



# The inflamed sputum in lower respiratory tract infection: L-lactate levels are correlated to neutrophil accumulation

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Fredman G, Kolpen M, Hertz FB, Petersen PT, Jensen AV, Baunbæk-Egelund G, Ravn P, Jensen PØ, Faurholt-Jepsen D. The inflamed sputum in lower respiratory tract infection: L-lactate levels are correlated to neutrophil accumulation. *APMIS* 2019; 127: 72–79.

Lower respiratory tract infections (LRTI) are common, but little is known about the response of biomarkers of inflammation in the lungs. Therefore, our primary aim was to compare the concentration of L-lactate to the concentration of neutrophils in sputum and systemic markers of infection. Because it is difficult to differentiate viral and bacterial infection on the basis of clinical presentation in LRTI, our secondary aim was to evaluate if L- and D-lactate may serve as markers of local inflammation as representatives of neutrophils and bacteria, respectively. *Methods* – Patients with acute LRTI were prospectively recruited. Sputum samples were collected and analysed for neutrophil count, L-lactate and D-lactate. We had data on pathogens from sputum cultures and polymerase chain reaction (PCR) (atypical bacteria, virus) and C-reactive protein (CRP) from blood. *Results* – In 44 sputum samples from 32 patients, the median (interquartile range (IQR)) sputum neutrophil granulocyte count was  $0.615 \times 10^7$  cells/mL (0.236–1.575). The sputum neutrophil granulocyte count was associated with sputum L-lactate ( $p = 0.011$ ) and CRP ( $p = 0.018$ ), but not with D-lactate ( $p = 0.177$ ). There was a strong association between sputum D-lactate and L-lactate ( $p < 0.0001$ ). *Conclusion* – As L-lactate in sputum is closely correlated to sequestration of neutrophils in the lungs, L-lactate is a marker for local inflammation in LRTI and a potential biomarker in clinical management of LRTI. On expectorated sputum, D-lactate had no clinical relevance.

**Key words:** lower respiratory tract infections; sputum neutrophil granulocytes; sputum L-lactate; sputum D-lactate; lung infections.

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Lower respiratory tract infections (LRTI) are common. The response of biomarkers of inflammation in the lungs is, however, far from fully understood. Markers of inflammation and pulmonary infection have been studied in patients with chronic obstructive pulmonary disease (COPD) and cystic

fibrosis (CF) (1–3). CF-related chronic airway inflammation and tissue remodelling are thought to be caused by neutrophils that are present in large numbers in the airways of CF patients (1). In a study of sputum from CF patients with pulmonary exacerbation, there was an association between sputum neutrophil count and sputum lactate (2). However, this study did not differentiate between the two

Received 16 July 2018. Accepted 26 November 2018

isomers of lactate. Increased markers of neutrophilic inflammation in sputum have been found to be associated with bacterial infection in COPD exacerbations (3), potentially leading to increased levels of both L-lactate and D-lactate.

In LRTI, the differentiation between virus and bacteria, based on the clinical presentation in LRTI, is difficult. In hospital settings, a causative pathogen is not detected in nearly half of pneumonia cases and often it is a mix of pathogens (4–7). To reduce unnecessary prescription of antibiotics in viral LRTI, there is a need for markers to differentiate viral from bacterial infections when detection fails.

In nature, lactate exists in two isoforms, L-lactate and D-lactate, produced from pyruvate by the isomeric-specific enzymes, L-lactate dehydrogenase (L-LDH) and D-lactate dehydrogenase (D-LDH) (8). L-Lactate is produced in human tissue from pyruvate during anaerobic metabolism of carbohydrate and is the primary form of lactate in humans (8). D-Lactate is only produced by lower organisms equipped with D-LDH such as bacteria (9). D-Lactate synthesis is possible in mammals via the methylglyoxal pathway, but this pathway only generates moderate amounts of D-lactate in the bloodstream (8). Blood D-lactate level in healthy humans is often undetected by appropriate laboratory analysis, because it is usually so low (8). In contrast, little is known about the response of lactate isomers towards bacteria and virus at the site of infection, namely in the lungs. Studies on the clinical utility of D-lactate as an indicator of ongoing bacterial infection in various compartments indicate that D-lactate may prove useful and be a supplement in assessing the effect of antimicrobial therapy (9, 10). In bacterially infected pleural fluid, the sensitivity of D-lactate as an indicator for ongoing bacterial infection was 60% and the specificity was 100% (10). Therefore, our secondary aim was to assess the utility of sputum D-lactate as marker of pulmonary bacterial invasion.

Markers of inflammation and pulmonary infection have been studied in patients with COPD and CF (1–3). CF-related chronic airway inflammation and tissue remodelling are thought to be caused by neutrophils that are present in large numbers in the airways of CF patients (1). In a study of sputum from CF patients with pulmonary exacerbation, there was an association between sputum neutrophil count and sputum lactate (2). However, this study did not differentiate between the two isomers of lactate. Increased markers of neutrophilic inflammation in sputum have been found to be associated with bacterial infection in COPD exacerbations (3),

potentially leading to increased levels of both L-lactate and D-lactate.

Altogether, we aimed to assess the utility of sputum L-lactate and sputum D-lactate as markers of pulmonary neutrophilic inflammation and bacterial invasion in the lungs of patients admitted with acute LRTI.

## MATERIALS AND METHODS

The study was a prospective observational study involving 32 patients admitted to the emergency ward with acute infection. Patients were enrolled prospectively soon after admission based on clinical suspicion of pneumonia or any other cause of LRTI if patients had new purulent expectoration. As participants at enrolment did not comply with the criteria for community-acquired pneumonia on admission X-ray would not be available to all eligible patients, the LRTI was defined as the working diagnosis based on clinical judgement involving purulent expectoration as well as minimum of one of the following: cough, fever, chest pain or (when available) an infiltrate on X-ray. Only patients who were  $\geq 18$  years old and able to expectorate could be enrolled. Participants were recruited consecutively during workdays (Monday to Friday) either during their stay in the emergency ward or after transfer to the Department of Lung and Infectious Diseases at University Hospital Nordsjælland between 1 November 2016 and 15 March 2017. Patients not able to expectorate, not willing to participate or unlikely to survive the following 72 h were excluded.

The study was approved by the Regional Scientific Ethical Committees (VEK H-16021931) and was in accordance with the Declaration of Helsinki. All study subjects gave written informed consent for their inclusion in the study.

Data on demography and co-morbidities were obtained from a questionnaire given to the participants on the day of inclusion. Same day, paraclinical parameters (e.g. blood leucocyte count and CRP) were obtained from medical records. Data on prior antibiotic treatment and corticosteroid use were obtained from both the questionnaire and the medical records.

### Sputum samples

Research samples were prioritized secondarily to routine samples for microbiological assessment. Sputum was collected during admission only on enrolment and repeated if the participant was able to produce a purulent expectorated sample on a subsequent day. Sputum samples were diluted (50 $\times$ ) and homogenized in phosphate-buffered saline (PBS), pH 7.4 (Substrate Department, Panum Institute, Denmark) and stored at  $-80$  °C until analysis. If the sputum was not already sent for routine analysis, the samples were divided into two aliquots, of which one was sent for routine analysis to the Department of Clinical Microbiology, Herlev University Hospital. Results were reported as the presence and identity of the pathogens detected in sputum.

Respiratory pathogens were identified from culture using biochemical methods and/or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Atypical bacteria (*Mycoplasma pneumoniae*, *Chlamydomphila pneumoniae*, *Chlamydomphila psittaci* and *Legionella pneumophila*) were identified using real-time PCR, and respiratory viruses were identified using PCR (e.g. *Metapneumovirus*, *Rhinovirus*, *Influenza A*, *Influenza B*, *Parainfluenza 1–3* and *Respiratory syncytial virus (RSV)*).

### Neutrophil count

The concentration of neutrophils in the sputum is shown as the exact numbers and was determined by adding 100  $\mu$ L of diluted sputum to a TruCount tube (BD 340334; Becton Dickinson). In addition, 5  $\mu$ L of each of the following mouse anti-human monoclonal antibodies (BD Biosciences, La Jolla, CA, USA) was added to the samples: peridinin chlorophyll A protein-labelled CD14 PerCP (BD345786), fluorescein isothiocyanate (FITC)-labelled CD15 (BD5554019) and allophycocyanin (APC)-labelled CD45 (BD555485). The samples were incubated for 30 min and fixated with 1 mL of 1 $\times$  fluorescence-activated cell sorter (FACS) lysis solution (BD349202) and stored overnight in dark at 5 °C before analysis by flow cytometry using a FACSCanto (BD Biosciences) equipped with a 15 mV argon-ion laser tuned at 488 nm, and a red laser-emitting diode at 635 nm for excitation. Light-scatter and exponentially amplified fluorescence parameters were recorded from at least 10 000 events. Leucocytes were identified by gating on CD45 and the polymorphonuclear leukocytes (PMN) were discriminated by gating on CD14 and CD15 as previously described (11). The instrument was calibrated with CS&T beads (BD Biosciences, Franklin Lakes, NJ, USA).

### Lactate measurement

The concentration of D-Lactate in sputum was measured in samples using a D-lactate colorimetric assay kit (MAK058, Sigma-Aldrich, Missouri, MO, USA) according to the manufacturer's recommendations. Samples were diluted (5 $\times$ ) if the useful concentration range of 0.1–10 mM D-lactate was exceeded. Frozen samples with 50 $\times$  diluted sputum were thawed and transferred to a 96-well microtitre plate. D-Lactate concentration was estimated by the addition of a reaction mix of D-lactate buffer, D-lactate enzyme mix and D-lactate substrate for 30 min in dark at room temperature, whereby the D-lactate was quantitated by the optical density at 450 nm measured with an enzyme-linked immunosorbent assay (ELISA) plate reader (Thermo Scientific Multiskan EX; Thermo Fisher Scientific Inc., BioImage, Søborg, Denmark). The D-lactate concentrations were determined by the interpolation of values generated from a standard curve.

The concentration of L-lactate in the sputum was measured in samples using a L-lactate assay kit (MAK064, Sigma-Aldrich) according to the manufacturer's recommendations. Samples were diluted (5 $\times$ ) if the useful concentration range of 1–2 mM L-lactate was exceeded. Frozen samples with 50 $\times$  diluted sputum were thawed and transferred to a 96-well microtiter plate. The L-lactate concentration was estimated by the addition of a master

reaction mix of lactate assay buffer, lactate enzyme mix and lactate probe for 30 min in dark at room temperature, whereby the L-lactate was quantitated by the optical density at 570 nm measured with an ELISA plate reader (Thermo Scientific Multiskan EX). The L-lactate concentrations were determined by the interpolation of values generated from a standard curve.

### Statistics

Normally distributed data are expressed as mean (standard deviation) and non-normally distributed expressed as median with 25–75th interquartile range (IQR). Categorical variables are expressed as number and percentage. Continuous variables not adhering to the normal distribution were analysed with non-parametric statistics. Correlation analyses were based on linear regression models presented as beta (B) coefficient and 95% confidence interval (95% CI). Regression analyses were performed to investigate the association between neutrophil granulocyte count in sputum with L-lactate and D-lactate. We used multilevel mixed models to adjust for clustering of individuals with more than one sputum sample to account for intra-individual correlation. Post-hoc q-q plots over standardized residuals were performed to verify the assumption of normally distributed data. Outliers were identified by plotting the data, but were kept in the final models, as the outliers did not alter the correlations reported (data not shown). A p-value  $\leq$  0.05 was considered statistically significant. Descriptive statistics and linear regression including q-q plots were performed in Stata (StataCorp LP, College Station, TX, USA), and graphs were made with GraphPad Prism 7.03 (GraphPad Software, La Jolla, CA, USA).

## RESULTS

Thirty-two participants met the criteria and participated in the study. Sixteen (50%) were females, and the median IQR age was 70 years (60–79) (Table 1). Data on pre-existing pulmonary disease were available for 30 (93.8%) participants, with 16 (50%) having COPD, eight (25%) having asthma and two (6.3%) having pulmonary fibrosis. A total of 44 sputum samples were collected from 32 participants. Although admitted to the emergency ward, participants were clinically stable with a mean (range) respiratory rate of 18 per minute (14–23), heart rate of 81 beats per minute (60–110), systolic blood pressure 127 mmHg (91–174), peripheral oxygen saturation of 94% (89–100) and temperature of 37.2 °C (35.9–38.2).

Antibiotic treatment had been initiated in all 32 (100%) participants when research samples were collected. Among 30 (93.8%) participants with data on corticosteroid treatment, 13 (43.3%) used oral prednisolone, 10 (33.3%) used inhaled corticosteroid and five (16.8%) with mixed use.

The neutrophil count was assessed in 44 sputum samples. The median neutrophil count was

**Table 1.** Characteristics of 32 patients admitted with lower respiratory tract infections (LRTI)

Age (years), median (IQR)	70 (60–79)
Female sex, % (n)	16 (50.0)
Body mass index (kg/m <sup>2</sup> ), median (IQR) (n = 30)	25.6 (23.4–29.3)
Cigarette smoking, n (%) (n = 30)	
Never	4 (13.3)
Current	7 (23.3)
Former	19 (63.3)
Package years n = 25, median (IQR)	39 (17–50)
Chronic respiratory diseases, n (%) (n = 30)	
Asthma	8 (25.0)
COPD	16 (50.0)
Pulmonary fibrosis	2 (6.3)
Diabetes n (%) (n = 29)	3 (10.3)
Blood neutrophil count (10 <sup>9</sup> /L) on admission (n = 27)	7.9 (5–12.5)

IQR, interquartile range; COPD, Chronic obstructive pulmonary disease.

0.615 × 10<sup>7</sup> cells/mL (IQR 0.236–1.575). Levels of measured parameters are shown in Table 2.

The measurement of L-lactate was performed in 41 sputum samples with a median of 1.939 mmol/L (IQR 0.800–3.101). The measurement of D-lactate was performed in 42 sputum samples with a median of 0.940 mmol/L (IQR 0.680–1.402). The sputum neutrophil count was associated with the level of sputum L-lactate (B = 31.811, 95% CI [7.276–56.345], p = 0.011) (Fig. 1A) and the level of CRP in blood (B = 1.390, 95% CI [0.241–2.538], p = 0.018) (Fig. 1C), but the neutrophil count was not associated with the level of D-lactate in sputum (B = 13.471, 95% CI [–6.067–33.010], p = 0.177) (Fig. 1B). We also found an association between sputum L-lactate and sputum D-lactate (B = 0.693, 95% CI [0.469–0.916], p < 0.0001) (Fig. 1D). Blood CRP was not associated with sputum L-lactate (B = 0.380, 95% CI [–8.270–9.029], p = 0.931) or sputum D-lactate (B = –0.412, 95% CI [–7.875–7.051], p = 0.914).

Among 26 (81.3%) participants with admission sampling for microbiological testing, 20 (76.9%) participants had a LRTI pathogen detected; 10 had

bacterial, 10 had viral and 2 of the participants had a mixed bacterial/viral infection (Table 3). Six (18.7%) of the samples for microbiology were either contaminated or lost between the emergency ward and the laboratory.

There was no difference in the level of L-lactate (B = –144.153, 95% CI [–1535.651–1247.34] n = 32, p = 0.839) and D-lactate (B = –2.320, 95% CI [–1156.545–1151.90] n = 32, p = 0.997) in sputum from participants with a negative sputum culture, compared to those with verified pathogenic bacteria detected.

There was a higher level of L-lactate (B = 1755.872, 95% CI [340.942–3170.801] n = 27, p = 0.015) and D-lactate (B = 1957.429, 95% CI [644.031–3270.826] n = 27, p = 0.003) in sputum from participants with a verified viral LRTI, compared to those without virus.

There was no difference in neutrophil count in sputum from participants with verified bacterial LRTI, compared to those without (B = 2.060, 95% CI [–12.103–16.225] n = 34, p = 0.776) and participants with a verified viral LRTI, compared to those without (B = –2.972, 95% CI [–21.075–15.130] n = 29, p = 0.748).

There was no difference in the level of blood CRP in participants with a negative sputum culture, compared to those with verified bacteria (B = 8.643, 95% CI [–38.624–55.911] n = 33, p = 0.720).

There was no difference in the level of blood CRP in participants with a verified viral LRTI, compared to those without (B = –12.175, 95% CI [–66.323–41.973] n = 29, p = 0.659).

## DISCUSSION

We measured the neutrophil count and the two isoforms of lactate (L- and D-lactate) in sputum in patients admitted with acute LRTI and found an association between the sputum neutrophil count and L-lactate, but not D-lactate. We saw an

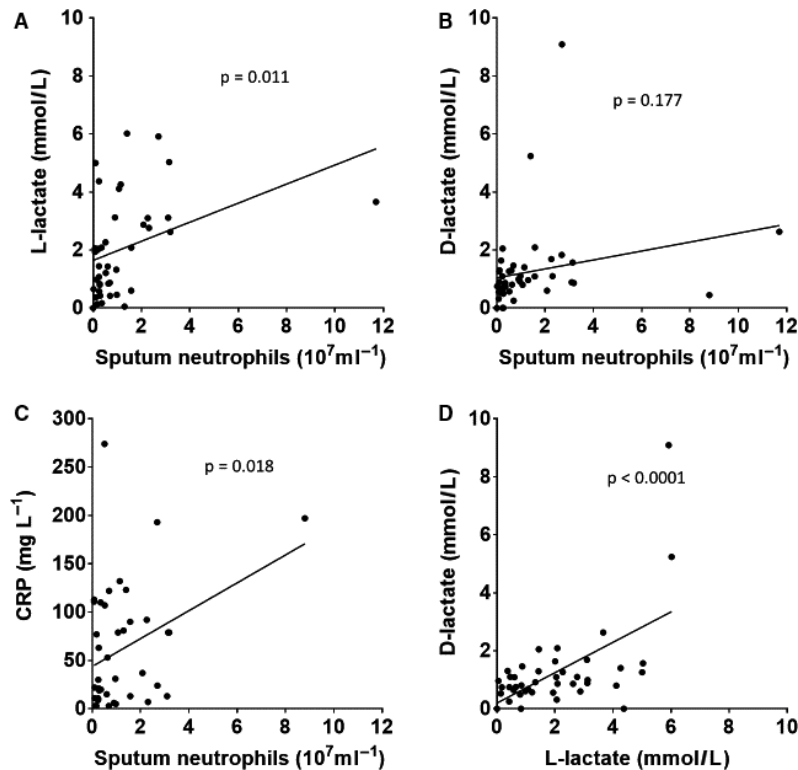
**Table 2.** Measured parameters

	All (n = 32)	Bacterial (n = 10) <sup>1</sup>	Virus (n = 10) <sup>2</sup>
Sputum			
Sputum neutrophil count (10 <sup>6</sup> /mL), mean ± SD	13.6 ± 9.7	13.8 ± 12.0	5.2 ± 7.1
L-lactate (mM), mean ± SD	2.0 ± 1.7	2.7 ± 1.6	2.0 ± 1.8
D-lactate (mM), mean ± SD	1.4 ± 1.5	1.5 ± 1.2	1.7 ± 2.4
Blood			
Blood neutrophil count (10 <sup>6</sup> /mL), mean ± SD	6.9 ± 2.6	6.7 ± 2.8	8.0 ± 2.5
CRP (mg/L), median (IQR)	30 (11–107)	22.5 (12–101.5)	15 (9–30)

CRP, C-reactive protein; IQR, interquartile range; SD, Standard deviation.

<sup>1</sup>Includes two samples of mixed bacterial and viral aetiology and includes three sputum samples with more than one bacterial pathogen.

<sup>2</sup>Includes two samples of mixed bacterial and viral aetiology.



**Fig. 1.** (A) The association between L-lactate and sputum neutrophil count in sputum from participants with suspected lower respiratory tract infections (LRTI). Unadjusted linear regression demonstrated a significant association ( $B = 31.811$ , 95% CI [7.276; 56.345],  $p = 0.011$ ). (B) The association between D-lactate and sputum neutrophil count in sputum from participants with suspected LRTI. Unadjusted linear regression did not demonstrate an association ( $B = 13.471$ , 95% CI [-6.067, 33.010],  $p = 0.177$ ). (C) The association between blood CRP and sputum neutrophil count in sputum from participants with suspected LRTI. Unadjusted linear regression demonstrated a significant association ( $B = 1.390$ , 95% CI [0.241; 2.538],  $p = 0.018$ ). (D) The association between sputum D-lactate and sputum L-lactate in sputum from participants with suspected LRTI. Unadjusted linear regression demonstrated a significant association ( $B = 0.693$ , 95% CI [0.469, 0.916],  $p < 0.0001$ ).

association between both L-lactate and D-lactate with viral infection, but not between L-lactate and D-lactate with bacterial infection. There was no difference in sputum neutrophil count among participants with and without verified pathogens. Earlier studies of sputum neutrophil count in COPD and CF patients have demonstrated that the augmented neutrophil count during exacerbation decreases to baseline values after eradication of the infection (2, 3). It is reasonable to believe that elevated sputum neutrophil count in acute LRTI declines when treated appropriately. The low detection rate of bacteria in the clinical laboratory may explain why we did not see an association between D-lactate and bacteria as we had expected.

During bacterial infection, neutrophil granulocytes are recruited from the bloodstream to the focus of infection (12). In pneumonia, this is expected to result in increased neutrophil count in the lungs and sputum. However, the inaccessibility of spontaneously expectorated sputum from non-inflamed

persons without infection prevents the inclusion of sputum samples from healthy persons as appropriate control.

Our finding of neutrophil count in the LRTI sputum being unaffected by the detection of virus or bacteria is in line with COPD patients during exacerbation (3). This indicates a strong contribution of viral infection to the lung inflammation in LRTI patients.

Earlier studies demonstrated that the lungs are capable of releasing lactate by metabolizing glucose, although the net balance of lactate is close to zero in healthy lungs (13–15). In the human neutrophil metabolism, lactate is produced in glycolysis by the cleavage from LDH (16). In disease states such as acute lung injuries, the net production of pulmonary lactate increases and correlates with the severity of the lung injury (17, 18). This lactate production is thought to be due to augmented cytokine effects on pulmonary and inflammatory cells (18, 19). In addition, viral infection of human cells may

**Table 3.** Detected bacterial and viral pathogens in 32 sputum samples<sup>1</sup>

	n (%)
<b>Bacterial</b>	
Negative sputum	15 (46.8)
Positive sputum <sup>2</sup>	13 (40.6)
Missing sputum for culturing	4 (12.5)
<i>S. pneumoniae</i>	2 (6.3)
<i>H. influenzae</i>	3 (9.4)
<i>Moraxella</i> sp.	3 (9.4)
<i>Mycoplasma</i> sp.	2 (6.3)
<i>S. marcescens</i>	1 (3.1)
<i>E. aerogenes</i>	1 (3.1)
<i>S. maltophilia</i>	1 (3.1)
<b>Virus</b>	
Negative nasopharyngeal swab/sputum	14 (43.8)
Positive nasopharyngeal swab/sputum	10 (31.3)
Missing sputum for culturing	8 (25.0)
<i>Respiratory syncytial virus</i> (RSV)	3 (9.4)
<i>Influenza A</i>	2 (6.3)
<i>Influenza B</i>	1 (3.8)
<i>Coronavirus</i>	2 (6.3)
<i>Rhinovirus/Enterovirus</i>	2 (6.3)

<sup>1</sup>Mixed bacterial/viral LRTI n = 2 (7.7%).

<sup>2</sup>Thirteen positive sputum samples from 10 patients.

induce the Warburg effect (20–22) leading to production of L-lactate (23).

The elevated levels of L-lactate in sputum and the association between sputum L-lactate and sputum neutrophil count strengthened our theory that L-lactate is a metabolite from human neutrophils and that raised levels of L-lactate may indicate ongoing airway inflammation in LRTI.

Previous studies of lactate production and increase in acute lung injuries have not differentiated between the two isoforms, L- and D-lactate (17, 18). In mammals, the amount of L-LDH exceeds the amount of D-LDH by far, and therefore L-lactate is the primary isoform of lactate in humans (8). The metabolic activity in bacteria halts immediately during the early phase of antibiotic treatment, and D-lactate has shown to be a potentially useful biomarker for successful therapy (9). Measuring D-lactate during antibiotic treatment in various body compartments has been promising as an indicator of decrease in bacterial load (2). In our study, D-lactate was detected in sputum, but we could not demonstrate an association between the sputum neutrophil count and sputum D-lactate, indicating that D-lactate does not originate from neutrophils. Instead, as we have assumed in this study, bacteria may be the source of D-lactate. However, this assumption is challenged by the lack of association between bacteria and D-lactate in our study. We speculate that the antibiotic treatment may have confounded the distribution of D-lactate in the sputum samples by reducing the bacterial production of D-lactate.

Virus and bacteria were detected in 41.7% and 40%, respectively, in sputum samples from patients

with acute LRTI in our study, which is in line with a previous study (5). However, we only detected 8% with a mixed bacterial/viral LRTI. This is in contrast with previous studies' finding of 26–81% with a mixed infection (5, 24, 25). This lack of detected microbes may have been prevented by using bronchoalveolar lavage, which is a very useful tool for diagnosing LRTI (26).

Despite that antibiotics had been initiated in all participants, the rate of positive cultures was relatively high in our study. One could consider if antibiotics, when eradicating sensitive bacteria leading to less diversity, allow overgrowth of other bacteria not involved in infection. That could explain why there is no association between bacterial infection and D-lactate in our study, but between viral infection and D-lactate. It has already been suggested that virus may disrupt the respiratory mucosal epithelium and impair the immune system as seen in influenza, where there is often a superinfection with *Staphylococcus aureus* (6, 27, 28).

That is supported by the strong association between sputum D-lactate and L-lactate. From the present study, D-lactate does not qualify as a good marker to differentiate bacterial from viral infection, while L-lactate seems to be strongly associated with severity of local pulmonary inflammation.

The research sampling was done at the first encounter with the participants already admitted and after initiation of antibiotics. Sampling should preferably have been done directly at admission before initiation of antibiotic treatment. We could not prioritize research samples over clinical samples, as this could impair detection rates, but unfortunately almost a fifth of samples for microbiology were contaminated or lost, which reduced the number of specimens detected. Sampling should preferably have been done before initiation of antibiotic treatment, as culture of common pathogens in LRTI (e.g. *Streptococcus pneumoniae*) is difficult after initiation. Thus, it is likely that some of the cultured samples from infected participants contained non-viable bacteria that could not be identified in the clinical laboratory and which potentially could have been found if a different PCR had been used. Further, culture on expectorated samples treated with antibiotics (e.g. penicillin) may cause opportunistic growth of mainly gram-negative bacteria (e.g. *Escherichia coli*), which are rarely a LRTI pathogen, but this was not seen.

## CONCLUSION

Our study has shown that L-lactate in sputum is associated with the neutrophil invasion in the lungs

in acute LRTI. Although D-lactate in sputum was a marker of specific interest, since it is unique to bacterial metabolism, it had no clinical relevance in the current study. Both sputum L-lactate and D-lactate were associated with viral LRTI but not bacterial, suggesting a greater inflammatory response in viral or mixed viral/bacterial than bacterial LRTI. This suggestion may be further verified by comparing to blood-derived biomarkers proposed to discriminate between bacterial and viral or non-bacterial LRTI such as procalcitonin (29).

As L-lactate is widely available to measure in laboratory settings, we recommend further studies to focus on the usefulness of this marker, and how it represents the invasion of leucocytes into the lungs. Furthermore, L-lactate should be evaluated as a marker to monitor treatment response directly in the lung, which may be more sensitive than systemic markers such as CRP. Finally, as patients admitted with LRTI often stop producing sputum early in the course of infection, L-lactate should be tested in patients who are intubated or having a tracheostomy, where routine tracheal suctioning is performed.

## FUNDING

The study was supported by the Nordsjællands Hospital.

## COMPETING INTERESTS

None.

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