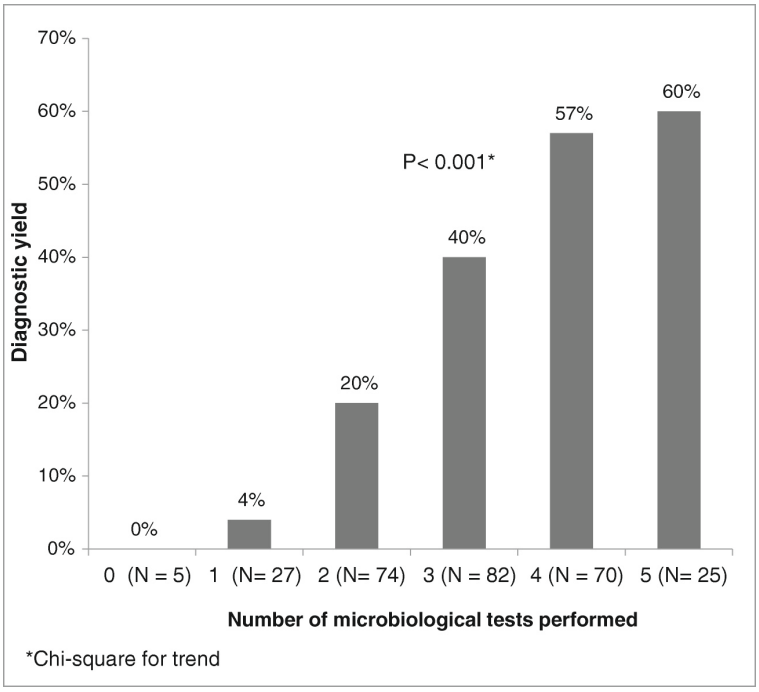


Figure 2 Number of performed microbiological tests and diagnostic yield

Antimicrobial alteration

Antibiotic regimens on day 1 and day 3 of admission are depicted in Figure 3. At day 3, 12 patients had already been admitted to the ICU, died or had been discharged. For these patients, no reliable data available on antibiotic use on day 3 could be retrieved. We therefore excluded them from further analyses concerning antibiotic alteration. Information on antibiotic use on day 3 of admission was available for 271 (96%) patients.

Antibiotic treatment was altered in 70 (26%) patients. Discontinuation of dual therapy (switch to monotherapy) was the most frequent change in antibiotic regimen (n = 53, 76%), followed by narrowing a cephalosporin to a small spectrum penicillin (n = 7, 10%). In 58 (21%) of the patients, the alteration involved removal or addition of atypical coverage. There was a stepwise increase in percentage of patients with an altered antibiotic regimen for each additional test performed (p = 0.001, Chi-squared test for trend) (Figure 4). In the multivariable analyses, performing a PCR assay for atypical pathogens was independently associated with both any alteration of antibiotic treatment on day 3 (OR 2.6 95% CI 1.4–4.9) and with an alteration regarding atypical coverage (OR 3.1 95% CI 1.6–6.0) (Table 2, Table 3).

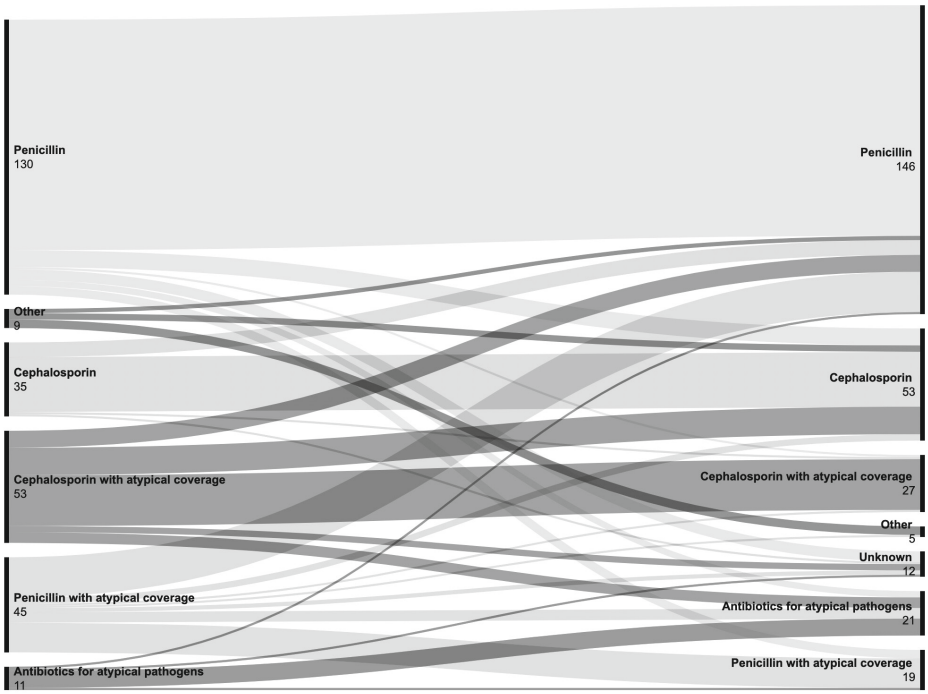


Figure 3 Antibiotic treatment and alterations. The first bar shows antibiotic treatment at the time of hospital admission, and the second bar shows antibiotic treatment at day 3 of hospital admission. The lines between both bars represent alteration in antibiotic regimens. Numbers represent the number of patients receiving a certain type of antibiotic

Table 2 Odds ratio for performing each individual microbiological test and any alteration of antibiotic treatment by day 3 of hospital admission

	Odds ratio (95% CI)	P value
Blood culture*	2.2 (1.0–4.9)	0.06
Sputum culture*	1.5 (0.8–2.8)	0.18
Urinary antigen test*	1.7 (0.7–4.0)	0.22
PCR for respiratory viruses*	0.8 (0.4–1.6)	0.53
PCR for atypical pathogens*	2.6 (1.4–4.9)	0.003
CURB-65 (≥ 2)**	1.7 (1.0–3.1)	0.06

*Reference category: test not performed within 2 days of admission
**Reference category: CURB-65 < 2

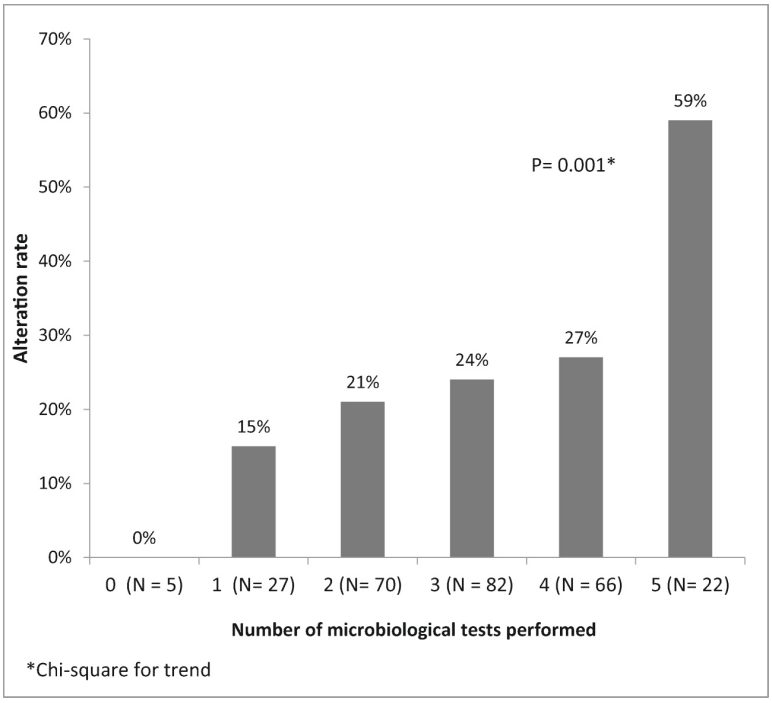


Figure 4 Number of microbiological tests performed and alteration rate

Table 3 Odds ratio for performing each individual microbiological test and alteration in atypical coverage by day 3 of hospital admission

	Odds ratio (95% CI)	P value
Blood culture*	1.8 (0.8–4.2)	0.18
Sputum culture*	1.5 (0.8–2.9)	0.21
Urinary antigen test*	2.2 (0.8–6.1)	0.13
PCR for respiratory viruses*	0.9 (0.5–1.9)	0.87
PCR for atypical pathogens*	3.1 (1.6–6.0)	0.001
CURB-65 (≥ 2)**	1.0 (0.5–1.8)	0.95

*Reference category: test not performed within 2 days of admission
**Reference category: CURB-65 < 2

Secondary outcomes

There was no significant difference between patients in whom 0–2 or 3–5 diagnostic tests were performed in either LOS nor negative outcomes (death within 30 days of admission and secondary ICU admission combined, due to low numbers) (Table 4).

Table 4 Number of microbiological tests performed and secondary endpoints

Number of tests	N	30-day mortality and/or secondary ICU admission, N (%)	Median LOS in days [IQR]
0-2	106	13 (12)	5 [3-8]
3-5	177	13 (7)*	6 [4-8]**
Total	283	226 (9)	5 [3-8]

*p = 0.158 (Chi-squared test): group with 0–2 performed tests compared to the group with 3–5 performed tests; **p = 0.126 (Mann–Whitney U): group with 0–2 performed tests compared to the group with 3–5 performed tests

DISCUSSION

The main finding of this study is the positive association between the number of microbiological tests performed within the first 2 days of hospital admission and the rate of antibiotic regimen alteration by day 3 in adults hospitalised with CAP. The antibiotic treatment alteration rate was almost three times higher by day 3 in patients in whom a PCR assay for atypical pathogens was performed. A change in atypical coverage was the most frequent alteration.

Regarding specific diagnostic tests, Oosterheert et al. investigated the addition of PCR assays for atypical pathogens and respiratory viruses to standard microbiological testing in day-to-day clinical practice in patients admitted with lower respiratory tract infections including, but not limited to, pneumonia.[17] The addition of these PCR assays to conventional diagnostics did increase diagnostic yield from 21 to 43%; however, antibiotic treatment was only modified based on PCR results in 11% of patients. We found a 26% overall alteration rate. A likely explanation for this difference is the higher frequency of dual therapy in our cohort (35% vs 16%) providing more opportunities for alterations.

More recently, Vestjens et al. retrospectively studied the association between the total costs of diagnostic testing and antimicrobial de-escalation in patients with CAP in three Dutch non-academic teaching hospitals.[18] It was demonstrated that the mean costs for microbiological testing per patient was the highest in the hospital where PCR assays were performed most frequently. In the study by Vestjens et al., the de-escalation rate was highest in the hospital with the lowest costs for testing. It was concluded that this seemingly counterintuitive finding could be explained by the presence of an automated iv-to-oral trigger alert in that specific hospital, guiding physicians to reconsider antibiotic regimens by drawing their attention to microbiological test results (including negative results). No such antibiotic stewardship intervention was in place in the hospital where the present study was performed.

However, to assess the potential added value of such an automated antibiotic stewardship intervention, we checked the medical records of the 15 patients receiving

dual therapy at admission, and in whom, a PCR assay for atypical pathogens was performed and whose antibiotic regimen was not altered, to assess the reasons for not switching antibiotic therapy. In the charts of four patients, the reason for continuing dual therapy was argued. However, in the 11 remaining patients, there was no note by the treating physician on the result of the PCR assay for atypical pathogens nor was a reason for continuing dual therapy argued. Considering that all these 11 patients had a negative PCR assay and did not have a positive UAT for Legionella implies that our observed frequency of alteration based on PCR is an underestimation of its true potential. It also supports the conclusion from Vestjens et al. that, apart from ordering a specific test, the way of communicating the results to physicians is also relevant towards the purpose of the test. Still, although its relatively (but decreasing) costliness compared to longer existing microbiological test methods, performing PCR assays for atypical pathogens clearly contributed to antibiotic therapy alteration in this single-centre study.

This study does have limitations, mainly due to its retrospective and single-centre design. First, we included the microbiological tests ordered on the day of hospital admission and the day after hospital admission. Inaccuracy of recorded time of sampling rendered it impossible to use a more exact timeframe (e.g. within 24 h or 48 h) in which microbiological tests were performed for every patient.

Second, we grouped patients receiving amoxicillin/clavulanic acid into the small spectrum penicillin group. As a result, we did not identify switches from amoxicillin/clavulanic acid to amoxicillin or penicillin as alteration. However, this only involved four patients with this scenario, making the impact on our findings rather small.

Third, due to low rates of antibiotic resistance in the Netherlands, guidelines for antibiotic treatment of CAP differ from countries with higher rates of resistance. As the antimicrobial resistance rates of *S. pneumoniae* for penicillin are low in the Netherlands, a small spectrum penicillin is the first line of treatment in patients with mild to moderate CAP.[16] Therefore, the number of patients receiving monotherapy with a small spectrum penicillin in our study is higher than it would be in other countries, thereby limiting the external validity of our findings for these countries. However, a strength of this study is that it reflects day-to-day clinical practice. Furthermore, our study included a well-defined cohort of patients with mainly mild to moderate–severe CAP. Median age, antibiotic use prior to hospital admission, level of pathogen identification and 30-day mortality were also very similar to those found in another large and recent Dutch cohort including non-ICU patients with CAP.[19]

In conclusion, for each additional microbiological test performed, we found a stepwise increase in alteration of antimicrobial therapy in patients admitted with CAP. Performing a PCR assay for atypical pathogens was most evidently associated with antibiotic alteration, most often being a switch from dual therapy to monotherapy.

FUNDING

This work was supported by a grant from the St. Antonius Research Fund via an earmarked donation from Verwelius Construction Corporation for research on CAP.

REFERENCES

1. van den Bosch CMA, Geerlings SE, Natsch S, Prins JM, Hulscher MEJL. Quality Indicators to Measure Appropriate Antibiotic Use in Hospitalized Adults. *Clin Infect Dis*. 2015;60(2):281-291. doi:10.1093/cid/ciu747
2. Pakhale S, Mulpuru S, Verheij TJM, Kochen MM, Rohde GGU, Bjerre LM. Antibiotics for community-acquired pneumonia in adult outpatients. *Cochrane Database Syst Rev*. 2014;2014(10):CD002109 doi:10.1002/14651858.CD002109
3. Kothe H, Bauer T, Marre R, Suttorp N, Welte T, Dalhoff K. Outcome of community-acquired pneumonia: influence of age, residence status and antimicrobial treatment. *Eur Respir J*. 2008;32(1):139 - 146. doi:10.1183/09031936.00092507
4. Garcia-Vidal C, Fernández-Sabé N, Carratalà J, et al. Early mortality in patients with community-acquired pneumonia: causes and risk factors. *Eur Respir J*. 2008;32(3):733 - 739. doi:10.1183/09031936.00128107
5. Schuts EC, Hulscher MEJL, Mouton JW, et al. Current evidence on hospital antimicrobial stewardship objectives: a systematic review and meta-analysis. *Lancet Infect Dis*. 2016;16(7):847-856. doi:10.1016/S1473-3099(16)00065-7
6. Carratalà J, Garcia-Vidal C, Ortega L, Al E. Effect of a 3-step critical pathway to reduce duration of intravenous antibiotic therapy and length of stay in community-acquired pneumonia: A randomized controlled trial. *Arch Intern Med*. 2012;172(12):922-928. doi:10.1001/archinternmed.2012.1690
7. Paterson DL. "Collateral Damage" from Cephalosporin or Quinolone Antibiotic Therapy. *Clin Infect Dis*. 2004;38:S341-S345. doi: 10.1086/382690
8. Musher DM, Thorner AR. Community-Acquired Pneumonia. *N Engl J Med*. 2014;371(17):1619-1628. doi:10.1056/nejmra1312885
9. Wiersinga WJ, Bonten MJ, Boersma WG, et al. *Management of Community-Acquired Pneumonia in Adults: 2016 Guideline Update from the Dutch Working Party on Antibiotic Policy (SWAB) and Dutch Association of Chest Physicians (NVALT)*. Accessed January 4, 2019. http://www.swab.nl/swab/cms3.nsf/uploads/6a6e127f9a2c1168c125816f004a013a/$file/cap_swab_2017-def_r5.pdf
10. File, TM. New Diagnostic Tests for Pneumonia: What is Their Role in Clinical Practice? *Clin chest med*. 2011;32(3):417-430. doi:10.1016/j.ccm.2011.05.011
11. van der Eerden MM, Vlaspolder F, de Graaff CS, Groot T, Jansen HM, Boersma WG. Value of intensive diagnostic microbiological investigation in low- and high-risk patients with community-acquired pneumonia. *Eur J of Clin Microbiol and Infect Dis*. 2005;24(4):241-249. doi:10.1007/s10096-005-1316-8
12. Johansson N, Kalin M, Tiveljung-Lindell A, Giske CG, Hedlund J. Etiology of Community-Acquired Pneumonia: Increased Microbiological Yield with New Diagnostic Methods. *Clin Infect Dis*. 2010;50(2):202-209. doi: 10.1086/648678
13. Andreo F, Domínguez J, Ruiz J, et al. Impact of rapid urine antigen tests to determine the etiology of community-acquired pneumonia in adults. *Respir Med*. 2006;100(5):884-891. doi: 10.1016/j.rmed.2005.08.020
14. Lim WS, van der Eerden MM, Laing R, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. *Thorax*. 2003;58(5):377. doi:10.1136/thorax.58.5.377
15. van de Garde EMW, Plouvier BC, Fleuren HWHA, et al. Pharmacotherapy within a learning healthcare system: rationale for the Dutch Santeon Farmadatabase. *Eur J Hosp Pharm*. 2019;26(1):46-50. doi: 10.1136/ejhpharm-2017-001329.

16. Wiersinga W, Bonten M, Boersma W, et al. SWAB/NVALT (Dutch Working Party on Antibiotic Policy and Dutch Association of Chest Physicians) guidelines on the management of community-acquired pneumonia in adults. *Neth J Med*. 2012;70(2):90-101.
17. Oosterheert JJ, van Loon AM, Schuurman R, et al. Impact of Rapid Detection of Viral and Atypical Bacterial Pathogens by Real-Time Polymerase Chain Reaction for Patients with Lower Respiratory Tract Infection. *Clin Infect Dis*. 2005;41(10):1438-1444. doi: 10.1086/497134
18. Vestjens SMT, Wittermans E, Spoorenberg SMC, et al. Inter-hospital variation in the utilization of diagnostics and their proportionality in the management of adult community-acquired pneumonia. *Pneumonia (Nathan)*. 2018;10:15. doi:10.1186/s41479-018-0059-0
19. Postma DF, van Werkhoven CH, van Elden LJR, et al. Antibiotic Treatment Strategies for Community-Acquired Pneumonia in Adults. *N Engl J Med*. 2015;372(14):1312-1323. doi:10.1056/nejmoa1406330

SUPPLEMENTARY MATERIAL

Supplementary Table 1 Baseline characteristics per group (0-5 tests)

Characteristic	0 tests	1 test	2 tests	3 tests	4 tests	5 tests
Median Age [IQR]	80 [50-89]	70 [60-81]	72 [62-84]	70[62-82]	68 [49-79]	66 [48-77]
Male N(%)	3 (60)	15 (56)	42 (57)	38 (46)	40 (57)	13 (52)
History of COPD N(%)	0 (0)	2 (7)	8 (11)	8 (10)	6 (9)	5 (20)
Antibiotic use prior to admission	1 (20)	9 (33)	20 (27)	27 (33)	22 (31)	11 (44)
Curb-65 score						
0	1 (20)	6 (22)	12 (16)	13 (16)	17 (24)	8 (32)
1	1 (20)	8 (30)	24 (32)	29 (35)	18 (26)	7 (28)
2	1 (20)	8 (30)	17 (23)	25 (31)	18 (26)	8 (32)
3	2 (40)	4 (15)	16 (22)	13 (16)	15 (21)	2 (8)
4	0 (0)	1 (4)	4 (5)	2 (2)	1 (1)	0 (0)
5	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)	0 (0)
Initial Antibiotic regimen N(%)						
Small spectrum penicillin	4 (80)	17 (63)	40 (54)	34 (42)	27 (39)	8 (32)
Cephalosporin	0 (0)	2 (7)	12 (16)	11 (13)	9 (13)	1 (4)
Small spectrum penicillin with coverage of atypical pathogens	1 (20)	2 (7)	6 (8)	13 (16)	16 (23)	7 (28)
Cephalosporin with coverage of atypical pathogens	0 (0)	3 (11)	11 (15)	17 (21)	13 (19)	9 (36)
Antibiotics for atypical pathogens	0 (0)	3 (27)	2 (18)	4 (36)	2 (18)	0 (0)
Other	0 (0)	0 (0)	3 (4)	3 (4)	3 (4)	0 (0)

CHAPTER 8

Summary, general discussion and future perspectives

SUMMARY

The aim of this thesis was to identify strategies to optimise the treatment of hospitalised community-acquired pneumonia patients (CAP) outside an intensive care unit (ICU) setting with a focus on corticosteroid treatment. First, this thesis focused on the question whether oral adjunctive corticosteroid treatment improves outcomes in hospitalised CAP patients and tries to identify a subgroup of CAP patients, based on inflammatory status at admission, in whom the beneficial effects of adjunctive corticosteroid treatment outweigh the disadvantages associated with corticosteroid use. Different methods were used to define subgroups. Next, this thesis investigated whether obesity and overweight were associated with worse clinical outcomes in a population of hospitalised COVID-19 patients who were all treated with the recommended fixed 6 mg dexamethasone dose. The aim was to test the hypothesis that 6 mg would be less effective in patients with obesity compared to patient with normal weight due to the pharmacokinetic properties of dexamethasone. Last, this thesis focused on optimising antibiotic treatment by exploring whether extensive microbiological testing facilitates early antibiotic alterations in CAP patients.

Chapter 2 describes the results of the multicentre placebo-controlled randomised Santeon-CAP trial. In this trial non-ICU patients with CAP were randomised to receive a 4-day course of 6 mg oral dexamethasone or placebo within 24 hours of hospital admission. Randomisation was stratified by disease severity (PSI risk class I-III vs class IV-V). Dexamethasone reduced median length of stay (LOS) by 0.5 days (5.0 vs 4.5 days; p =0.033) and reduced ICU admission rate (3% vs 7%; p = 0.03). Mortality rates did not differ between intervention and placebo group. Within both disease severity subgroups dexamethasone did not significantly reduce LOS. In the mild-moderate disease subgroup dexamethasone reduced ICU admission rate, the same was not found in the severe pneumonia group. Though not statistically significant, the rate of hospital readmission tended to be twice as high in the dexamethasone group compared to the placebo group.

In **Chapter 3** a post hoc-analysis of the Santeon-CAP study was performed in which white blood cell (WBC) differential parameters were used to define CAP subgroups. It was observed that in patients with a high WBC count (≥ 15.6 10^9 cells/l), high neutrophil count (≥ 13.2 10^9 cells/l) and high neutrophil-to-lymphocyte ratio (≥ 15.5) dexamethasone reduced LOS by 2 days, while there was no effect of dexamethasone on LOS in patients with a lower WBC count, lower neutrophil count or lower NLR ratio. White blood cell differential parameters did not modify the effect of dexamethasone on ICU admission or mortality.

In **Chapter 4** latent class analysis (LCA), a statistical method to identify 'hidden' subgroups in a population, was used to define subgroups using multiple inflammatory and clinical parameters. LCA was performed in two independent CAP cohorts: A Swiss cohort with patients from a multicentre trial investigating adjunctive prednisone

treatment (STEP trial), and a Dutch cohort with patients from a prospective observational study (Triple-P study) and a multicentre trial investigating adjunctive dexamethasone treatment (Ovidius trial). In both cohorts LCA identified two clinically distinct subgroups. One subgroup with more excessive inflammation and worse prognosis (class 2) and one subgroup with less exuberant inflammation and a better prognosis (class 1). In patients who participated in the Ovidius trial, the effect of corticosteroids on LOS was greater in Class 2 compared to Class 1. The same was not observed in the STEP trial.

Chapter 5 aimed to validate the findings described in **Chapter 4** in a third independent CAP cohort. Therefore, the LCA was repeated in the Santeon-CAP cohort. The LCA model used for the Ovidius-TripleP cohort was replicated as closely as possible. Again, LCA was able to identify the same two clinically distinct subgroups as in **Chapter 4**. Thus, proving LCA identified subgroups robustly. Yet the finding of a greater effect of dexamethasone in class 2 compared to class 1 patients could not be replicated.

Chapter 6 examined whether overweight and obesity are associated with worse clinical outcomes in COVID-19 patients treated with fixed-dose dexamethasone. In a population of patients admitted with COVID-19 to the general ward and treated with dexamethasone according to protocol (6 mg dexamethasone daily for 10 days of until discharge), overweight and obesity were not associated with worse clinical outcomes.

Chapter 7 explores the relationship between the extent of microbiological testing and early antibiotic treatment alterations in hospitalised CAP patients. There was a stepwise increase in the percentage of patients with altered antibiotic regimens by day three of hospitalisation for each additional type of microbiological test performed. A PCR assay for atypical pathogens was most strongly associated with antibiotic treatment alterations.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The aim of the studies presented in this thesis was to identify strategies to optimise the treatment of patients hospitalised with community-acquired pneumonia with a focus on corticosteroids. In this general discussion the implications of the studies presented in this thesis will be discussed and perspectives for future research will be provided.

ADJUNCTIVE CORTICOSTEROID TREATMENT FOR CAP

Adjunctive corticosteroid treatment in CAP has been a much researched and debated topic. The rationale being that corticosteroids attenuate the systemic inflammatory response and could thereby prevent an unfavourable clinical course caused by an excessive or dysregulated immune response.[1] A considerable number of studies have investigated whether adjunctive corticosteroid treatment improves outcomes for hospitalised CAP patients.[2–9]

Positive effects of adjunctive corticosteroid treatment in CAP

Interpreting the results of adjunctive corticosteroid trials is somewhat of a challenge as trials differ greatly in patient population and intervention (e.g., corticosteroid type, dose and treatment duration). An individual patient data meta-analysis (IPDMA) of six corticosteroid in CAP trials showed that adjunctive corticosteroid treatment reduced length of hospital stay (LOS) by 1.0 day and reduced time to clinical stability. However, corticosteroids did not reduce ICU admission or 30-day mortality rates.[10] A meta-analysis by Stern et al. did find a mortality benefit of adjunctive corticosteroid treatment in patients with severe CAP, yet this was based on the results of a small single-centre, single blinded study in which criteria for disease severity were not reported. Furthermore, re-analysis of the baseline characteristics of that study showed that kidney function was significantly worse in the control group compared to the intervention group at randomisation.[8,11,12] Table 1 provides an overview of the characteristics and results of the studies included in the IPDMA[10], the Santeon-CAP study (**Chapter 2**) and the most recent corticosteroid trial by Meduri et al.[13]

Most corticosteroid studies investigated intravenous corticosteroid treatment. In **Chapter 2** (The Santeon-CAP study), the effect of oral corticosteroid treatment was studied in a non-ICU population. This study showed that 4 days of 6 mg oral dexamethasone reduced LOS by 0.5 days. In addition, dexamethasone also reduced the risk of ICU admission after initial admission to a general ward. The Swiss STEP trial is the only other study that has investigated adjunctive treatment with oral corticosteroids (prednisone 50 mg). This study also showed that adjunctive corticosteroid treatment reduced LOS but it did not find a beneficial effect of prednisone on ICU admission or mortality.[3]

Table 1 Overview of characteristics and results of corticosteroid trials

Study	Country	No	Population	Intervention	Primary outcome	Median LOS (Corticosteroid vs placebo)	ICU admission* (Corticosteroid vs placebo)	Mortality (Corticosteroid vs placebo)
Confalonieri (2005)	Italy	46	Severe CAP (ATS criteria)	Hydrocortisone 200mg iv bolus followed by 10mg/h iv for 7d	Improvement PaO2/FiO2#	21 vs 13†	N/A	38% vs 0%† (60d)
Snijders (2010)	The Netherlands	204	Hospitalised CAP	Prednisone 40mg iv or po for 7d	Clinical cure day 7	10.0 vs 10.6§	N/A	5.8% vs 5.5% (30d)
Meijvis (2011)	The Netherlands	302	Hospitalised CAP (non-ICU)	Dexamethasone 6mg iv for 4d	LOS#	6.5 vs 7.5†	5% vs 7%	6% vs 7% (30d)
Fernandez-Serrano (2011)	Spain	52	Severe CAP (consolidation of ≥2 lobes and PO_2/FIO_2<300)	mPRED 200 mg iv bolus followed by tapering (3.3–0.8 mg/h) over 9 d	Respiratory failure requiring MV or NPPV	10 vs 12	17% vs 23%	4% vs 5% (>9d)
Blum (2015)	Switzerland	785	Hospitalised CAP	Prednisone 50mg po for 7d	Time to clinical stability#	6.0 vs. 7.0†	4% vs 6%	4% vs 3% (30d)
Torres (2015)	Spain	120	Severe CAP (ATS or PSI criteria and CRP >150 mg/L)	mPRED 0.5 mg/kg iv twice daily for 5d	Treatment failure#	11 vs 10.5	0% vs 0%	10% vs 15% (In hospital)
Briel (2018)‡	-	1506			All-cause 30d mortality	7.0 vs 8.0†	5.6% vs 6.3%	5.0% vs 5.9% (30d)
Wittermans (2021)	The Netherlands	401	Hospitalised CAP (non-ICU)	Dexamethasone 6mg po for 4d	LOS#	4.5 vs 5.0†	3% vs 7%†	2% vs 4% (30d)
Meduri (2022)	United States	584	Severe CAP (ATS criteria or ICU or intermediate care unit admission).	mPRED 40mg/day 7 days followed by tapering over 20d	60d all-cause mortality	7 vs 8	N/A	16% vs 18% (60d)

*Intensive care unit admission after initial admission to a general ward.
†Statistically significant difference between treatment groups.
§LOS reported as mean
‡Individual data meta-analysis of the studies by Confalonieri, Snijders, Meijvis, Blum, Fernandez-Serrano and Torres.
#Statistically significant difference between intervention and placebo group for the primary outcome
Abbreviations: d days; ATS American Thoracic society; PSI pneumonia severity index; ICU intensive care unit; mPRED Methylprednisolone; LOS length of hospital stay; MV mechanical ventilation; NPPV non-invasive positive pressure ventilation

From these findings, one can conclude that both iv and oral adjunctive corticosteroid treatment reduce LOS in hospitalised CAP patients. Yet, a reduction in LOS is the only benefit of adjunctive corticosteroids that has consistently been reported across multiple studies. Because results regarding ICU admission and mortality are conflicting, the effect of corticosteroids on ICU admission and mortality remains uncertain. Furthermore, over the years both LOS and mortality rate have decreased for hospitalised CAP patients (Table 1). For example, in the Ovidius trial[2] (2011) which had a similar population to that of the Santeon-CAP study, median LOS in the control group was 7,5 days compared to 5 days in the control group of the Santeon-CAP study. This means that, assuming a constant relative effect, the absolute effect of corticosteroids will become smaller compared to the time in which the first large corticosteroid trials were conducted. In studies showing that corticosteroids reduce LOS, LOS was reduced by approximately 10%. In earlier trials this translated into a 1-day reduction in LOS whereas in the Santeon-CAP study, the most recent trial, this translated into a 0.5-day reduction. The important question is whether the beneficial effect of adjunctive corticosteroid treatment on LOS outweighs the risks of corticosteroid treatment.

Adverse effects of adjunctive corticosteroid treatment in CAP

Because corticosteroids stimulate the gluconeogenesis, hyperglycaemia is a common side effect of corticosteroid treatment. Not surprisingly, hyperglycaemia is the most reported adverse effect of corticosteroid therapy in CAP trials.[10] In **Chapter 2**, the observed risk of hyperglycaemia was higher in patients treated with dexamethasone compared to placebo (7% vs 1%; p =0.001).

Another concern is a possible increased risk of hospital readmission in patients treated with corticosteroids. This concern was first raised in the IPDMA by Briel et al., where a higher percentage of CAP-related readmissions was observed in patients treated with corticosteroids compared to those treated with placebo (5.0% vs 2.7%).[10] In **Chapter 2**, readmission rate was twice as high in the dexamethasone group compared to the placebo group (5% vs 10%, p = 0.051). Even though differences between intervention and placebo group were not statistically significant in **Chapter 2**, it does further complicate the question if a 10% reduction in LOS is enough to justify adjunctive corticosteroid treatment for CAP patients. The above suggests that adjunctive corticosteroids should not be routinely prescribed in all hospitalised CAP patients, which is in line with the current recommendations in national and international guidelines.[14,15]

CAN CAP SUBGROUPS BE DEFINED IN WHICH THE BENEFICIAL EFFECTS OF ADJUNCTIVE CORTICOSTEROID TREATMENT OUTWEIGH THE RISKS?

High serum levels of inflammatory mediators, which are indicative of a high systemic inflammatory response, are associated with disease severity in CAP.[16–21] It has been hypothesised that corticosteroids might be beneficial in patients with severe CAP with an excessive immune response, but not in patients with non-severe CAP and a low or well-balanced immune response.[22] Yet, identifying clinical and/or laboratory parameters that can distinguish between these groups upon hospital admission has proven a challenge. Several options will be discussed below.

Disease severity based on clinical prediction scores

The Santeon-CAP study (**Chapter 2**) was the first trial specifically designed to assess whether the effect of adjunctive corticosteroids differs between patients with mild-moderate CAP (PSI I-III) and severe CAP (PSI IV-V).[23] Randomisation was therefore stratified by disease severity at time of hospital admission. There was no statistically significant difference in effect of dexamethasone between both groups, though the effect of dexamethasone on LOS seemed greater in the patients with mild-moderate pneumonia compared to those with severe pneumonia. However, due to the early termination of the trial combined with a shorter LOS and lower mortality rate than expected, there might not have been enough statistical power to show a difference between treatment arms within the disease severity subgroups. The IPDMA also assessed whether the effect of corticosteroids differed according to PSI score. Contrary to the results in the Santeon-CAP study, the IPDMA suggested that the effect of corticosteroids might be greater in patients with severe CAP compared to those with mild-moderate CAP, yet the effect did not differ significantly between groups.[10] These results indicate that the PSI score does not adequately distinguish between patients who do and patients who do not benefit from corticosteroid treatment. This might be due to the fact that the PSI score does not necessarily correspond to level of inflammation.

The PSI score is a predictor for mortality, it is therefore heavily influenced by age and comorbidities[23]; clinical signs and symptoms of excessive inflammation or severe disease contribute less to the PSI score. Other clinical scores for identifying patients with severe disease are the American Thoracic Society (ATS) criteria and the CURB-65 (acronym for confusion, urea, blood pressure and >65 years of age). These scores rely more on clinical criteria than on age and co-morbidities and thus might be more appropriate to identify patients with severe disease based on inflammation.[15,24] In a sensitivity analysis of the Santeon-CAP study (**Chapter 2**) in patients aged ≤65, the largest reduction in LOS was observed in patients with a high CURB-65 score. Further prospective research is necessary to confirm these results.

C-reactive protein

C-reactive protein (CRP) is a readily available inflammatory biomarker which is widely used in day-to-day clinical practice. In a trial investigating adjunctive methylprednisolone, Torres et al. only included ICU patients with a CRP >150mg/L. They observed less treatment failure in the intervention group compared to the control group.[5] **Chapter 2** also showed a shorter LOS and lower rate of ICU admission in patients with a CRP above the median (≥210 mg/L) who were treated with dexamethasone compared to those who received placebo. However, the IPDMA subgroup analyses based on CRP did not show a differential treatment effect of corticosteroids on LOS or mortality between patients with a CRP above the median (≥188 mg/L) and those with a CRP below the median.[10] An issue with CRP is that it has slower kinetics compared to other inflammatory biomarkers. Mendez et al. showed that serum CRP concentrations were dependent on days since symptom onset. Patients who presented to hospital within 3 days of symptom onset had lower levels CRP concentrations than patients presenting after 3 days, while procalcitonin, interleukin-6 (IL-6) and interleukin-8 (IL-8) were already elevated.[25]

White blood cell count differential parameters

Neutrophil-to-lymphocyte ratio (NLR) and lymphocyte count are easily obtainable parameters upon hospital admission. Both have been associated with level of inflammation and clinical outcomes in CAP.[26,27] In **Chapter 3** a greater effect of dexamethasone was observed in patients with higher peripheral neutrophil counts and a higher NLR. In patients with a WBC count ≥ 15.6 10^9 cells/l, a neutrophil count ≥ 13.2 10^9 cells/l and NLR ≥ 15.5 dexamethasone reduced LOS by 2 days, while there was no effect of dexamethasone on LOS in patients with a lower WBC count, lower neutrophil count or lower NLR. In all white blood cell differential parameter subgroups, both high and low, the percentage of hospital readmission was higher in patients treated with dexamethasone compared to those who received a placebo. For the high WBC count, neutrophil count and NLR subgroups a 2-day decrease in LOS should be weighed against a possible higher risk of hospital admission.

No other studies have assessed for effect modification by WBC differential parameter subgroups on the effect of corticosteroids on clinical outcomes in CAP. A recent study in COVID-19 patients found that patients with a NLR > 6.11 had a greater effect of low dose corticosteroids than patients with a NLR <6.11.[28] Though COVID-19 is a different disease than CAP.

Because there was no effect modification by lymphocyte count in **Chapter 3**, the greater effect of dexamethasone in patients with a high NLR was presumably driven by a high neutrophil count rather than by a low lymphocyte count. Ebrahimi et al. showed that CAP is associated with pronounced neutrophil extracellular trap (NET) formation.[29] Furthermore, the authors found that NETosis was modulated by prednisone and that the effect of adjunctive prednisone treatment on time to clinical stability was

modified by NET marker levels. The authors hypothesised that the beneficial effects of corticosteroid treatment in CAP may be due to modulation of NET formation or neutrophil pre-activation. Neutrophil function was not assessed in **Chapter 3**, though this hypothesis may explain why the effect of dexamethasone was modified by neutrophil count. This is further supported by the fact that higher peripheral neutrophil counts were associated with higher levels of NET markers in the study by Ebrahimi et al. It would be interesting to further elucidate the effects of corticosteroids on neutrophil function in CAP. A better understanding might aid in the identification of patients who would benefit from corticosteroid treatment.

For now, neutrophil count or NLR might be promising parameters for guiding corticosteroid treatment in CAP. Specifically because a leukogram is cheap, easy to perform and in many cases is routinely performed as part of the initial patient work-up. Yet, the results of **Chapter 3** should be interpreted with caution due to its retrospective nature and it being a single centre study. The findings need to be validated in a separate CAP cohort after which prospective validation would be necessary.

Multiple systemic inflammatory biomarkers

The above focuses on single, readily available parameters as predictor for corticosteroid response in CAP (clinical prediction scores, WBC differential counts, CRP). Yet the inflammatory response in CAP is a complex interaction between numerous mediators. Furthermore, not one but several clinical parameters are associated with systemic inflammation. Thus, there are numerous laboratory and clinical parameters that determine inflammation. Therefore, combining multiple parameters that are indicative of systemic inflammation might be a more appropriate approach to identify a CAP subgroup more likely to benefit from corticosteroid treatment. Latent class analysis (LCA) is a statistical method that uses multiple patient characteristics to identify 'hidden subgroups' in a population (**Chapter 1** provides a brief description of LCA). In acute respiratory distress syndrome LCA has successfully identified two clinically distinct subgroups, a hypoinflammatory and hyperinflammatory subgroup, both requiring different strategies for fluid and ventilator management.[30,31] **Chapter 4** and **Chapter 5** explored whether LCA could also identify clinically distinct subgroups in CAP and if so, whether these subgroups respond differently to adjunctive corticosteroids.

In **Chapter 4** and **Chapter 5**, LCAs of baseline inflammatory and clinical parameters were performed in three independent cohorts of CAP patients. In **Chapter 4** LCA was performed in two independent cohorts: the combined Ovidius-Triple P cohort and the Swiss Step cohort.[2,3,32] In both cohorts LCA identified a subgroup with more excessive systemic inflammation and worse prognosis (class 2), and a subgroup with less systemic inflammation and better prognosis (class 1). In Ovidius trial patients, a greater effect of adjunctive dexamethasone treatment on LOS in class 2 compared to class 1 was observed. The same was not found in the STEP cohort. **Chapter 5** aimed

to validate the finding from the Ovidius cohort in the Santeon-CAP cohort. Similar to the Ovidius-TripleP and STEP cohorts, two clinically distinct subgroups of CAP patients were identified. Yet, the finding of a larger effect of corticosteroids in Class 2 compared to class 1 could not be replicated despite a similar population and the use of the same dexamethasone dose as in the Ovidius trial. Taken together, LCA of baseline clinical and inflammatory parameters can identify clinically relevant CAP subgroups with different inflammatory profiles and different clinical outcomes. Yet, a differential effect of corticosteroids between classes was only found in one out of three analysed cohorts. Thus, LCA defined classes did not prove robust in the identification of patients in whom corticosteroids have a greater positive effect. It is important to note that the sample size of class 2 in the Santeon-CAP cohort (n=84) may have been too small to show differences between classes.

Besides a possible sample size issue, there are also other hypotheses that may explain why the response to corticosteroid only differed between classes in one out of three cohorts. First, unmeasured parameters such as the pulmonary inflammatory response might influence the corticosteroid response. The pulmonary inflammatory response and degree of pulmonary damage were not measured in **Chapter 4** and **Chapter 5** nor in other chapters in this thesis. Several studies have shown that the cytokine response is more intense in the lung than in serum and that for most cytokines the levels in serum and in bronchoalveolar lavage fluid are not correlated.[18,19,33] It is plausible that the nature or the extent of pulmonary inflammatory response or the degree of pulmonary damage in CAP may influence the response to corticosteroids.

Second, it has been shown that the inflammatory response in CAP can exhibit signs of concurrent hyperinflammation and immune suppression.[34] One could hypothesise that corticosteroids would not benefit patients with concurrent immune suppression. Third, it has been proposed that corticosteroid resistance might be the cause of the negative or conflicting findings in trials investigating corticosteroids for sepsis.[35] Last, it is possible that high concentrations of some inflammatory mediators contribute to pulmonary damage and sepsis whilst others are necessary for clearing infection. Because corticosteroids downregulate a broad range of inflammatory mediators, it is possible that they also downregulate vital parts of the inflammatory response.

ADJUNCTIVE CORTICOSTEROIDS FOR CAP: CONCLUSION AND FUTURE PERSPECTIVES

Based on the borderline significant results of the Santeon-CAP study and a general trend of decreasing LOS and mortality for CAP patients, the routine use of corticosteroids for all non-ICU patients hospitalised with CAP is not recommended. Due to a lack of a clearly defined and validated subgroup of CAP patients for whom the benefits of adjunctive corticosteroid treatment outweigh the risk, adjunctive corticosteroid treatment is also not recommended for a specific subgroup.

Corticosteroids might still be beneficial for a specific subgroup of patients for whom the benefits outweigh the risks. Yet, further research would be necessary to define and validate such a subgroup. In this thesis, LCA defined subgroups based on inflammatory parameters only modified corticosteroid response in one out of three cohorts. Though there may have been insufficient power to show a difference in corticosteroid effect in the 3rd cohort due to the relatively small number of patients in class 2. Analysis of a larger cohort would be necessary to definitively determine whether LCA of baseline clinical and inflammatory data can identify a subgroup of CAP patients for whom the beneficial effects of corticosteroids outweigh the risks. This thesis also showed that dexamethasone had a greater effect in patients with high peripheral neutrophil counts and a high NLR, but these results require validation. It would be interesting to further elucidate the role of corticosteroids on neutrophil function in CAP and see if this could lead to new insights in guiding corticosteroid therapy. Furthermore, the IPDMA by Briel et al.[10] is currently being updated. The updated IPDMA will include data from the Santeon-CAP study (**Chapter 2**) and the recently published corticosteroid in CAP trial by Meduri et al.[13] The addition of patients from two additional studies may increase statistical power sufficiently to identify CAP subgroups for which corticosteroid treatment is more effective.

DEXAMETHASONE FOR COVID-19

In **Chapter 6**, it was postulated that COVID-19 patients with overweight or obesity would benefit less from the fixed 6 mg dexamethasone dose compared to patients with normal weight. This was based on the assumption that systemic exposure of dexamethasone would be lower in patients with a higher BMI due to the lipophilic nature of dexamethasone. To test this hypothesis, the outcomes between patients with overweight and obesity were compared to those with normal weight in a population of hospitalised COVID-19 patients who were all treated with the recommended 6 mg dexamethasone dose. The results showed that overweight and obesity were not associated with an unfavourable clinical course in this population. Hence, the hypothesis could not be confirmed.

After the study described in **Chapter 6** was completed, a trial comparing the pharmacokinetics of 6 mg dexamethasone between COVID-19 patients with normal weight and COVID-19 patients with obesity was published by Abouir et al.[36] This trial confirmed that COVID-19 patients with obesity had lower serum concentrations of dexamethasone compared to patients with normal weight. This indicates that different dosing should be used in patients with obesity to achieve similar exposure to patients with normal weight. However, in agreement with the results in **Chapter 6**, this trial noted that despite lower serum dexamethasone concentrations in patients with obesity there was no difference in LOS or duration of ICU stay between patients with obesity and those with normal weight.

An explanation for why clinical outcomes were not worse for patients with overweight or obesity compared to those with normal weight despite lower systemic exposure to dexamethasone may be the finding in **Chapter 6** of a lower inflammatory state in patients with obesity compared to those with normal weight. Perhaps, the lower inflammatory state of patients with obesity indicates that lower serum peak dexamethasone concentrations are sufficient in patient with obesity compared to patients with normal weight as there is less inflammation to dampen.

Two randomised trials have compared high dose vs low dose dexamethasone in COVID-19; In the COVID steroid-2 study (6 mg vs 12 mg) days alive without life support and 28-day mortality did not differ significantly between study arms.[37] In the study by Taboada et al. higher dose dexamethasone (20 mg) decreased clinical worsening within 11 days (primary endpoint) but did not improve time to recovery, ICU admission or mortality.[38] Unfortunately, these trials did not report subgroup analyses based on BMI. To definitely answer the question whether outcomes for COVID-19 patients with overweight or obesity can be improved by using higher doses of dexamethasone subgroup analyses in prospective randomised trials are necessary. However, based on the fact that obesity was not associated with worse clinical outcomes in **Chapter 6** nor in the trial by Abouir et al., for now it seems unlikely that patients with obesity or overweight require a higher dexamethasone dose.

MICROBIOLOGICAL TESTING AND ANTIBIOTIC TREATMENT ALTERATIONS IN CAP

Chapters 2 through 5 aimed to optimise CAP treatment by adding corticosteroids to standard treatment with antibiotics. Yet, antimicrobial treatment remains the basis of the treatment of CAP. **Chapter 7** explored whether extensive microbiological testing could improve antibiotic treatment.

An important indicator of appropriate antimicrobial treatment is the alteration of empirical antibiotics based on microbiological test results.[39] In clinical practice, no causative micro-organism is identified in >60% of CAP patients, which can hamper appropriate adjustment of antibiotic treatment.[14] **Chapter 7** showed that, in day-to-day clinical practice, more extensive microbiological testing within the first 2 days of hospital admission was associated with a higher frequency of early antibiotic alterations. A PCR assay for atypical pathogens contributed most to antibiotic treatment alterations, the odds of antibiotic alteration were 2.6 times higher if a PCR assay for atypical pathogens was performed. The influence of PCR assays on antibiotic modification was less outspoken in a different Dutch study of patients with lower respiratory tract infections (not limited to CAP). Oosterheert et al. found that antibiotic treatment was only modified in 11% of patients based on PCR assays for atypical pathogens and respiratory viruses when these were added to the standard microbiological testing protocol.[40] This is most likely caused by the difference in proportion of patients receiving dual therapy (beta-lactam combined with an antibiotic to cover of atypical bacteria) at admission. The majority of alterations in **Chapter 7** were switches from dual- to monotherapy, both positive and negative PCR results provide opportunities for this alteration. In the study by Oosterheert et al. only 16% received dual therapy compared to 35% in **Chapter 7**, thus PCR assays would have less potential to alter treatment. The above illustrates that differences in local antimicrobial treatment protocols and patient population may lead to different results in different centres. Nonetheless, a general recommendation would be to at least perform a PCR assay for atypical pathogens in all patients for whom the empiric antibiotic regimen includes an antibiotic with the purpose of covering atypical bacteria.

CONCLUSIONS

This thesis discussed several strategies to improve the treatment for community-acquired pneumonia outside an ICU setting. It showed that more extensive microbiological testing facilitates early alteration of antibiotic treatment in CAP. A general recommendation would be to at least perform a PCR for atypical pathogens in patients treated with dual therapy upon admission.

Based on the results of the corticosteroid studies presented in this thesis and other available evidence, the routine prescription of adjunctive corticosteroids for all CAP patients is not recommended. Currently, the benefits of corticosteroid treatment do not seem to outweigh possible risks associated with corticosteroid use in CAP. This thesis could not identify a robust subgroup of CAP patients in whom the risk-benefit ratio is acceptable. Peripheral neutrophil counts or NLR seem promising parameters for possible guiding of corticosteroid therapy as the effect of dexamethasone on LOS was greater in patients with high neutrophil counts or a high NLR. Yet, validation of these findings is required. LCA could only identify a subgroup benefiting more from corticosteroid treatment in one out of three analysed cohorts. Because there may have been insufficient power to show a difference in corticosteroid effect in the third cohort, analysis of a larger cohort would be necessary to definitively determine whether LCA of baseline clinical and inflammatory data can identify a subgroup of CAP patients for whom the beneficial effects of corticosteroids outweigh the risks. Furthermore, regarding COVID-19, based on the currently available evidence patients with overweight or obesity do not require higher doses of dexamethasone compared to patients with normal weight.

REFERENCES

1. Martin-Loeches I, Torres A. Corticosteroids for CAP, influenza and COVID-19: when, how and benefits or harm? *Eur Respir Rev*. 2021;30(159):1-9. doi:10.1183/16000617.0346-2020
2. Meijvis SC, Hardeman H, Remmelts HH, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. *The Lancet*. 2011;377(9782):2023-2030. doi:10.1016/S0140-6736(11)60607-7
3. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: A multicentre, double-blind, randomised, placebo-controlled trial. *The Lancet*. 2015;385(9977):1511-1518. doi:10.1016/S0140-6736(14)62447-8
4. Confalonieri M, Urbino R, Potena A, et al. Hydrocortisone Infusion for Severe Community-acquired Pneumonia. *Am J Respir Crit Care Med*. 2005;171(3):242-248. doi:10.1164/rccm.200406-808OC
5. Torres A, Sibila O, Ferrer M, et al. Effect of Corticosteroids on Treatment Failure Among Hospitalized Patients With Severe Community-Acquired Pneumonia and High Inflammatory Response: A Randomized Clinical Trial. *JAMA*. 2015;313(7):677-686. doi:10.1001/jama.2015.88
6. Snijders D, Daniels JMA, de Graaff CS, van der Werf TS, Boersma WG. Efficacy of Corticosteroids in Community-acquired Pneumonia. *Am J Respir Crit Care Med*. 2010;181(9):975-982. doi:10.1164/rccm.200905-0808OC
7. Fernández-Serrano S, Dorca J, Garcia-Vidal C, et al. Effect of corticosteroids on the clinical course of community-acquired pneumonia: a randomized controlled trial. *Crit Care*. 2011;15(2):R96-R96. doi:10.1186/cc10103
8. Nafae R, Ragab M, Amany F, Rashed S. Adjuvant role of corticosteroids in the treatment of community-acquired pneumonia. *Egypt J Chest Dis Tuberc* . 2013;62:439-445.
9. Sabry N, Omar E. Corticosteroids and ICU Course of Community Acquired Pneumonia in Egyptian Settings. *Pharmacol Pharm*. 2011;2(2):73-81. doi:10.4236/pp.2011.22009.
10. Briel M, Spoorenberg SMC, Snijders D, et al. Corticosteroids in Patients Hospitalized With Community-Acquired Pneumonia: Systematic Review and Individual Patient Data Metaanalysis. *Clin Infect Dis*. 2017;66(3):346-354. doi:10.1093/cid/cix801
11. Stern A, Skalsky K, Avni T, Carrara E, Leibovici L, Paul M. Corticosteroids for pneumonia. *Cochrane Database of Syst Rev*. 2017;(12):CD007720. doi:10.1002/14651858.CD007720.pub3
12. Waterer G, Metersky ML. Corticosteroids for Community-Acquired Pneumonia: Overstated Benefits and Understated Risks. *Chest*. 2019;156(6):1049. doi:10.1016/j.chest.2019.06.017
13. Meduri GU, Shih MC, Bridges L, et al. Low-dose methylprednisolone treatment in critically ill patients with severe community-acquired pneumonia. *Intensive Care Med*. 2022;48(8):1009-1023. doi:10.1007/s00134-022-06684-3
14. Wiersinga W, Bonten MJM, Boersma W, et al. Management of community-acquired pneumonia in adults: 2016 guideline update from the Dutch Working Party on Antibiotic Policy (SWAB) and Dutch Association of Chest Physicians (NVALT). *Neth J Med*. 2018;76(1):4-13.
15. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am J Respir Crit Care Med*. 2019;200(7):E45-E67. doi:10.1164/RCCM.201908-1581ST
16. Igonin AA, Armstrong VW, Shipkova M, Lazareva NB, Kukes VG, Oellerich M. Circulating cytokines as markers of systemic inflammatory response in severe community-acquired pneumonia. *Clin Biochem*. 2004;37(3):204-209. doi:10.1016/j.clinbiochem.2003.11.001
17. Ramírez P, Ferrer M, Martí V, et al. Inflammatory biomarkers and prediction for intensive care unit admission in severe community-acquired pneumonia. *Crit Care Med*. 2011;39(10):2211-2217. doi:10.1097/CCM.0B013E3182257445

18. Lee YL, Chen W, Chen LY, et al. Systemic and bronchoalveolar cytokines as predictors of in-hospital mortality in severe community-acquired pneumonia. *J Crit Care*. 2010;25(1):176.e7-e13. doi:10.1016/J.JCRC.2009.05.002

19. Paats MS, Bergen IM, Hanselaar WEJJ, et al. Local and systemic cytokine profiles in nonsevere and severe community-acquired pneumonia. *Eur Respir J*. 2013;41(6):1378-1385. doi:10.1183/09031936.00060112

20. Antunes G, Evans SA, Lordan JL, Frew AJ. Systemic cytokine levels in community-acquired pneumonia and their association with disease severity. *Eur Respir J*. 2002;20(4):990-995. doi:10.1183/09031936.02.00295102

21. Zobel K, Martus P, Pletz MW, et al. Interleukin 6, lipopolysaccharide-binding protein and interleukin 10 in the prediction of risk and etiologic patterns in patients with community-acquired pneumonia: results from the German competence network CAPNETZ. *BMC Pulm Med*. 2012;12:6. doi:10.1186/1471-2466-12-6

22. Sibila O, Rodrigo-Troyano A, Torres A. Nonantibiotic Adjunctive Therapies for Community-Acquired Pneumonia (Corticosteroids and Beyond): Where Are We with Them? *Semin Respir Crit Care Med*. 2016;37(06):913-922. doi:10.1055/s-0036-1593538

23. Fine MJ, Auble TE, Yealy DM, et al. A Prediction Rule to Identify Low-Risk Patients with Community-Acquired Pneumonia. *N Engl J Med*. 1997;336(4):243-250. doi:10.1056/NEJM199701233360402

24. Lim W, van der Eerden MM, Laing R, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. *Thorax*. 2003;58(5):377-382. doi:10.1136/thorax.58.5.377

25. Méndez R, Menéndez R, Cillóniz C, et al. Initial inflammatory profile in community-acquired pneumonia depends on time since onset of symptoms. *Am J Respir Crit Care Med*. 2018;198(3):370-378. doi:10.1164/RCCM.201709-1908OC

26. de Jager CPC, Wever PC, Gemen EFA, et al. The Neutrophil-Lymphocyte Count Ratio in Patients with Community-Acquired Pneumonia. *PLoS One*. 2012;7(10):4-11. doi:10.1371/journal.pone.0046561

27. Cataudella E, Giraffa CM, di Marca S, et al. Neutrophil-To-Lymphocyte Ratio: An Emerging Marker Predicting Prognosis in Elderly Adults with Community-Acquired Pneumonia. *J Am Geriatr Soc*. 2017;65(8):1796-1801. doi:10.1111/jgs.14894

28. Cai J, Li H, Zhang C, et al. The Neutrophil-to-Lymphocyte Ratio Determines Clinical Efficacy of Corticosteroid Therapy in Patients with COVID-19. *Cell Metab*. 2021;33(2):258-269.e3. doi:10.1016/j.cmet.2021.01.002

29. Ebrahimi F, Giaglis S, Hahn S, et al. Markers of neutrophil extracellular traps predict adverse outcome in community acquired pneumonia: Secondary analysis of a randomised controlled trial. *Eur Respir J*. 2018;51(4):1701389. doi:10.1183/13993003.01389-2017

30. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med*. 2014;2(8):611-620. doi:10.1016/S2213-2600(14)70097-9

31. Famous KR, Delucchi K, Ware LB, et al. Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy. *Am J Respir Crit Care Med*. 2017;195(3):331-338. doi:10.1164/rccm.201603-0645OC

32. Endeman H, Schelfhout V, Voorn GP, van Velzen-Blad H, Grutters JC, Biesma DH. Clinical features predicting failure of pathogen identification in patients with community acquired pneumonia. *Scand J Infect Dis*. 2008;40(9):715-720. doi: 10.0.4.56/00365540802014864

33. Montón C, Torres A, El-Ebiary M, Filella X, Xaubet A, de La Bellacasa JP. Cytokine expression in severe pneumonia: A bronchoalveolar lavage study. *Crit Care Med*. 1999;27(9):1745-1753. doi:10.1097/00003246-199909000-00008

34. Brands X, Haak BW, Klarenbeek AM, et al. Concurrent Immune Suppression and Hyperinflammation in Patients With Community-Acquired Pneumonia. *Front Immunol*. 2020;11:796. doi:10.3389/FIMMU.2020.00796

35. Vandewalle J, Libert C. Glucocorticoids in Sepsis: To Be or Not to Be. *Front Immunol*. 2020;11:1318. doi:10.3389/fimmu.2020.01318

36. Abouir K, Gosselin P, Guerrier S, et al. Dexamethasone exposure in normal-weight and obese hospitalized COVID-19 patients: An observational exploratory trial. *Clin Transl Sci*. 2022;15(7):1796-1804. doi:10.1111/cts.13297

37. Munch MW, Myatra SN, Kumar B, et al. Effect of 12 mg vs 6 mg of Dexamethasone on the Number of Days Alive Without Life Support in Adults With COVID-19 and Severe Hypoxemia: The COVID STEROID 2 Randomized Trial. *JAMA*. 2021;326(18):1807-1817. doi:10.1001/jama.2021.18295.

38. Taboada M, Rodríguez N, Varela PM, et al. Effect of high versus low dose of dexamethasone on clinical worsening in patients hospitalised with moderate or severe COVID-19 pneumonia: an open-label, randomised clinical trial. *Eur Respir J*. 2022;60(2): 2102518. doi:10.1183/13993003.02518-2021

39. van den Bosch CMA, Geerlings SE, Natsch S, Prins JM, Hulscher MEJL. Quality Indicators to Measure Appropriate Antibiotic Use in Hospitalized Adults. *Clin Infect Dis*. 2015;60(2):281-291. doi:10.1093/cid/ciu747

40. Oosterheert JJ, van Loon AM, Schuurman R, et al. Impact of Rapid Detection of Viral and Atypical Bacterial Pathogens by Real-Time Polymerase Chain Reaction for Patients with Lower Respiratory Tract Infection. *Clin Infect Dis*. 2005;41(10):1438-1444. http://dx.doi.org/10.1086/497134

Nederlandse samenvatting

Dankwoord

Curriculum vitae

List of publications

NEDERLANDSE SAMENVATTING

INLEIDING

Community-acquired pneumonia, afgekort CAP, is de Engelse term voor een longontsteking die buiten het ziekenhuis wordt opgelopen. Een bacteriële longontsteking komt het vaakst voor, waarbij *Streptococcus Pneumoniae* in Nederland de meest voorkomende verwekker is. Een longontsteking door een virus (zoals het influenzavirus) komt veel minder vaak voor (<5%). In de periode waarin het onderzoek voor dit proefschrift werd uitgevoerd, deed het coronavirus (SARS-CoV-2) zijn intrede als nieuwe verwekker van CAP. Dit leidde tot de COVID-19 pandemie. COVID-19 wordt in dit proefschrift apart besproken. Voor CAP die niet wordt veroorzaakt door het coronavirus, het hoofdonderwerp van dit proefschrift, zal de term CAP gebruikt worden. Voor een infectie met het coronavirus zal de term COVID-19 worden gebruikt.

Antibiotica zijn de hoeksteen van de behandeling van CAP. Twee belangrijke onderdelen van een adequaat antibioticabeleid zijn het zo snel mogelijk starten van antibiotica gericht op de meest waarschijnlijke verwekker (empirische therapie) en het aanpassen van deze empirische therapie zodra de oorzakelijke ziekteverwekker wordt geïdentificeerd (gerichte therapie). In Nederland bestaat de empirische therapie vaak uit een ß-lactam antibioticum (bijv. amoxicilline). Bij zieke patiënten kan deze gecombineerd worden met een antibioticum dat atypische bacteriën (bijv. legionella) dekt. Dit wordt duotherapie genoemd. Om de ziekteverwekker te achterhalen wordt bij opname materiaal, zoals sputum of een keeluitstrijk, voor microbiële diagnostiek afgenomen. Een adequate antibiotische behandeling zorgt voor betere klinische uitkomsten en vermindert het risico op antibioticaresistentie.

De behandeling van CAP kent uitdagingen. Ten eerste wordt bij 60% van de patiënten geen oorzakelijke verwekker gevonden waardoor antibiotica niet aangepast kunnen worden. Daarnaast wordt een deel van de CAP-patiënten toch ernstig ziek ondanks adequate behandeling met antibiotica. Zij ontwikkelen een sepsis, moeten op de Intensive Care (IC) worden opgenomen of komen te overlijden. Er wordt gedacht dat een overmatige reactie van het immuunsysteem (immuunrespons) hier mogelijk de oorzaak van is. Het is van belang dat de immuunrespons gebalanceerd is. Bij een gebalanceerd respons wordt de ziekteverwekker effectief opgeruimd met zo min mogelijk schade aan het longweefsel. Echter, als de immuunrespons te sterk of ongebalanceerd is, kan er schade ontstaan aan het longweefsel en andere organen. Het toevoegen van corticosteroïden, krachtige ontstekingsremmende medicijnen, zou door het remmen van de immuunrespons deze schade aan de long mogelijk kunnen verminderen of voorkomen. Toch laten studies naar corticosteroïden bij CAP tot nu toe wisselende resultaten zien. Het is de vraag of de voordelen van een aanvullende behandeling met

corticosteroïden opwegen tegen de nadelen ervan. Daarom wordt behandeling met corticosteroïden bij CAP momenteel niet aanbevolen in de behandelrichtlijnen.

Anders dan bij CAP toonde onderzoek al snel na de uitbraak van de COVID-19 pandemie aan dat corticosteroïden een effectieve behandeling zijn voor COVID-19. Dexamethason, een type corticosteroïd, is sindsdien de standaardbehandeling voor patiënten die met ernstige COVID-19 in het ziekenhuis worden opgenomen. Echter is het onduidelijk of de standaarddosering van 6mg voor elke patiënt even effectief is.

Doel van het proefschrift

Het doel van dit proefschrift was het identificeren van strategieën om de behandeling van opgenomen CAP-patiënten te optimaliseren. In dit proefschrift werd enerzijds onderzocht of het toevoegen van orale corticosteroïden aan de behandeling van CAP leidt tot betere klinische uitkomsten en of er een subgroep met CAP-patiënten bestaat voor wie de voordelen van corticosteroïdbehandeling opwegen tegen de nadelen ervan. Anderzijds werd onderzocht of het inzetten van uitgebreidere microbiële diagnostiek leidt tot frequentere identificatie van een verwekker en frequentere aanpassing van het antibioticabeleid. Tot slot onderzocht dit proefschrift of het effect van de standaarddosering dexamethason bij COVID-19 patiënten wordt beïnvloed door overgewicht of obesitas. De onderzoeken in dit proefschrift richtten zich alleen op patiënten die op een reguliere verpleegafdeling (dus niet op de intensive care) werden opgenomen.

CORTICOSTEROÏDEN VOOR CAP

Hoofdstuk 2 beschrijft de Santeon-CAP studie. In deze studie werd onderzocht of aanvullende behandeling met orale dexamethason de opnameduur van CAP-patiënten in het ziekenhuis verkort. Er werd gekozen voor orale medicatie omdat toediening van studiemedicatie via het infuus mogelijk leidt tot een langere opnameduur door het vertragen van de overstap van intraveneuze antibiotica naar orale antibiotica. Daarnaast werd de Santeon-CAP studie op zo'n manier opgezet dat ook onderzocht kon worden of het effect van dexamethason afhankelijk is van de ernst van de longontsteking. De hypothese was dat patiënten die ernstiger ziek zijn een hevigere immuunrespons hebben en daardoor meer baat zouden hebben van dexamethason dan patiënten met een mildere longontsteking.

Aan de Santeon-CAP studie namen CAP-patiënten uit vier Nederlandse ziekenhuizen deel. Patiënten werden willekeurig verdeeld in een placebogroep en een dexamethasongroep. Patiënten in de dexamethasongroep werden behandelend met 1 tablet (6 mg) dexamethason voor 4 dagen, patiënten in de placebogroep werden behandeld met 1 placebotablet per dag voor 4 dagen. Deze behandelgroepen werden verder onderverdeeld naar ernst van de longontsteking. Hierbij werd onderscheid gemaakt tussen milde tot matige CAP en ernstige CAP. Deze indeling werd gemaakt

op basis van de pneumonia severity index (PSI) score. De PSI-score is ontwikkeld om op basis van patiëntkenmerken en klinische gegevens het risico op overlijden te voorspellen. De PSI-score wordt gebruikt om ziekte-ernst in te schatten.

De uitkomsten van de Santeon-CAP studie lieten zowel positieve als negatieve effecten van dexamethason zien. Het belangrijkste positieve effect van dexamethason was dat dexamethason de opnameduur met 0,5 dag (10%) verkortte. De mediane opnameduur was 4,5 dagen in de dexamethasongroep en 5,0 dagen in de placebogroep. Daarnaast was de kans op IC-opname in de dexamethasongroep kleiner dan in de placebogroep (3% vs. 7%). Een negatief effect van dexamethason was dat het percentage heropnames hoger was in de dexamethasongroep vergeleken met de placebogroep (10% vs. 5%). Verder was het risico op overlijden gelijk tussen de placebogroep en de dexamethasongroep. Er werd geen verschil gevonden in het effect van dexamethason tussen patiënten met ernstige CAP en met patiënten met milde tot matige CAP.

De uitkomsten van de Santeon-CAP studie komen grotendeels overeen met de resultaten van eerdere onderzoeken naar corticosteroïden bij CAP. Een gecombineerde analyse van deze onderzoeken liet zien dat het toevoegen van corticosteroïden aan de behandeling van CAP de opnameduur met 1 dag (+/- 10%) verkort, maar het risico op overlijden of IC-opname niet vermindert en het risico op heropname mogelijk vergroot. Een opnameduurverkorting van 0,5-1,0 dag (+/- 10%) lijkt niet op te wegen tegen een hoger risico op heropname. De vraag is of er een specifieke subgroep met CAP-patiënten bestaat voor wie de positieve effecten van behandeling met corticosteroïden wel opwegen tegen de risico's ervan. De PSI-score bleek in de Santeon-CAP studie in ieder geval geen onderscheid te kunnen maken tussen patiënten die wel en die geen baat hebben van behandeling met corticosteroïden. Omdat de PSI-score ontwikkeld is om het risico op overlijden te voorspellen, wordt deze score flink beïnvloed door leeftijd en de aanwezigheid van andere (chronische) aandoeningen. Klinische aanwijzingen voor inflammatie tellen minder zwaar mee. Daarom werden in de volgende hoofdstukken van dit proefschrift twee methoden gebruikt om CAP-patiënten in inflammatoire subgroepen in te delen.

CAP-subgroepen op basis van aantal witte bloedcellen

In **Hoofdstuk 3** werden CAP-patiënten in inflammatoire subgroepen verdeeld op basis van het aantal witte bloedcellen (leukocyten) in het bloed bij opname. Er bestaan verschillende soorten leukocyten. Het aantal leukocyten en de samenstelling van de leukocyten in het bloed kan worden bepaald met een leukocytendifferentiatie. Neutrofielen vormen het grootste deel van de leukocyten, zij spelen een belangrijke rol bij de afweerreactie bij CAP. Neutrofielen migreren naar de plek van de ontsteking waar ze bacteriën elimineren. Een toename van het aantal neutrofielen in het bloed duidt op een actieve ontstekingsreactie als reactie op een infectie. Het aantal neutrofielen in het bloed is geassocieerd met de uitgebreidheid van de immuunrespons. Een ander

type witte bloedcel is de lymfocyt, deze vormt een veel kleiner deel van het totaal aantal leukocyten. Bij CAP kan er sprake zijn van een normaal, verhoogd of verlaagd lymfocytengetal. Recent is gebleken dat een laag aantal lymfocyten geassocieerd is met ernstigere ziekte en een uitgebreidere immuunrespons bij CAP. Daarnaast blijkt de verhouding tussen het aantal neutrofielen en het aantal lymfocyten (neutrofiel-lymfocyt ratio) geassocieerd te zijn met de mate van inflammatie bij verschillende ziekten. Specifiek bij CAP blijkt een hoge neutrofiel-lymfocyt ratio geassocieerd te zijn met slechtere klinische uitkomsten.

In **hoofdstuk 3** werd onderzocht of het aantal leukocyten, neutrofielen, lymfocyten en de neutrofiel-lymfocyt ratio invloed hebben op het effect van dexamethason op klinische uitkomsten bij CAP-patiënten. Hiervoor werden patiënten die hadden deelgenomen aan de Santeon-CAP studie (**hoofdstuk 2**) ingedeeld in subgroepen op basis van de hoogte van het aantal leukocyten, aantal neutrofielen, aantal lymfocyten en de neutrofiel-lymfocyt ratio in het bloed bij opname. Bij patiënten met een hoog totaal aantal leukocyten, een hoog aantal neutrofielen of een hoge neutrofiel-lymfocyt ratio verkortte dexamethason de opnameduur met 2 dagen. Dexamethason had geen effect op de opnameduur van patiënten met een laag aantal leukocyten, laag aantal neutrofielen of een lage neutrofiel-lymfocyt ratio. Het aantal lymfocyten leek geen invloed te hebben op het effect van dexamethason. In alle subgroepen was het percentage heropnames hoger bij patiënten die met dexamethason werden behandeld dan bij patiënten die met placebo werden behandeld. Bij de subgroepen met een hoog aantal leukocyten, hoog aantal neutrofielen en hoge neutrofiel-lymfocyt ratio zal een vermindering van de opnameduur van 2 dagen dus afgewogen moeten worden tegen een hoger risico op heropname.

Het aantal neutrofielen en de neutrofiel-lymfocyt ratio in het bloed lijken mogelijk dus veelbelovende waarden om CAP-patiënten te identificeren voor wie behandeling met corticosteroïden zinvol is. Het voordeel van een leukocytendifferentiatie is dat het goedkoop en makkelijk uit te voeren is. Daarnaast wordt deze vaak standaard uitgevoerd bij CAP-patiënten op de spoedeisende hulp. Toch moeten de resultaten uit **hoofdstuk 3** met een slag om de arm worden geïnterpreteerd vanwege de retrospectieve aard van de studie en het feit dat de studie slechts in één ziekenhuis is uitgevoerd. De bevindingen uit **hoofdstuk 3** moeten eerst bevestigd worden in een ander cohort met CAP-patiënten.

CAP-subgroepen op basis van latente klasse analyse

In **hoofdstuk 2** en **hoofdstuk 3** werden losse variabelen gebruikt om patiënten in subgroepen te verdelen (PSI-score, aantal witte bloedcellen). Echter, de immuunrespons is een complexe interactie tussen de vele onderdelen van het immuunsysteem. Er bestaan dan ook veel verschillende stoffen in het bloed die gerelateerd zijn aan inflammatie (inflammatoire markers). Daarnaast zijn er ook meerdere klinische verschijnselen (zoals een lage bloeddruk of een laag zuurstofgehalte in het bloed) die

duiden op inflammatie of ernstige ziekte. Daarom werd in **hoofdstuk 4** en **hoofdstuk 5** een combinatie van patiëntkenmerken, klinische verschijnselen, bloedwaarden en inflammatoire markers gebruikt om inflammatoire subgroepen te identificeren. Hiervoor werd gebruik gemaakt van een latente klasse analyse (afgekort LCA). Een LCA is een statistische methode die wordt gebruikt om patiënten te groeperen op basis van vergelijkbare eigenschappen of kenmerken, ook als die groepen niet direct zichtbaar zijn. Hierdoor kunnen verborgen subgroepen in een populatie of groep worden ontdekt. Subgroepen die door LCA worden geïdentificeerd worden klassen genoemd. In **hoofdstuk 4** en **hoofdstuk 5** werden in totaal drie LCAs uitgevoerd, elk in een ander cohort met CAP-patiënten.

In **hoofdstuk 4** werden twee LCAs uitgevoerd. Eén in het Ovidius-TripleP cohort en één in het Zwitserse STEP cohort. In het Ovidius-TripleP cohort zaten CAP-patiënten uit de Triple-P studie en de Ovidius studie. In de Ovidius studie werden patiënten behandeld met dexamethason of een placebo. De Triple-P studie was een observationeel onderzoek, er vond dus geen interventie plaats naast de reguliere behandeling van CAP. In het STEP cohort zaten patiënten uit de STEP studie die in meerdere Zwitserse ziekenhuizen werd uitgevoerd. In de STEP studie werden patiënten behandeld met prednison (een type corticosteroïd) of een placebo.

In zowel het Ovidius-TripleP cohort als het STEP-cohort bleek LCA in staat om CAP-patiënten in twee klinisch relevante CAP-subgroepen (klassen) in te delen, klasse 1 en klasse 2. Patiënten in klasse 1 hadden minder tekenen van inflammatie en waren minder ernstig ziek. Patiënten in klasse 2 waren ernstiger ziek en hadden meer tekenen van inflammatie. De klinische uitkomsten van patiënten in klasse 2 waren slechter dan die van patiënten in klasse 1. Patiënten in klasse 2 hadden een langere opnameduur en hadden een hoger risico op overlijden. Analyse van de patiënten uit het Ovidius-TripleP cohort die deelgenomen hadden aan de Ovidius studie liet zien dat het effect van dexamethason op de opnameduur groter was in klasse 2 dan in klasse 1. In het STEP cohort werd geen verschil waargenomen tussen de klassen wat betreft het effect van prednison op klinische uitkomsten. In **hoofdstuk 5** werd een derde LCA uitgevoerd in het Santeon-CAP cohort (**hoofdstuk 2**). Voor de LCA in het Santeon-CAP cohort werden, waar mogelijk, dezelfde patiëntkenmerken, klinische verschijnselen, bloedwaarden en inflammatoire markers gebruikt als in de LCA van het Ovidius-TripleP cohort. In het Santeon-CAP cohort werden dezelfde twee klassen geïdentificeerd als in de Ovidius-TripleP cohort. Echter, het effect van dexamethason op klinische uitkomsten verschilde niet tussen beide klassen.

Hoewel LCA in elk cohort dezelfde twee (klinisch relevante) klassen vond, werd er maar in één van de drie cohorten een verschil gevonden in het effect van corticosteroïden tussen deze klassen. Echter, de steekproefgrootte in **hoofdstuk 5** was mogelijk te klein om een verschil aan te tonen. Daarom zou de LCA herhaald moeten worden in een groter

cohort met CAP-patiënten. Voor deze LCA dienen dan dezelfde variabelen gebruikt te worden als in het Ovidius-TripleP cohort en het Santeon-CAP cohort.

Conclusie

Op basis van de resultaten van de Santeon-CAP studie (**hoofdstuk 2**) gecombineerd met de resultaten van eerder onderzoek naar corticosteroïden bij CAP, wordt het niet aanbevolen om corticosteroïden te gebruiken als aanvullende behandeling voor CAP-patiënten die op een reguliere verpleegafdeling worden opgenomen. Omdat er ook geen duidelijk gedefinieerde en gevalideerde CAP-subgroep is voor wie de voordelen van corticosteroïden opwegen tegen de nadelen ervan, wordt behandeling met corticosteroïden ook niet voor een specifieke CAP-subgroep aanbevolen. Het is mogelijk dat er alsnog een CAP-subgroep bestaat die wel baat zou hebben bij aanvullende behandeling met corticosteroïden. Echter, er is verder onderzoek nodig om deze groepen te definiëren en te valideren. Een mogelijkheid voor toekomstig onderzoek zou het uitvoeren van een LCA in groter cohort zijn, zoals hierboven besproken. Op basis van de resultaten in **hoofdstuk 3** lijkt het aantal neutrofielen of de neutrofiel-lymfocyt ratio veelbelovend als hulpmiddel bij de beslissing om corticosteroïden wel of niet toe te passen. Verder onderzoek zou nodig zijn om deze bevindingen te bevestigen. Tot slot zou het interessant zijn om meer inzicht te krijgen in hoe de functie van neutrofielen wordt beïnvloed door corticosteroïden.

DEXAMETHASON VOOR COVID-19

Binnen enkele maanden na het uitroepen van de COVID-19 pandemie lieten studies zien dat behandeling met corticosteroïden het risico op invasieve beademing en het risico op overlijden verminderde. Op basis van deze studies werd een dagelijkse dosis van 6 mg dexamethason gedurende 10 dagen (of tot ontslag) de standaardbehandeling voor opgenomen COVID-19-patiënten. Vanwege de eigenschappen van dexamethason is het mogelijk dat de concentratie van dexamethason in het bloed lager is bij patiënten met overgewicht of obesitas in vergelijking met patiënten met een normaal gewicht bij dezelfde dexamethason dosering. De vraag is of de standaarddosering van 6 mg even effectief is bij patiënten met overgewicht of obesitas in vergelijking met patiënten met een normaal gewicht. Daarom werd in **hoofdstuk 6** een retrospectieve cohortstudie uitgevoerd onder patiënten die met COVID-19 in het St. Antonius ziekenhuis werden opgenomen op een reguliere verpleegafdeling én met de standaarddosering dexamethason werden behandeld. Het risico op intensive care opname en het risico op overlijden werd vergeleken tussen patiënten met een normaal gewicht, overgewicht en obesitas. Het bleek dat patiënten met overgewicht of obesitas geen hoger risico hadden op intensive care opname en overlijden vergeleken met patiënten met een normaal gewicht. Er waren dus geen aanwijzing dat 6 mg dexamethason minder effectief zou zijn bij patiënten met overgewicht of obesitas.

MICROBIËLE DIAGNOSTIEK EN AANPASSINGEN IN HET ANTIBIOTICABELEID

In hoofdstuk 2 t/m 5 werden corticosteroïden onderzocht als aanvullende behandeling voor CAP. Echter, een adequaat antibioticabeleid blijft de basis van een goede behandeling van CAP. Zoals eerder benoemd, is het aanpassen van antibiotica op basis van de uitslag van microbiële diagnostiek een belangrijk onderdeel van een adequate antibiotische behandeling van CAP. Echter, hiervoor moet eerst een verwekker door middel van diagnostiek worden aangetoond.

In **hoofdstuk 7** wordt een retrospectieve cohortstudie beschreven waarin werd onderzocht of het antibioticabeleid bij CAP-patiënten vaker wordt aangepast als er meer microbiële diagnostiek wordt uitgevoerd. In deze analyse werden de volgende soorten microbiële diagnostiek meegenomen: sputumkweken, bloedkweken, PCR voor respiratoire virussen, PCR voor atypische bacteriële verwekkers en urine antigeentests voor Legionella en *S. pneumoniae*. De resultaten lieten zien dat er vaker een verwekker werd gevonden als er meer verschillende tests werden uitgevoerd binnen de eerste twee dagen van opname. Daarnaast lieten de resultaten zien dat hoe meer microbiële diagnostiek er binnen de eerste twee dagen werd ingezet, hoe vaker het antibioticabeleid werd aangepast binnen de eerste drie dagen van opname. Met name het uitvoeren van een PCR voor atypische verwekkers was sterk geassocierd met aanpassingen in het antibioticabeleid. Dit wordt verklaard door het feit dat zowel een negatieve als een positieve uitslag kan zorgen voor aanpassingen in het antibioticabeleid, met name bij patiënten die met duotherapie worden behandeld. Bij een positieve PCR kan het ß-lactam antibioticum gestaakt worden en bij een negatieve PCR kan het antibioticum dat atypische verwekkers dekt gestaakt worden.

CONCLUSIE

Dit proefschrift onderzocht verschillende manieren om de behandeling van patiënten die met CAP op een reguliere verpleegafdeling worden opgenomen te optimaliseren. Dit proefschrift liet zien dat het uitbreiden van de microbiële diagnostiek leidt tot meer aanpassingen in het antibioticabeleid. Een algemene aanbeveling zou zijn om bij patiënten die bij opname starten met duotherapie ten minste een PCR-test voor atypische verwekkers uit te voeren. Verder laat dit proefschrift zien dat de voordelen van corticosteroïden bij CAP niet opwegen tegen de nadelen ervan. Daarom dienen corticosteroïden niet standaard toegevoegd te worden aan de behandeling van CAP-patiënten die op een reguliere verpleegafdeling worden opgenomen. Er kon geen goed gedefinieerde subgroep met CAP-patiënten worden geïdentificeerd voor wie behandeling met corticosteroïden wel gunstig zou zijn. Op basis van de resultaten in **hoofdstuk 3** lijkt het aantal neutrofielen of de neutrofiel-lymfocyt ratio veelbelovend als hulpmiddel bij de beslissing om corticosteroïden wel of niet toe te passen. Echter, eerst

is verder onderzoek nodig om deze bevindingen te bevestigen. Ook zou het herhalen van de LCA uit **hoofdstuk 5** in een groter cohort mogelijk kunnen helpen om in de toekomst een CAP-subgroep te identificeren voor wie behandeling met corticosteroïden zin heeft. Tenslotte werden corticosteroïden voor COVID-19 ook behandeld in dit proefschrift. COVID-19 patiënten met overgewicht of obesitas die werden behandeld met de standaarddosering dexamethason hadden geen hoger risico op intensive care opname of overlijden in vergelijking met COVID-19 patiënten met een normaal gewicht die met de standaarddosering werden behandeld. Er waren dus geen aanwijzingen dat de standaarddosering van 6mg dexamethason minder effectief zou zijn bij patiënten met overgewicht of obesitas in vergelijking met patiënten met een normaal gewicht.

DANKWOORD

Dit was proefschrift niet tot stand gekomen zonder de bijdrage van vele anderen. Ik wil dan ook iedereen die direct of indirect aan dit proefschrift heeft bijgedragen ontzettend bedanken voor alle begeleiding, hulp en steun die ik de afgelopen 5,5 jaar heb mogen ontvangen. Een aantal mensen wil ik graag in het bijzonder bedanken.

Allereerst mijn promotoren, prof. dr. Willem Jan Bos en prof. dr. Jan Grutters, en mijn co-promotor dr. Ewoudt van de Garde. Beste Willem Jan, ondanks je drukke agenda wist je altijd tijd voor mij vrij te maken. Ik heb je betrokkenheid, je kritische blik en het vertrouwen dat jij mij hebt gegeven heel erg gewaardeerd. Ontzettend bedankt voor je fijne begeleiding. Beste Jan, ik heb heel veel bewondering voor de eindeloze hoeveelheid ideeën en nieuwe invalshoeken die jij altijd inbracht. Deze zijn erg waardevol geweest bij het tot stand komen van dit proefschrift. Beste Ewoudt, ik heb ontzettend veel gehad aan je statistische kennis, je geduld en je hulp met het schrijven en reviseren van manuscripten. Ik waardeer het heel erg dat jij altijd beschikbaar was voor overleg op de momenten dat ik even vastliep. Zonder jou was dit niet gelukt.

(Oud) leden van Santeon-CAP studiegroep: Paul, Stefan en Simone. Paul, bij jou kon ik altijd terecht met vragen over alles wat met de microbiologie te maken had. Stefan en Simone, mijn voorgangers, jullie hebben de Santeon-CAP studie op poten gezet. Jullie harde werk om de Santeon-CAP studie van de grond te krijgen (Simone) en draaiende te houden (Stefan) heeft een goede basis gelegd waar ik op voort kon bouwen. Ik ben jullie hier heel veel dank voor verschuldigd.

Heel veel dank aan iedereen die betrokken is geweest bij de Santeon-CAP studie. Zonder de inspanningen van de lokale hoofdonderzoekers, A(N)IOS en de researchverpleegkundigen in de deelnemende centra waren we nooit zover gekomen. Dit geldt ook voor de medewerkers van de Klinische Chemie, de Medische Microbiologie en Immunologie en de Academie. In het bijzonder wil ik graag Yvonne van Schaik, Anne Reiners, Ben de Jong†, Bob Meek, Maria de Vries, Sandra Otte en Petra Goené (Klinische Farmacie), en natuurlijk Noortje Koppelman bedanken. Daarnaast ook veel dank aan Kitty en Bianca. Ik ben jullie niet alleen dankbaar voor de praktische hulp rondom het includeren van patiënten voor de Santeon-CAP studie maar ook voor de gezelligheid, de koffiemomenten en jullie luisterend oor.

Ook ontzettend veel dank aan de patiënten die deelgenomen hebben aan de Santeon-CAP studie, zonder hun bereidheid om deel te nemen was er überhaupt geen Santeon-CAP studie geweest.

Oud-collega A(N)IOS van de beschouwende poule en de longziekten, internisten en longartsen heel erg bedankt voor de fijne samenwerking in de periodes dat ik in de kliniek werkzaam was.

Mijn mede-AIOS van de huisartsopleiding, bedankt voor jullie oprechte interesse in alle promotieperikelen tijdens de laatste loodjes van dit traject. Speciale dank aan Irene voor het ontwerpen van de mooie omslag van dit proefschrift.

Daniëlle, heel erg bedankt voor jouw flexibiliteit tijdens mijn eerste jaar van de huisartsopleiding. Dit gaf mij de ruimte die ik nodig had om dit proefschrift af te ronden. Daarnaast heeft jouw enthousiasme als huisarts en als opleider gezorgd voor een ontzettend fijn, gezellig en leerzaam jaar.

Judith en Iris, samen begonnen we in Leidsche Rijn aan onze eerste baan. Sindsdien zijn jullie ontzettend waardevolle vriendinnen. Wat hebben we de afgelopen jaren samen veel gelachen (en soms gehuild). Jullie zijn er altijd voor mij en daar ben ik jullie ontzettend dankbaar voor.

Tessa en Fleur, sinds de eerste dag van onze studie zijn wij al met z'n drieën samen. De eerste coschappen, afstuderen, onze eerste baan, veel grote stappen hebben we samen gedeeld. Ik ben dan ook trots en dankbaar dat jullie nu mijn paranimfen zijn.

Papa, Mama, Astrid en Isabel bedankt dat jullie er altijd voor mij zijn. Bij jullie kan ik altijd terecht voor advies en jullie hebben mij altijd onvoorwaardelijk gesteund. Zonder jullie was ik nooit gekomen waar ik nu ben. Ik hou ontzettend veel van jullie.

Bobby, al 10 jaar sta jij aan mijn zijde. Zonder jouw liefde, flexibiliteit en onvoorwaardelijk steun was ik nooit zover gekomen. Maar ook jouw praktische hulp was van onschatbare waarde (o.a. als mijn persoonlijke ICT-helpdesk). Ik ben zo gelukkig dat ik het leven met jou mag delen en kijk uit naar alle mooie momenten die we samen nog mogen beleven.

CURRICULUM VITAE

Esther Wittermans was born on January 11th 1992 in St. Leonards on Sea, United Kingdom. She attended secondary school at Lorentz Casimir Lyceum in Eindhoven. After graduating in 2010, she spent a year studying at Lewis & Clark College (Portland, OR, USA) through the Fulbright Campus Scholarship Program. After returning to the Netherlands in 2011, she enrolled in medical school at Utrecht University. During her final year of medical school, she worked on her first community-acquired pneumonia research project during a research internship at St. Antonius Hospital in Nieuwegein. She graduated medical school in 2017 after which she started as a PhD student at St. Antonius Hospital under supervision of Prof. dr. W.J.W. Bos, Prof. dr. J.C. Grutters and Dr. E.M.W. van de Garde. She combined research activities with clinical work as a resident (non-training) in the internal medicine department and the pulmonary medicine department. In March 2022, she commenced training to become a general practitioner at the University Medical Center Utrecht.

LIST OF PUBLICATIONS

This Thesis

Wittermans E, Vestjens SMT, Bos WJW, Grutters JC, van de Garde EMW, Vlaminckx BJM. The extent of microbiological testing is associated with alteration of antibiotic therapy in adults with community-acquired pneumonia. Eur J Clin Microbiol Infect Dis. 2019;38(7):1359-1366.

Wittermans E, Vestjens SMT, Spoorenberg SMC, Blok WL, Grutters JC, Janssen R, Rijkers GT, Smeenk FWJM, Voorn GP, van de Garde EMW, Bos WJW; Santeon-CAP Study Group. Adjunctive treatment with oral dexamethasone in non-ICU patients hospitalised with community-acquired pneumonia: a randomised clinical trial. Eur Respir J. 2021;12;58(2):2002535.

Wittermans E, van de Garde EMW, Voorn GP, Aldenkamp AF, Janssen R, Grutters JC, Bos WJW; Santeon-CAP study group. Neutrophil count, lymphocyte count and neutrophil-to-lymphocyte ratio in relation to response to adjunctive dexamethasone treatment in community-acquired pneumonia. Eur J Intern Med. 2022;96:102-108.

Wittermans E, van der Zee PA, Qi H, van de Garde EMW, Blum CA, Christ-Crain M, Gommers D, Grutters JC, Voorn GP, Bos WJW, Endeman H. Community-acquired pneumonia subgroups and differential response to corticosteroids: a secondary analysis of controlled studies. ERJ Open Res. 2022;10;8(1):00489-2021.

Wittermans E, Grutters JC, Moeniralam HS, Ocak G, Paul Voorn G, Bos WJW, van de Garde EMW. Overweight and obesity are not associated with worse clinical outcomes in COVID-19 patients treated with fixed-dose 6 mg dexamethasone. Int J Obes (Lond). 2022;46(11):2000-2005.

Wittermans E, van der Zee PA, Qi H, Grutters JC, Voorn GP, Bos WJW, van de Garde EMW, Endeman H. Latent class analysis-based subgroups and response to corticosteroids in hospitalised community-acquired pneumonia patients: a validation study. ERJ Open Res. 2023;9(2):00577-2022.

Other publications

Vestjens SMT, **Wittermans E**, Spoorenberg SMC, Grutters JC, van Ruitenbeek CA, Voorn GP, Bos WJW, van de Garde EMW. Inter-hospital variation in the utilization of diagnostics and their proportionality in the management of adult community-acquired pneumonia. Pneumonia (Nathan). 2018 Dec 25;10:15.